Retinoid-Induced Modulation of Immunoglobulin M Secretion by Bovine Mononuclear Leukocytes In Vitro[1]

BRIAN J. NONNECKE,[2] TIMOTHY A. REINHARDT, and SHARON T. FRANKLIN
USDA, Agricultural Research Service
National Animal Disease Center
Metabolic Diseases and Immunology Research Unit
Ames, IA 50010-0070

ABSTRACT

Effects of *trans* and *cis* isomers of retinol and retinoic acid on IgM secretion by bovine peripheral blood mononuclear leukocytes were evaluated in vitro. Mononuclear leukocyte cultures that were unstimulated or stimulated by pokeweed mitogen were supplemented with isomers of retinol and retinoic acid at 10^{-10} to 10^{-6} *M*. Concentrations of polyclonal IgM in supernatants from 14-d cultures were measured by an ELISA. Cultures stimulated by pokeweed mitogen consistently secreted more IgM than parallel, unstimulated cultures. Retinoid supplementation did not affect basal IgM secretion by unstimulated cultures. However, each retinoid affected IgM secretion by cultures stimulated by mitogen. The nature of the effect was dependent on the concentration of the specific retinoid. All-*trans*-retinoic acid enhanced secretion at 10^{-10} *M* and inhibited secretion at 10^{-6} *M*. The other retinoids, however, did not inhibit IgM secretion at any concentration. Each retinoid enhanced IgM secretion at one or more concentrations, although enhancement was produced by much lower concentrations of retinoic acid isomers than retinol isomers. These results indicate that retinol and retinoic acid modulate polyclonal IgM secretion by cultures of bovine mononuclear leukocytes stimulated by mitogen. Future research will determine which subsets of the mononuclear leukocyte population are affected and whether *trans*-retinoic acid is the metabolite that produces these effects.

(**Key words**: retinol, retinoic acid, immunoglobulin, bovine lymphocytes)

Abbreviation key: **FBS** = fetal bovine serum, **MNL** = mononuclear leukocytes, **PWM** = pokeweed mitogen, **RAR** = retinoic acid receptors, **RXR** = retinoic X receptor.

INTRODUCTION

Vitamin A (retinol) and its metabolites are essential for growth and survival of all vertebrates. Collectively referred to as retinoids, they are required for normal vision, growth, and reproduction and have recently been shown (6, 27, 28) to modulate immune function and infectious disease susceptibility. Naturally occurring and experimentally induced vitamin A deficiencies are associated with depressed immunity (28). Documented effects of vitamin A deficiency include impaired antibody production (7, 26, 29, 31), decreased contact sensitivity to antigen (5), depressed natural killer cell activity (23), reduced lymphocyte proliferation induced by mitogens and antigens (5), and increased susceptibility to bacterial and viral diseases (22, 29). In general, repletion of vitamin A in deficient animals partially or completely restored immune function and disease resistance (7, 28).

Clinical and experimental data suggest that vitamin A status influences the susceptibility of dairy cows to economically important infectious diseases such as mastitis (6). Plasma vitamin A (retinol) concentrations decline during the periparturient period, when the risk of new IMI is high (11, 15, 17), pointing to a potential role for vitamin A in the maintenance of disease resistance. During this period, the in vitro functions of maternal peripheral blood lymphocytes and polymorphonuclear leuko-

Received December 14, 1992.
Accepted March 15, 1993.
[1]No endorsements are herein implied.
[2]To whom reprint requests should be addressed.

cytes are suppressed (10, 16). Studies by Chew (6) have suggested that supplemental vitamin A may enhance mastitis resistance. A more recent study (25) found that dietary supplementation with a high concentration of vitamin A, as retinol acetate, during the dry period and early lactation had no effect on udder health. In vitro studies by Tjoelker et al. (32, 33) indicated that vitamin A modulates the function of bovine polymorphonuclear leukocytes and lymphocytes.

Mechanisms by which retinoids exert their effects on immune function remain to be determined. However, many of the effects clearly are mediated via nuclear receptors (8, 12). Indeed, hematopoietic cells of different lineage and stages of differentiation constitutively express at least the α form of the retinoic acid receptor (**RAR**), and expression is unaffected either by differentiation or cell cycle (18). Retinoids, primarily as retinoic acid, appear to exert their effects through inducer and effector immune cell populations (28). Functional changes induced by retinoic acid are manifested by changes in secretion of cytokines. In this manner, retinoic acid increased production of human and murine interleukins (9, 34), suppressed interferon-γ production by human mononuclear leukocytes (**MNL**) (1), and promoted the release, but not the expression, of human tumor necrosis factor (36). It has been proposed (3, 4) that a retinol metabolite, distinct from retinoic acid, is essential for B-lymphocyte growth. In those studies (3, 4), 12-hydroxy-4,14-retro-retinol was a selective and essential factor, or cofactor, in B-cell proliferation.

