INFECTION AND IMMUNITY, Nov. 1985, p. 566–571
0019-9567/85/110566-06$02.00/0

Vol. 50, No. 2

Lymphokine-Induced Inhibition of Growth of *Eimeria bovis* and *Eimeria papillata* (Apicomplexa) in Cultured Bovine Monocytes†

C. A. SPEER,[1]* D. W. REDUKER,[1] D. E. BURGESS,[1] W. M. WHITMIRE,[1] AND G. A. SPLITTER[2]

Veterinary Research Laboratory, Montana State University, Bozeman, Montana 59717,[1] and Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin 53706[2]

Received 17 June 1985/Accepted 17 August 1985

Sporozoites of *Eimeria bovis* penetrated and developed normally to first-generation meronts in bovine monocytes (BM) and Madin-Darby bovine kidney (MDBK) cells that had been pretreated with culture medium (CM) or supernatant (NS) from nonstimulated bovine T cells. At 240 h after sporozoite inoculation (ASI), the mean percent development (meronts/[sporozoites + meronts]) in CM- and NS-pretreated BM was 52 and 28%, respectively; values for MDBK cells were 36 and 35%, respectively. Pretreatment of BM and MDBK cells with supernatant (ConAS) from concanavalin A-stimulated bovine T cells had no effect on the ability of sporozoites to penetrate cells; however, at 240 h ASI, only 1% of the sporozoites in ConAS-pretreated BM cultures had developed to meronts. In contrast, ConAS had no adverse effect on the ability of *E. bovis* sporozoites to develop to first-generation meronts in MDBK cells. At 240 h ASI, *E. bovis* meronts in ConAS-pretreated BM were abnormal in appearance and retarded in development, whereas sporozoites appeared structurally normal by light microscopy. Pretreatment of BM with ConAS had no effect on the ability of sporozoites of *Eimeria papillata* (Apicomplexa) to penetrate cells. Sporozoites of *E. papillata* did not develop to meronts in ConAS-pretreated BM and, in contrast to *E. bovis*, most sporozoites were destroyed intracellularly.

The invasion and development of *Eimeria* species in the intestinal tract of various animals may result in coccidiosis, a complex intestinal disease characterized by mortality, morbidity, and weight loss. Although virtually cosmopolitan in distribution and host species affected, coccidiosis probably has its greatest impact on the poultry and cattle industries around the world.

Although active infection in various animals results in immunity to subsequent challenge with the same *Eimeria* species and resistance to some *Eimeria* species appears to be predominantly cell mediated and T cell dependent (for a review, see reference 22), the mechanisms by which macrophages, sensitized T cells, or their soluble products (lymphokines) may function in providing protection against these parasitic protozoa are unknown. In other systems, lymphokines from T cells sensitized by specific antigens or heterologous agents (e.g., *Mycobacterium bovis* BCG, concanavalin A [ConA]) have been found to confer on macrophages enhanced antimicrobial activities against a variety of obligate and facultative intracellular parasites (3–5, 10, 16–21, 28). For example, peritoneal macrophages treated with lymphokines will inhibit intracellular replication of amastigotes of *Leishmania tropica* (10, 16, 18, 20).

We report herein the ability of a lymphokine(s) obtained from ConA-stimulated bovine T cells to induce cultured bovine monocytes (BM) to inhibit intracellular development of first-generation meronts of *Eimeria bovis* (a bovine parasite) and *Eimeria papillata* (Apicomplexa; a murine parasite).

MATERIALS AND METHODS

Bovine lymphocytes and T cells. Bovine peripheral blood leukocytes (PBL) were obtained from a 2-year-old Hereford steer as described previously (2) with some modification. Briefly, the buffy coat from 500 ml of heparinized (10 IU of heparin per ml) venous blood was removed onto 4 ml of Ficoll-Hypaque (Histopaque; Sigma Chemical Co., St. Louis, Mo.) and centrifuged at 350 × *g* for 35 to 45 min. The PBL-rich layer was removed and washed twice in phosphate-buffered saline, and the number of cells was determined by a Coulter electronic cell counter. Cell suspensions were washed twice with phosphate-buffered saline and suspended to 1.6×10^7 cells per ml in culture medium (CM; Eagle minimal essential medium containing 10% fetal calf serum, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM pyruvate, 50 U of penicillin G per ml, and 50 μg of gentamycin per ml). To enrich for T cells, 25 ml was loaded onto a 35-cc nylon wool (NW) column and incubated in 5% CO_2–95% air at 37°C for 1.5 h. The nonadherent (NA) cells were eluted with CM at 37°C. The effectiveness of NW T-cell enrichment was monitored by ConA stimulation in vitro. Approximately 10^5 NWNA cells or 5×10^4 NWNA cells plus 5×10^4 NW-adherent cells were cultured for 48 h in 200 ml of CM containing 0.5 μg of ConA per well with a 4-h pulse of 0.5 μCi of [^{3}H]thymidine per well. NA cells typically exhibited approximately (e.g., 148,961 cpm) 1.5 times the ConA supernatant (ConAS) stimulation levels of a 1:1 mixture of NA cells plus adherent cells (compared with 93,328 cpm), indicating substantial enrichment of T cells comparable to previously reported results (29).

Lymphokine production. CM samples (5 ml) containing 10^6 PBL or NWNA cells (T cells) per ml were incubated for 2, 3, or 4 days at 37°C in 5% CO_2–95% air in 25-cm^2 flasks in CM with or without ConA (4 μg of ConA per 2×10^6 cells per ml of medium). After incubation, the culture suspension was removed from each flask and centrifuged at 250 × *g* for 10 min to remove cells. The supernatant obtained from each culture flask was diluted to a volume of 9 ml with RPMI 1640 culture medium containing all the additives indicated above for minimal essential medium. Supernatants were stored at −20°C until used.

Parasites. *E. bovis* and *E. papillata* oocysts were sepa-

* Corresponding author.

† Journal Series no. J-1709, Montana State University Agricultural Station.

TABLE 1. Effects of T-cell supernatants on *E. bovis* in BM and MDBK cells

Cell type	Group	No. of intracellular sporozoites at the following times (h) ASI[a]: 1	6	24	48	240	No. of meronts (240 h ASI)	Meront development (%[b]; 240 h ASI)
BM	ConAS	125 ± 23 (n = 16)	190 ± 14[c]	156 ± 24[c]	137 ± 18[c]	62 ± 16[c]	1 ± 1[c]	1 ± 1[c]
	NS	121 ± 21	241 ± 17[c]	215 ± 14[c]	216 ± 32[c]	90 ± 18[c]	68 ± 15[c]	28 ± 7[c]
	RPMI 1640 medium	122 ± 21 (n = 16)	286 ± 39[c]	267 ± 37[c]	248 ± 42[c]	38 ± 8[c]	80 ± 11[c]	52 ± 5[c]
MDBK	ConAS	146 ± 14	132 ± 19[d]	121 ± 14[d]	166 ± 31[d]	40 ± 7[d]	40 ± 10	49 ± 7[d]
	NS	152 ± 14	168 ± 24	145 ± 16[d]	219 ± 40	68 ± 12	37 ± 9	35 ± 4
	RPMI 1640 medium	151 ± 20	183 ± 27	172 ± 12[d]	249 ± 44	73 ± 13	41 ± 8	36 ± 3

[a] Sample size, 12 counts unless otherwise indicated. Values are means ± standard deviation.
[b] Percent meront development = meronts/(sporozoites + meronts).
[c] Significantly different ($P \leq 0.05$) from other BM groups within each sample time.
[d] Significantly different ($P \leq 0.05$) from other MDBK groups within each sample time.

rated from feces by sugar flotation, sporulated in aerated aqueous 2.5% $K_2Cr_2O_7$, and stored at 4°C in 2.5% $K_2Cr_2O_7$. All oocysts used in these experiments were less than 5 months old. *E. bovis* or *E. papillata* oocysts were suspended in phosphate-buffered saline (pH 7.2) and broken by grinding in a motor-driven Teflon-coated tissue grinder, and sporozoites were excysted from sporocysts as described previously (27).