However, studies evaluating effects of retinoids on ruminant immune cell function and disease susceptibility are needed. The present study compared the effects of the *cis* and *trans* isomers of retinol and retinoic acid on in vitro IgM secretion by bovine MNL that were unstimulated or stimulated by pokeweed mitogen (**PWM**).

MATERIALS AND METHODS

Cows and MNL Isolation

Eight female, age-matched, nonpregnant, adult Holstein cows were housed at the USDA, Agricultural Research Service, National Animal Disease Center. A pelleted diet containing ground corn cobs, wheat middlings, and corn, in addition to hay and pasture, was fed to meet energy and protein requirements. Water was provided for ad libitum intake. The cattle remained clinically normal throughout the experimental period. Plasma retinol and β-carotene concentrations, as measured by reverse-phase HPLC (11), averaged 345.8 and 427.9 ng/ml, respectively, during the experimental period. These values were within normal ranges for adult dairy cattle (11, 25). All animal-related procedures were approved by the Institutional Animal Care and Use Committee of the National Animal Disease Center.

The peripheral blood MNL were isolated from each of the eight cattle, as previously described (24). The MNL were enumerated (Celltrak-3B automated cell counter; Angel Engineering, Waltham, MA) and adjusted to 3.0×10^6 cells/ml in RPMI-1640 medium (Gibco, Grand Island, NY). The MNL-enriched cell population typically consisted of >95% MNL, of which approximately 90% were lymphocytes, and the remainder were monocytes. Cell viability in MNL suspensions was typically >90%. Cell suspension (500 μl) was used to seed IgM-secreting MNL cultures.

Vitamin A Metabolites

13-*Cis*-retinol, all-*trans*-retinol, 13-*cis*-retinoic acid, and all-*trans*-retinoic acid were from Sigma Chemical Co. (St. Louis, MO), and 9-*cis*-retinoic acid was generously provided by Milan Uskokovic (Hoffman-LaRoche, Nutley, NJ). Spectrophotometric and HPLC analysis indicated the purity of the retinoids to be >98%. Individual retinoids were solubilized in 100% HPLC-grade ethanol and diluted in heat-inactivated fetal bovine serum (**FBS**; Hyclone Laboratories, Inc., Logan, UT) with low endogenous concentrations of retinol (undetectable) and β-carotene (34 ng/ml). Analysis indicated retinoid concentration in FBS-retinoid stock solutions to be 1.5×10^{-5} *M*. When this method was used to prepare retinoids, the concentration of ethanol in control and retinoid-supplemented cultures was <.05%. This concentration of ethanol does not affect bovine MNL function in vitro (24). Retinoid-FBS preparations were stored at

–80°C until needed. Retinoids were exposed to light of minimum duration and intensity to minimize degradation. All retinoid preparations were submitted to a single cycle of freezing and thawing prior to use.

MNL Cultures

Cultures of unstimulated MNL and MNL stimulated by PWM (.08 μg/ml; Sigma Chemical Co.) were established concurrently in 24-well tissue culture plates (Costar, Cambridge, MA). The total volume of individual cultures was 1.5 ml, and cell density was maintained at 1.0×10^6 cells/ml for all experiments. All cultures contained a total of 6.7% by volume of FBS. Culture conditions (i.e., volume, cell density, mitogen concentration, and incubation period) providing optimal secretion of polyclonal IgM by PWM-stimulated MNL from donor cattle were established prior to the study. Retinoids in FBS were added to cultures at the initiation of the incubation period at concentrations ranging from 10^{-10} to 10^{-6} *M*. Parallel, control cultures received an equivalent volume of FBS without retinol or retinoic acid. The concentration of ethanol was the same (<.1%) in control and retinoid-supplemented cultures. Duplicate cultures were incubated for 14 d at 39°C in a humidified atmosphere consisting of 5% CO_2. Supernatants from cultures were harvested and frozen at –80°C until IgM analysis could be performed.

Quantitative Measurement of Polyclonal IgM

The concentration of polyclonal IgM in culture supernatants was determined by an ELISA performed as described (24). Reagents consisted of IgM standards (ICN Biochemical, Costa Mesa, CA), isotype-specific murine anti-bovine IgM (Ultimate Concentrations, Etna, NH), goat anti-murine Ig, biotinylated $F(ab')_2$ fragment (Amersham, Arlington Heights, IL), and streptavidin-biotinylated peroxidase complex (Amersham). Absorbance of test values and standards at 405 (test wavelength) and 490 nm (reference wavelength) was determined spectrophotometrically using an automated ELISA plate washer and reader (Dynatech, Guernsey Channel Islands, England). The IgM concentration in supernatants was quantitatively measured by comparison of absorbance of supernatants with absorbance of standards within a linear curve fit. Secretion was expressed as the concentration (micrograms per milliliter) of IgM in supernatants or as a percentage of the amount of IgM secreted by control cultures. Individual experiments evaluating effects of specific retinoids (at 10^{-10} to 10^{-6}) on cells from all donors were performed at least twice.