Continuous cell lines. Madin-Darby bovine kidney (MDBK) cells (American Type Culture Collection, Rockville, Md.) and BM are established cell lines. The BM line was originally obtained from blood monocytes of a 6-year-old Guernsey cow. BM have been found to be esterase positive and phagocytic, to have a normal karyotype, and to not express class II antigens of the major histocompatibility complex (G. A. Splitter, unpublished data). The CM used to maintain MDBK cells consisted of RPMI 1640 medium plus 10% fetal calf serum, 2 mM L-glutamine, 50 U of penicillin G per ml, and 50 μg of dihydrostreptomycin per ml. The CM used to maintain BM was the same as that for MDBK cells, except for the addition of 5×10^{-2} mM 2-mercaptoethanol per ml. All cultures were incubated at 38°C in 5% CO_2–95% air.

***Eimeria* spp. inhibition assay.** Each chamber on an eight-chamber slide (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) was inoculated with 0.3 ml of CM containing 3×10^4 cells of MDBK or BM. After incubation for 6 h, the CM was removed and replaced as follows. Two chambers on each slide were inoculated with CM only (0.3 ml), two chambers were inoculated with supernatant from nonstimulated PBL cultures (NS; 0.3 ml), and four chambers were inoculated with supernatant from ConAS-stimulated PBL cultures (ConAS; 0.3 ml). BM and MDBK cells were incubated for 24 h, the CM was removed by aspiration, and each well was inoculated with 0.3 ml of RPMI 1640 medium–2% fetal calf serum containing 1.9×10^4 freshly excysted sporozoites of *E. bovis* or *E. papillata*. At 1, 6, 24, 48, 96, and 240 h after sporozoite inoculation (ASI), cultures were fixed in Bouin's fluid, stained with Heidenhain's hematoxylin, and examined by bright-field microscopy.

The numbers of parasites present at various intervals ASI were determined as follows. All intracellular sporozoites and meronts of *E. bovis* or *E. papillata* present within five fields of view at a magnification of ×500 were recorded as one count. One or two counts were made on each of the two or four chambers treated with CM, ConAS, or NS. All experiments were performed in triplicate, with some portions in quadruplicate.

To determine whether rinsing the BM cells after the addition of ConAS (and before parasite inoculation) with CM would affect the inhibition of development of *E. bovis* sporozoites, BM cultures were prepared as indicated above, and four chambers containing BM cultures on each of two eight-chamber slides were treated with CM containing ConAS. Four chambers of BM cultures were treated with CM only. After incubation for 24 h, all eight chambers on one slide were rinsed twice with CM and then inoculated with CM containing 1.9×10^4 sporozoites of *E. bovis*. On the other slide, the CM was removed from each chamber without rinsing. Each chamber was then inoculated with CM containing 1.9×10^4 sporozoites of *E. bovis*. Both slides were incubated as above for 240 h and then fixed, stained, and examined by light microscopy.

The following experiment was performed to determine whether the addition at 120 h ASI of ConAS would enhance the inhibition of development of *E. bovis* sporozoites in BM cells. Cultures of BM in four chambers on an eight-chamber slide were prepared as described above and treated with CM only; four chambers with BM were treated with CM containing ConAS. After incubation for 24 h, the CM was removed, and each chamber was inoculated with CM containing 1.9×10^4 sporozoites of *E. bovis*. At 120 h ASI, the CM was removed from each chamber and replaced with the corresponding CM (i.e., CM only or CM containing ConAS). At 240 h ASI, the cultures were fixed, stained, and examined by light microscopy. The nonrinsed cultures of previous experiments served as controls.

Effects of IL-2. The effect of bovine interleukin 2 (IL-2) on the ability of *E. bovis* sporozoites to develop in BM was evaluated by the following experiment. IL-2 was obtained from the Gibbon cell line MLA-144 as previously described (15) and calibrated with the Jurkat IL-2 standard provided by the National Cancer Institute (Frederick, Md.). IL-2 (1 U/1 ml of CM) was applied to each monolayer of BM in four chambers on eight-chamber slides. The other four chambers were treated with CM only. After cultures were incubated for 24 h, the CM was removed, and each well was inoculated with CM containing 1.9×10^4 sporozoites of *E. bovis* plus 1 U of IL-2. Cultures were incubated for 240 h, after which they were fixed, stained, and examined by light microscopy.

Statistical analysis. Numbers of intracellular sporozoites and meronts and percent meront development were square root transformed and arcsine of the square root transformed, respectively. Differences between groups in total number of intracellular parasites and percent development (meronts/[sporozoites + meronts]) were tested for significance ($P \leq 0.05$) by using single-factor analysis of variance with Duncan's multiple range or with Student's t test (30). Meronts were considered to be those parasites at the stage in which they contain two or more nuclei.

RESULTS

At 1 h ASI, there were no significant differences among the numbers of *E. bovis* intracellular sporozoites in BM and MDBK cell cultures that had been treated previously with ConAS or NS from PBL or CM (Table 1). At 6, 24, and 48 h ASI, however, significantly fewer sporozoites occurred in BM cells treated with ConAS than in those treated with NS or CM. Significantly fewer sporozoites were present at 6, 24, and 48 h ASI in BM cultures treated with NS than in those treated with CM. At 240 h ASI, significantly more sporozoites were present in BM treated with NS than in those treated with ConAS, which in turn contained significantly more sporozoites than those treated with CM. At 6, 24, 48, and 240 h ASI, there were significantly fewer sporozoites in MDBK cells treated previously with ConAS than in those treated with NS or CM. Except at 24 h ASI, there was no significant difference in numbers of sporozoites in MDBK cells treated previously with NS and those treated with CM.

At 240 h ASI, significantly fewer *E. bovis* meronts were present in BM cultures treated previously with ConAS (1% development) than in those treated with NS (28% development) or CM (52% development) (Table 1; Fig. 1). There were also significantly fewer meronts in BM cultures treated with NS than in those treated with CM. A similar inhibitory effect was observed with ConAS on BM cultures inoculated with *E. papillata* (Fig. 2).

At 240 h ASI, most intracellular sporozoites in ConAS-treated BM had not begun to develop and yet appeared structurally normal (Fig. 3 and 4). Crescent bodies that have been found to appear within a parasitophorous vacuole or the host cell cytoplasm with certain *Eimeria* spp. (26) were present in ConAS-, NS-, and CM-treated BM and MDBK cells containing sporozoites and meronts of *E. bovis* (Fig. 4 and 5). NS- and CM-treated BM that contained immature and mature meronts each had an enlarged nucleus and nucleolus (Fig. 6), whereas ConAS-treated BM harboring immature meronts never had enlarged nuclei and nucleoli. Most host cells harboring mature meronts were spheroid and elevated above the bottom of the culture vessel (Fig. 7). Mature *E. bovis* meronts each contained several thousand merozoites. Numerous extracellular merozoites were present at 240 h ASI in NS- and CM-treated BM (Fig. 7) and all MDBK cell cultures. Even though some parasite development occurred in BM cultures treated previously with ConAS, all meronts were retarded in development; no mature meronts were observed. Retarded meronts were irregular in shape, had few nuclei, which were small and irregular in shape, and were usually not surrounded by a prominent parasitophorous vacuole.