Data were presented ($\overline{X} \pm$ SEM), and statistical differences between control and retinoid-supplemented cultures were determined by Student's *t* test. Results were considered to be significant at $P \leq .05$.

RESULTS

Effects of Individual Retinoids on IgM Secretion by Bovine MNL Cultures

Secretion of IgM by unstimulated cultures was unaffected by retinol supplementation (Figure 1a). Concentrations of IgM in cultures supplemented with all-*trans*-retinol ranged from 1.9 to 2.1 μg/ml and in cultures supplemented with 13-*cis*-retinol from 1.3 to 1.5 μg/ml. Cultures stimulated with PWM and supplemented with all-*trans*-retinol at 10^{-6} *M* produced significantly ($P \leq .05$) more IgM than control cultures (13.8 vs 8.1 μg/ml) (Figure 1b). Cultures stimulated by PWM and supplemented with 13-*cis*-retinol at 10^{-6} *M* also produced significantly more ($P \leq .05$) IgM than control cultures (9.6 vs. 6.0 μg/ml). Retinol isomers at 10^{-10} to 10^{-7} *M*, however, did not affect IgM secretion by cultures stimulated with PWM.

Retinoic acid isomers, like retinol isomers, had no effect on IgM secretion by unstimulated cultures (Figure 2a). Concentrations of IgM ranged from .7 to 1.0 μg/ml in cultures supplemented with all-*trans*-retinoic acid, from .7 to 1.2 μg/ml in cultures supplemented with 13-*cis*-retinoic acid, and from .3 to 1.0 μg/ml in cultures supplemented with 9-*cis*-retinoic acid.

Trans-retinoic acid, at 10^{-10} *M*, significantly ($P \leq .05$) enhanced IgM secretion by cultures stimulated by PWM (Figure 2b). The concentration of IgM in supplemented cultures was 12.8 μg/ml compared with 8.6 μg/ml in unsupplemented, control cultures. Increasing all-*trans*-retinoic acid from 10^{-9} to 10^{-6} *M*

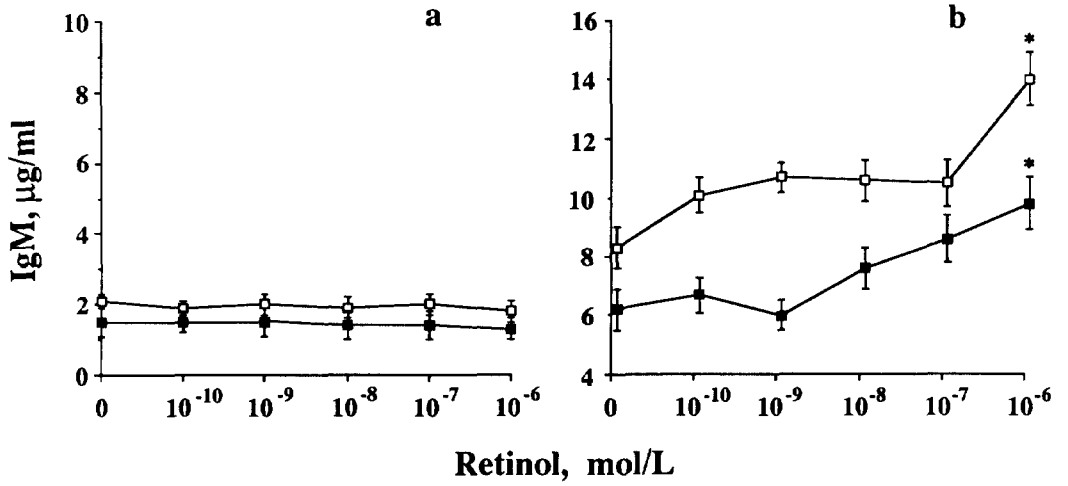

Figure 1. Effects of all-*trans*- (□) and 13-*cis*- (■) retinol isomers on IgM secretion by cultures of bovine peripheral blood mononuclear leukocytes that were unstimulated (a) or stimulated with pokeweed mitogen (PWM) (b). Cultures were unsupplemented or supplemented with retinol isomers at 10^{-10} to 10^{-6} *M*. Individual isomers were evaluated on separate days using cells from eight Holstein cows. * = Concentration of a specific isomer that caused a significant ($P \leq .05$; n = 8) change from unsupplemented controls in the amount of IgM secreted.

progressively decreased the concentration of IgM and, at 10^{-6} *M*, significantly ($P \leq .05$) inhibited IgM secretion relative to that of controls (4.4 vs 8.6 µg/ml). Neither 13- nor 9-*cis*-retinoic acids, unlike all-*trans*-retinoic acid, inhibited IgM secretion. Concentrations of IgM in cultures supplemented with 13-*cis*-retinoic acid at 10^{-9} and 10^{-8} *M* were 17.4 and 16.2 µg/ml, respectively, and were significantly higher ($P \leq .05$) than the concentration