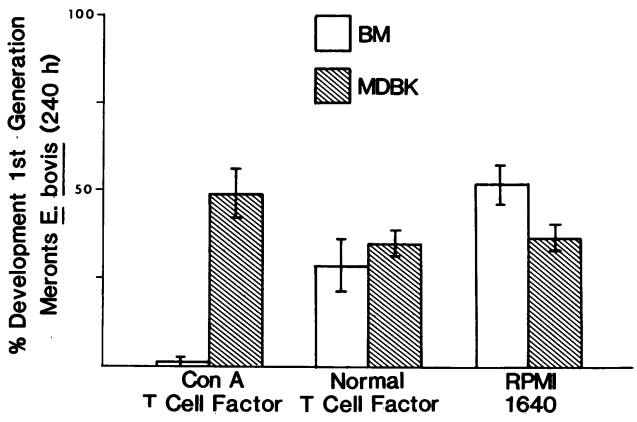

FIG. 1. Effects of T-cell supernatants on the percent development of first-generation meronts of *E. bovis* in BM and MDBK cells 240 h ASI.

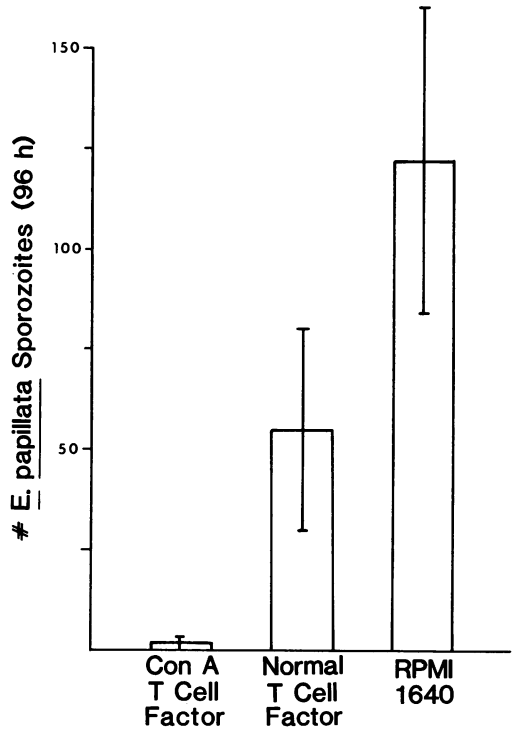

FIG. 2. Effects of T-cell supernatants on the number of intracellular sporozoites of *E. papillata* 96 h ASI of BM.

No difference in development was found between MDBK cells treated with NS or CM (Table 1; Fig. 1). Even though there was a greater percent development of *E. bovis* in MDBK cells treated with ConAS than in those treated with NS or CM, there were fewer parasites in ConAS-treated cultures than in NS- or CM-treated cultures. The mean numbers of parasites in these cultures (± standard deviation) were 80 ± 14, 97 ± 31, and 114 ± 20, respectively. Similar results were obtained in experiments that used supernatants from NWNA cells.

There were no significant differences in numbers of intracellular sporozoites of *E. bovis* between BM cultures treated with ConAS (from NWNA cells) and rinsed twice with CM (73 ± 21) and those that were not rinsed (67 ± 17). Meront development was slightly greater in the rinsed (8 ± 2) than in the nonrinsed (4 ± 2; $P \leq 0.05$) wells.

There was no significant difference in percent meront development of *E. bovis* between ConAS-treated cultures that had ConAS (from NWNA cells) added twice (24 h