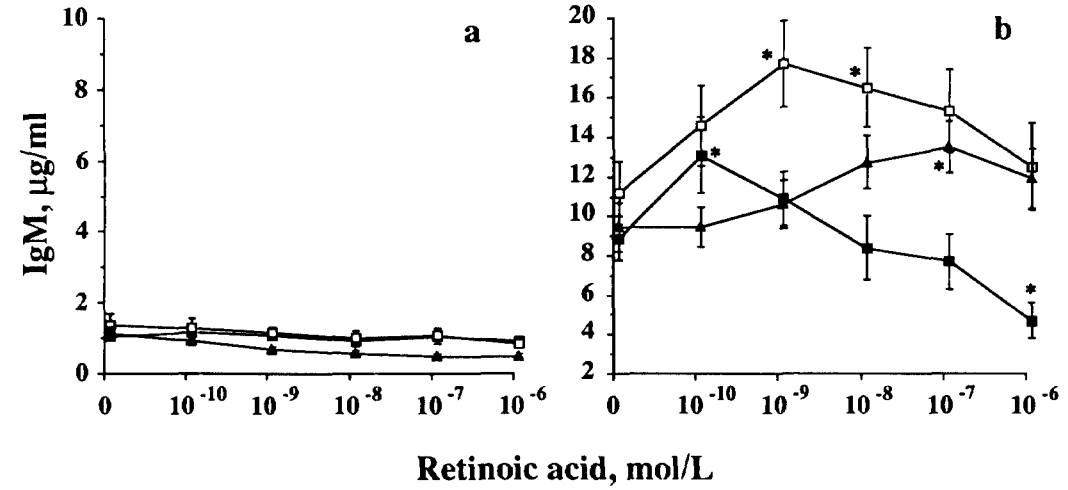

Figure 2. Effects of all-*trans*- (■), 13-*cis*- (□), and 9-*cis*- (▲) retinoic acid isomers on IgM secretion by cultures of bovine peripheral mononuclear leukocytes that were unstimulated (a) or stimulated with pokeweed mitogen (PWM) (b). Cultures were unsupplemented or supplemented with retinoic acid isomers at 10^{-10} to 10^{-6} *M*. Individual retinoic acid isomers were evaluated on separate days using cells from eight Holstein cows. * = Concentration of a specific isomer that caused a significant ($P \leq .05$; n = 8) change from controls in the amount of IgM secreted.

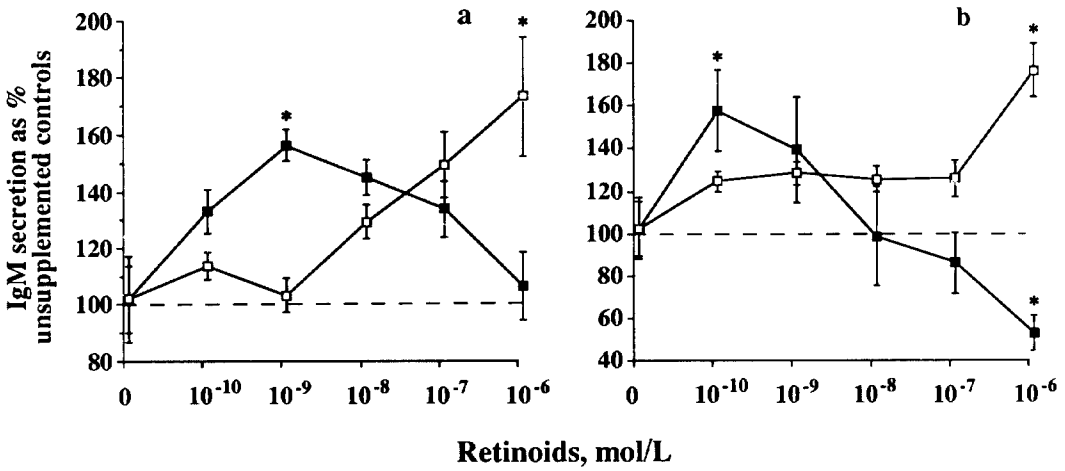

Figure 3. Comparison of the effects of 13-*cis*-retinoic acid (■) and retinol (□) isomers (a) and all-*trans*-retinoic acid (■) and retinol (□) isomers (b) on the relative amount of IgM secreted by cultures of bovine peripheral blood mononuclear leukocytes that were stimulated with pokeweed mitogen (PWM). Cultures were unsupplemented or supplemented with retinoids at 10^{-10} to 10^{-6} *M*. * = Concentration at which the amount of IgM secreted by cultures supplemented with retinoic acid differed ($P \leq .05$; n = 3) from IgM secretion by cultures supplemented with retinol.

(10.9 μg/ml) in unsupplemented cultures. At 10^{-7} *M*, 9-*cis*-retinoic acid significantly ($P \leq .05$) enhanced IgM secretion (13.2 μg/ml compared with 9.1 μg/ml secreted by control cultures).

Relative Amounts of IgM Secreted by Supplemented MNL Cultures

Cultures supplemented with 13-*cis*-retinoic acid at 10^{-9} *M* produced significantly more ($P \leq .05$) IgM than cultures supplemented with 13-*cis*-retinol at 10^{-9} *M* (154 vs. 101%, respectively, compared with that of parallel control cultures, Figure 3a). Concentrations of retinoic acid increased from 10^{-9} to 10^{-6} *M*, progressively inhibiting IgM secretion in cultures supplemented with 13-*cis*-retinoic acid and, in contrast, enhancing IgM secretion in cultures supplemented with 13-*cis*-retinoic acid. At 10^{-6} *M*, the concentration of IgM in cultures supplemented with 13-*cis*-retinoic acid was significantly greater ($P \leq .05$) than that in cultures supplemented with 13-*cis*-retinoic acid (171 vs 105%, respectively, compared with that of parallel control cultures).

The IgM concentration in cultures supplemented with all-*trans*-retinoic acid at 10^{-10} *M* was significantly greater ($P \leq .05$) than in cultures supplemented with all-*trans*-retinol at 10^{-10} *M* (155 vs. 122%, respectively, compared with that of parallel control cultures, Figure 3b). Increasing the concentration of these retinoids from 10^{-10} to 10^{-6} *M* produced a dramatic decrease in IgM concentrations in cultures supplemented with all-*trans*-retinoic acid and a contrasting increase in IgM concentrations in cultures supplemented with all-*trans*-retinol. When the retinoids were at 10^{-7} and 10^{-6} *M*, IgM concentrations in cultures supplemented with all-*trans*-retinol (123 and 174%, relative to control cultures) were significantly greater ($P \leq .05$) than in cultures supplemented with all-*trans*-retinoic acid (83 and 50%, relative to control cultures).

Simultaneous Evaluation of Retinoids on IgM Secretion

Effects of a single concentration of each retinoid previously shown (Figures 1 to 3) to enhance IgM secretion were examined concurrently. This approach was used to validate results from experiments evaluating retinoids independently. Data from these experiments are shown in Figure 4. Specific concentrations of retinoic acid and retinol isomers that were independently shown to enhance IgM secretion produced comparable, significant increases ($P \leq .05$) in IgM concentration when evaluated