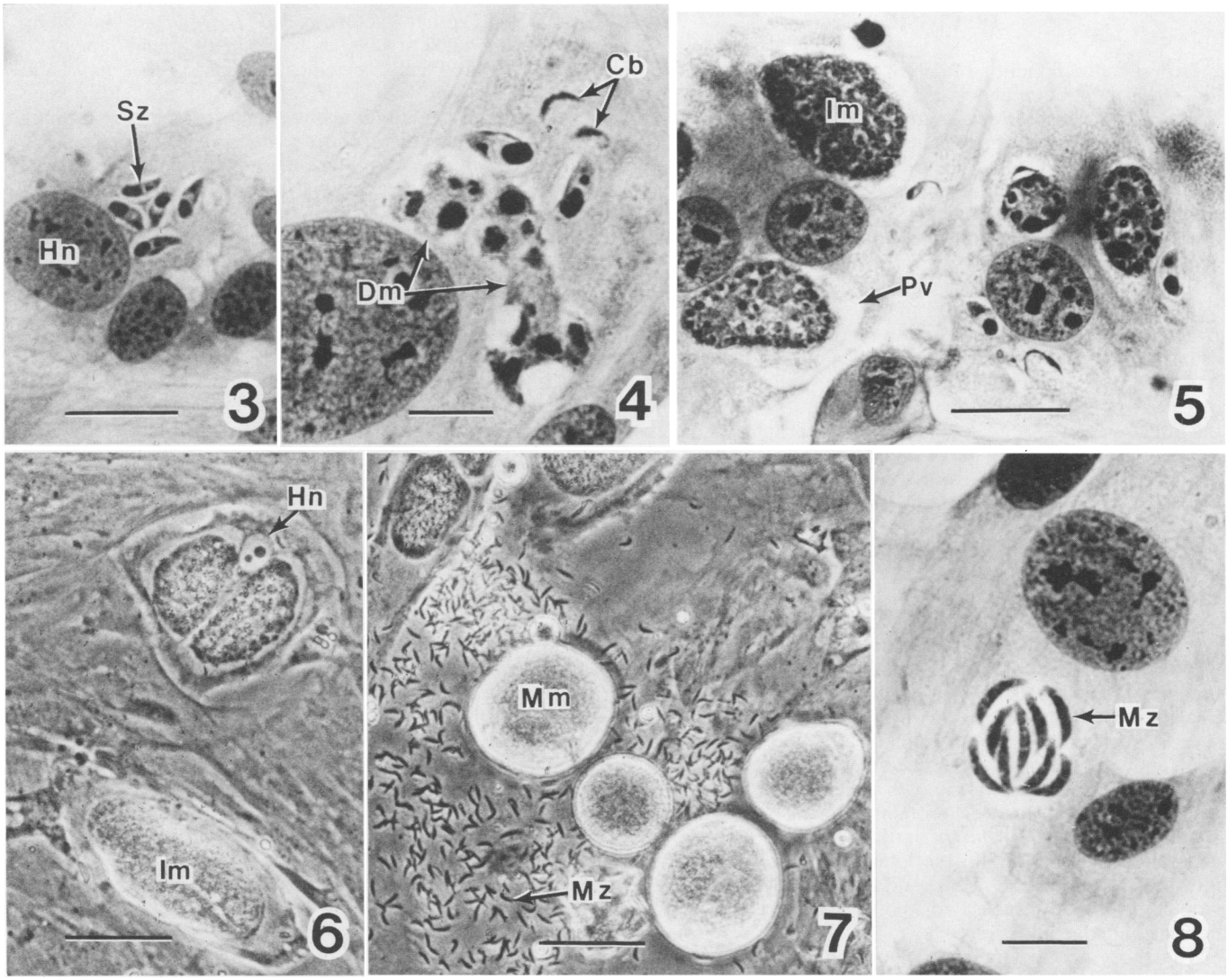

FIG. 3 to 8. Photomicrographs of *E. bovis* (Fig. 3 to 7) and *E. papillata* (Fig. 8) in BM. Fig. 3 to 5 and 8 are bright-field photomicrographs of fixed and stained specimens; Fig. 6 and 7 are of live specimens photographed with phase-contrast microscopy. Types of pretreatment and interval ASI of BM are listed below in parentheses. FIG. 3. Five intracellular sporozoites (Sz); Hn, host cell nucleus. (ConAS; 240 h.) Bar = 20 μm; magnification, ×800. FIG. 4. Two sporozoites and two degenerate meronts (Dm) in one BM; note crescent bodies (Cb) in host cell cytoplasm. (ConAS; 240 h.) Bar = 10 μm; magnification, ×1,170. FIG. 5. Sporozoites and immature meronts (Im) in several BM; note that Im's are surrounded by a prominent parasitophorous vacuole (Pv). (CM; 240 h.) Bar = 20 μm; magnification, ×860. FIG. 6. BM infected with immature meronts (Im); note that one BM that has an enlarged nucleus (Hn) and nucleolus contains two immature meronts. (CM; 240 h.) Bar = 50 μm; magnification, ×300. FIG. 7. Several mature meronts (Mm); note numerous free, first-generation merozoites (Mz). (NS; 240 h.) Bar = 50 μm; magnification, ×300. FIG. 8. Mature, first-generation meront with 9 merozoites (Mz). (CM; 96 h.) Bar = 10 μm; magnification, ×1,170.