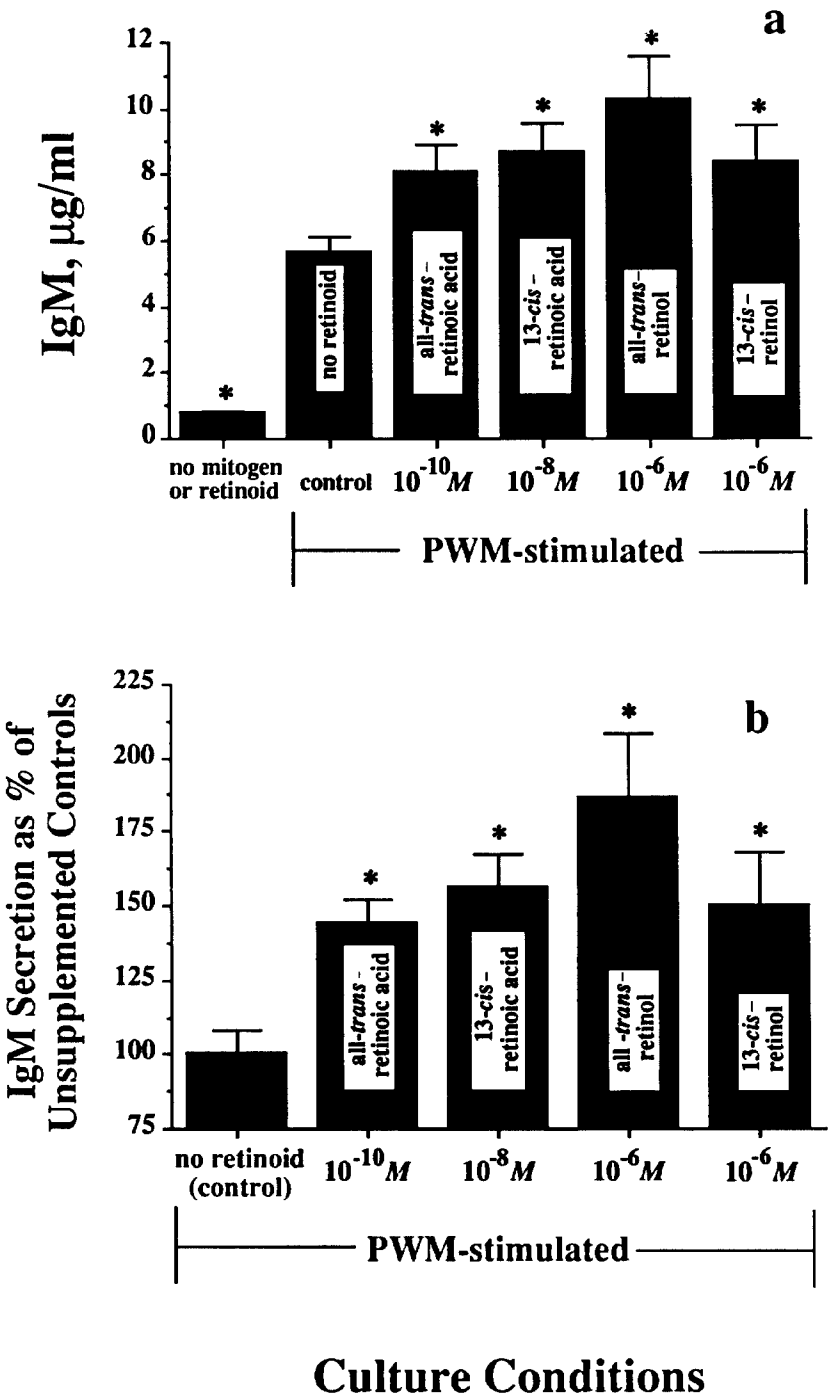

Figure 4. Results from experiments concurrently evaluating a single concentration of each retinoid shown previously in separate experiments to enhance IgM secretion. Effects of all-*trans*- and 13-*cis*-retinoic acid and retinol isomers on the actual (a) and relative (b) amounts of IgM secreted by cultures that were stimulated with pokeweed mitogen (PWM) and unsupplemented or supplemented with retinoid. The amount of IgM secreted by parallel unsupplemented, unstimulated cultures (a). * = Differences ($P \leq .05$; n = 8) between test and control cultures in the amount of IgM secreted.

simultaneously. The IgM concentration in unstimulated cultures without retinoids was significantly less ($P \leq .05$) than the concentration in parallel cultures stimulated by PWM.

DISCUSSION

Data presented in this study demonstrate that vitamin A (retinol) and its metabolites (retinoic acid) enhance polyclonal IgM secretion by bovine MNL stimulated by PWM. To our knowledge, this study is the first to document the enhancing effects of retinoids on IgM secretion by activated bovine lymphocytes in vitro. The unresponsiveness of resting bovine MNL cultures to retinoid supplementation suggests that leukocyte activation is essential for retinoids to produce their effects. This observation is supported by previous studies (14, 30) indicating that an initiation signal provided by a B-cell activator or antigen that is T cell-dependent is necessary for retinoic acid to induce augmentation of B-cell responses.

Enhancement of polyclonal IgM secretion by activated bovine MNL cultures supplemented with retinol or retinoic acid at physiologic concentrations is in general agreement with previous studies. Retinoic acid supplementation at concentrations similar to those evaluated in the present study increased the number of antibody plaque-forming cells stimulated with antigen (30), augmented IgM production by human-human B-cell hybridomas (2), and enhanced IgM production by human cord blood mononuclear cells stimulated with a polyclonal B cell activator that is T cell-dependent (14). The pivotal observation from those studies and from the present study is that retinoic acid and retinol augment Ig secretion by B cells stimulated by antigens or mitogens. Additional research is necessary to determine whether retinoid-induced enhancement of IgM secretion by bovine MNL cultures is due to direct actions of retinoids on the B cell or to indirect effects on other leukocyte subsets (T lymphocytes and monocytes).

Retinoic acids are more potent inducers, quantitatively and qualitatively, of cellular growth and differentiation than are retinol or retinal isomers (13, 20). Retinoids mediate these effects in a variety of cell types through nuclear RAR that bind all-*trans*-retinoic acid with high affinity (8). A previous study by Kizaki et al. (18) indicated that hematopoietic cells (monoblasts, myeloblasts, premyeloblasts, erythroblasts, and T lymphocytes) constitutively express RARα-mRNA, and the authors suggested that the immunological effects of retinoids might be mediated by the binding of all-*trans*-retinoic acid to RAR in leukocytes. Our results indicate that the all-*trans*- and 13-*cis*-retinoic acids were more potent at low concentrations than the retinol isomers in enhancing IgM secretion and suggest that the retinoic acids may be the principal mediators of retinoid-induced effects in bovine MNL cultures. The retinol likely is oxidized to retinoic acid in culture, thus explaining why more retinol is required to produce similar enhancement of IgM secretion. Unlike the other retinoids, all-*trans*-retinoic acid, the ligand for RAR, had a negative impact on IgM secretion at the highest concentrations tested. Further research is necessary to determine whether one or more of the RAR isoforms are present in bovine MNL and whether all-*trans*-retinoic acid is the metabolite responsible for the enhancement of IgM secretion in bovine MNL cultures supplemented with retinoid.

A second class of nuclear receptor for retinoic acid has been characterized that differs from RAR in primary structure, sensitivity to synthetic retinoid ligands, and ability to regulate expression of different target genes. Designated **RXR** (retinoic X receptor), this receptor is activated more by 9-*cis*-retinoic acid than by all-*trans*-retinoic acid (12). All-*trans*-retinoic acid does not bind RXR (21). All-*trans*-retinoic acid is isomerized to 9-*cis*-retinoic acid prior to binding to RXR. In contrast, 9-*cis*-retinoic acid is an equally effective ligand for the activation of RAR or RXR (19, 21, 35, 37). In the present study, 9-*cis*-retinoic acid was less potent than all-*trans*- and 13-*cis*-retinoic acids in enhancing IgM secretion by bovine MNL. This effect is likely due to the complex interactions between RXR and RAR on the regulation of gene transcription (19, 21, 35, 37). Specific roles for all-*trans*- and 9-*cis*-retinoic acids in the regulation of IgM secretion will be defined by future experiments in which their signals are present together.

CONCLUSIONS

These data indicate that retinol and retinoic acid isomers can alter the function of bovine MNL in vitro and suggest that changes in plasma and tissue concentrations of these reti-

noids may influence the function of the bovine immune system. Additional experiments are necessary to determine which MNL subpopulations are affected. Regulation of the expression of RAR and RXR in resting and activated MNL populations also should be considered so that the action of retinoids on the bovine immune system can be more fully understood.

ACKNOWLEDGMENTS

The authors thank D. McDorman and N. C. Eischen for their excellent technical expertise.

REFERENCES

1 Abb, J., H. Abb, and F. Deinhardt. 1982. Retinoic acid suppression of human leukocyte interferon production. Immunopharmacology 4:303.
2 Aotsuka, Y., and M. Naito. 1991. Enhancing effects of retinoic acid on monoclonal antibody production by human-human hybridomas. Cell Immunol. 133:498.
3 Buck, J., F. Derguini, E. Levi, K. Nakanishi, and U. Hammerling. 1991. Intracellular signaling by 14-hydroxy-4,14-retro-retinol. Science (Washington, DC) 254:1654.
4 Buck, J., A. Myc, A. Garbe, and G. Cathomas. 1991. Differences in the action and metabolism between retinol and retinoic acid in B-lymphocytes. J. Cell Biol. 115:851.
5 Carmen, J. A., S. M. Smith, and C. E. Hayes. 1989. Characterization of a helper T-lymphocyte defect in vitamin A-deficient mice. J. Immunol. 142:388.
6 Chew, B. P. 1987. Vitamin A and β-carotene on host defense. J. Dairy Sci. 70:2732.
7 Chun, T. U., J. A. Carmen, and C. E. Hayes. 1992. Retinoid repletion of vitamin A-deficient mice restores IgG responses. J. Nutr. 122:1062.
8 Giguere, V., E. S. Ong, P. Segui, and R. M. Evans. 1987. Identification of a receptor for the morphogen retinoic acid. Nature (Lond.) 330:624.
9 Goettsch, W., Y. Hatori, and R. P. Sharma. 1992. Adjuvant activity of all-*trans*-retinoic acid in C57/6 mice. Int. J. Immunopharmacol. 14:143.
10 Goff, J. P., M. E. Kehrli, Jr., and R. L. Horst. 1989. Periparturient hypocalcemia in cows: prevention using intramuscular parathyroid hormone. J. Dairy Sci. 72: 1182.
11 Goff, J. P., and J. R. Stabel. 1990. Decreased plasma retinol, α-tocopherol, and zinc concentrations during the periparturient period: effect of milk fever. J. Dairy Sci. 73:3195.
12 Heyman, R. A., D. J. Mangelsdorf, J. A. Dyck, R. B. Stein, G. Eichele, R. M. Evans, and C. Thaller. 1992. 9-Cis-retinoic acid is a high affinity ligand for retinoid X receptor. Cell 68:397.
13 Honna, Y., K. Takenaga, T. Kasukabe, and M. Hozumi. 1980. Induction of differentiation of cultured human promyelocytic leukemia cells by retinoids. Biochem. Biophys. Res. Commun. 95:507.
14 Israel, H., C. Odziemiec, and M. Ballow. 1991. The effects of retinoic acid on immunoglobulin synthesis by human cord blood mononuclear cells. Clin. Immunol. Immunopathol. 59:417.
15 Johnston, L. A., and B. P. Chew. 1984. Peripartum changes in plasma and milk β-carotene among dairy cows with or without mastitis. J. Dairy Sci. 67:1832.
16 Kehrli, M. E., Jr., B. J. Nonnecke, and J. A. Roth. 1988. Alterations in bovine neutrophil function during the periparturient period. Am. J. Vet. Res. 50:207.
17 Kendall, K. A., and K. E. Harshbarger. 1953. Plasma carotene and vitamin A levels of dairy cows before and after parturition. J. Dairy Sci. 36:583.
18 Kizaki, M., P. Koeffler, C. Lin, and C. W. Miller. 1990. Expression of retinoic acid receptor mRNA in hematopoietic cells. Leuk. Res. 14:645.
19 Kliewer, S. A., K. Umesono, D. J. Mangelsdorf, and R. E. Evans. 1992. Retinoid x receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D_3 signalling. Nature (Lond.) 355:446.
20 Lee, Q., M. R. Juchau, and J. C. Kraft. 1991. Microinjection of cultured rat embryos: studies with retinol, 13-cis- and all-trans-retinoic acid. Teratology 44:313.
21 Mangelsdorf, D. J., U. Boignaeyer, R. A. Heyman, Z. J. Yang, E. S. Ong, A. E. Oro, A. Kakizuka, and R. M. Evans. 1992. Characterization of three RXR genes that mediate the action of 9-*cis*-retinoic acid. Gene Dev. 6:329.
22 McMurray, D. N., R. A. Bartow, C. E. Mintzer, and E. Hernandez-Frontera. 1990. Micronutrient status and immune function in tuberculosis. Ann. New York Acad. Sci. 587:59.
23 Nays, K. M., and P. M. Newberne. 1985. Local and regional immune function of vitamin A-deficient rats with ocular herpes simplex virus (HSV) infections. J. Nutr. 115:1316.
24 Nonnecke, B. J., and R. L. Horst. 1992. 1,25-Dihydroxyvitamin D_3 enhancement of in vitro immunoglobulin secretion by mitogen-stimulated bovine peripheral blood lymphocytes. Immunol. Infect. Dis. 2:185.
25 Oldham, E. R., R. J. Eberhart, and L. D. Muller. 1991. Effects of supplemental vitamin A or β-carotene during the dry period and early lactation on udder health. J. Dairy Sci. 74:3775.
26 Pasatiempo, A. M., C. E. Taylor, and A. C. Ross. 1991. Vitamin A status and the immune response to pneumococcal polysaccharide: effects of age and early stages of retinol deficiency in rats. J. Nutr. 121:556.
27 Prabhala, R. H., S. Harinder, M. D. Garewal, F. L. Meyskens, and R. R. Watson. 1990. Immunomodulation in humans caused by beta carotene and vitamin A. Nutr. Res. 10:1473.
28 Ross, A. C. 1992. Vitamin A status: relationship to immunity and the antibody response. Proc. Soc. Exp. Biol. Med. 200:303.
29 Sijtsma, S. R., C. E. West, J. Rombout, and A. J. Van Der Zijpp. 1989. The interaction between vitamin A status and Newcastle disease virus infection in chickens. J. Nutr. 119:932.
30 Sidell, N., E. Fanatiga, and S. Golub. 1984. Immunological aspects of retinoids in humans. II. Retinoic acid enhances induction of hemolytic plaque-forming cells. Cell. Immunol. 88:374.
31 Smith, S. M., and C. Hayes. 1987. Contrasting impair-