before sporozoite inoculation and 120 h ASI) compared with those that had ConAS added once (24 h before sporozoite inoculation) (4 ± 5 and 4 ± 2, respectively). However, there were significantly fewer intracellular sporozoites in BM cultures treated twice with ConAS than in those treated once with ConAS (47 ± 3 and 67 ± 17, respectively).

IL-2 had no effect on the ability of *E. bovis* sporozoites to develop to first-generation meronts. There was no difference in parasite development in IL-2-treated BM cultures (68 ± 10) and those treated with CM (55 ± 11).

The effect of ConAS on the development of *E. papillata* in BM cultures was markedly different from the effect on *E. bovis*. At 96 h ASI, significantly fewer intracellular sporozoites of *E. papillata* were present in BM cultures treated with ConAS (from NWNA cells) than in NS- or CM-treated cultures (Fig. 2). Also, there were significantly fewer sporozoites in NS-treated BM cultures than in those treated with CM. Periodic microscopic examination of live cultures at 24 to 96 h ASI revealed few extracellular sporozoites in ConAS- and NS-treated BM cultures. Microscopic examination of fixed and stained preparations showed that most *E. papillata* intracellular sporozoites in ConAS-treated BM cells were structurally altered. Occasionally, sporozoites had fragmented into two or three pieces within the cytoplasm of ConAS-treated BM cells. In CM-treated BM, some *E. papillata* sporozoites developed to mature first-generation meronts, each of which contained 8 to 14 merozoites (Fig. 8). No meronts were present in ConAS-treated BM cultures.

DISCUSSION

Experimental infection of calves with *E. bovis* has shown that the parasite completes its endogenous development in about 3 to 4 weeks after oocyst inoculation (7–9). *E. bovis* sporozoites excyst from oocysts and penetrate endothelial cells of the central lacteal of the small intestine where they develop into first-generation meronts, each of which contains about 120,000 merozoites (7). During weeks 3 and 4 of development, a small second-generation meront, gametocytes, and oocysts are formed in the glandular epithelium of the cecum and large intestine. Hammond et al. (6) found that the small intestines of calves immunized against *E. bovis* had fewer first-generation meronts and merozoites at 14 to 16 days after a single inoculation of oocysts than did nonimmunized calves. At 4 weeks after the peak oocyst discharge of the immunizing infection, oocysts retained from the infection were found surrounded by macrophages within the lamina propria of the large intestine. Thus, the developmental cycle of *E. bovis* from sporozoite to oocyst allows ample time for exposure to effector cells such as macrophages.

In the present study, supernatant obtained from ConAS-stimulated bovine T cells induced an established cell line of BM to inhibit development of *E. bovis* sporozoites to first-generation meronts. This finding indicates that macrophages, activated by a soluble product(s) from T cells which we term *Eimeria* inhibitory factor, may be a protective immune effector mechanism against *E. bovis* infections. Based on previous studies with several different *Eimeria* species, resistance to reinfection appears to be T cell dependent and predominately cell mediated (for a review, see reference 22). It is still not known which T cell factor(s) or effector cells may provide protection against any *Eimeria* species. Because *E. bovis* initially develops to first-generation meronts within the lamina propria of the small intestine, sensitized T cells and activated macrophages would have ready access to sporozoites and merozoites during challenge infections.