ments in IgM and IgG responses of vitamin A-deficient mice. Proc. Natl. Acad. Sci. USA 84:5878.
32 Tjoelker, L. W., B. P. Chew, T. S. Tanaka, and L. R. Daniel. 1988. Bovine vitamin A and β-carotene intake and lactational status. 1. Responsiveness of peripheral blood polymorphonuclear leukocytes to vitamin A and β-carotene challenge in vitro. J. Dairy Sci. 71:3112.
33 Tjoelker, L. W., B. P. Chew, T. S. Tanaka, and L. R. Daniel. 1988. Bovine vitamin A and β-carotene intake and lactational status. 2. Responsiveness of mitogen-stimulated peripheral blood lymphocytes to vitamin A and β-carotene challenge in vitro. J. Dairy Sci. 71: 3120.
34 Trechsel, U., V. Evequoz, and H. Fleisch. 1985. Stimulation of interleukin-1 and -3 production by retinoic acid in vitro. Biochem. J. 230:339.
35 Tu, V., C. Delsert, B. Andersen, J. Holloway, O. Dejary, A. Naar, S. Kim, J. Boutin, C. Glass, and M. Rosenfeld. 1991. RXRβ: coregulation that enhances binding of retinoic acid, thyroid hormone and vitamin D receptors to their cognate elements. Cell 67:1251.
36 Turpin, J., K. Mehta, M. Blickk, J. P. Hester, and G. Lopez-Berestein. 1990. Effect of retinoids on the release and gene expression of tumor necrosis factor-α in human peripheral blood monocytes. J. Leuk. Biol. 48:444.
37 Zhang, X., B. Hoffman, P. Tran, G. Gräupner, and M. Pfahl. 1992. Retinoid x receptor is an auxiliary protein for thyroid hormone and retinoic receptors. Nature (Lond.) 355:441.