The substance in stimulated T-cell supernatants that induces macrophage activation has been called macrophage-activating factor and has generally been associated with a protein of 40,000 molecular weight (22). Recent biochemical and immunological evidence strongly indicates that this activity is associated with gamma interferon (23) and may be identical to it. Another lymphokine of 22,000 molecular weight secreted by a continuous T-cell line has also been shown to activate macrophages (14). Because the lymphokine used in the present study had no inhibitory effect on the growth of *E. bovis* in MDBK cells (a nonphagocytic, epithelioid cell line), it appears that the inhibitory effect on the growth of *E. bovis* in BM may not be due solely to gamma interferon. In fact, ConAS appeared to have a stimulatory effect on the growth of *E. bovis* in MDBK cells, because a significantly greater percent parasite growth occurred in these cultures than in those treated with NS or CM (Fig. 1). However, there were more parasites present in NS- or CM-treated MDBK cell cultures, respectively, than in ConAS-treated ones. Thus, it is likely that a greater percent growth occurred in ConAS-treated cultures because the parasite burden in these cultures was much smaller than in those treated with NS or CM.

Recent studies have shown that there are at least two lymphokines that are capable of protecting cultured macrophages, fibroblasts, and kidney cells against *Toxoplasma gondii*. One lymphokine appears to be gamma interferon (21, 24, 25). The other factor, called *Toxoplasma* inhibiting factor, has a 4,000 to 5,000 molecular weight and appears to protect cultured cells specifically against *T. gondii* and *Besnoitia jellisoni* (4, 5). Interferons do not show such specificity for infectious agents, and recombinant gamma interferon has been found to protect cells almost equally against *T. gondii* and vesicular stomatitis virus (21). All interferons are known to have antiproliferative and direct cytotoxic effects on their homologous host cells. Such adverse effects by gamma interferon may indirectly restrict the growth of *Toxoplasma* sp. in cultured cells.

Although we do not know the precise nature of the *Eimeria* inhibitory factor, it does not appear to be IL-2, because no inhibition of *E. bovis* growth occurred in IL-2-treated BM cultures. This was expected, because Baker et al. (1) showed that *Toxoplasma* inhibiting factor and IL-2 are distinct molecules with different biological activities. Further experiments are needed to identify and characterize the lymphokine responsible for the inhibition of intracellular development of *E. bovis*.

Regardless of the identity of the T-cell product that mediates the inhibition of *E. bovis* development, it is fast acting and long lasting. Rinsing BM cultures treated once with ConAS to remove excess ConAS resulted in only a slight decrease in the inhibitory effect of ConAS on the development of *E. bovis*. Even though the cells were treated for only 24 h with ConAS before sporozoite inoculation, the antiparasite effect was still present at 10 days ASI. Also, an additional treatment of BM with ConAS at 5 days ASI did not significantly enhance the antiparasite effect by BM on *E. bovis* sporozoites, indicating a single-hit activation process. In contrast, Nacy et al. (18) found that mouse peritoneal macrophages treated with lymphokines after infection exhibited potent microbicidal activity against *L. tropica*, whereas lymphokine-pretreated cells were only microbistatic. Such differences are probably due to variations in the *Leishmania* spp.-murine and *Eimeria* spp.-bovine in vitro systems.

Treatment of cultures with ConAS or NS had no adverse effect on the ability of *E. bovis* sporozoites to penetrate BM, and most intact *E. bovis* sporozoites remaining within ConAS-treated BM appeared normal; only a few were morphologically abnormal. However, at 6, 24, and 48 h ASI, significantly fewer intracellular sporozoites of *E. bovis* were present in ConAS-treated BM cultures than in those treated with NS or CM. This indicates that some sporozoites were destroyed intracellularly by BM or that they escaped from BM and were lost in the medium. *E. papillata* sporozoites also easily penetrated ConAS-treated BM but, in contrast to *E. bovis*, ConAS had an adverse effect on the intracellular development of its sporozoites. Cultures treated with NS and CM had approximately 30- and 70-fold more intracellular sporozoites of *E. papillata*, respectively, than ConAS-treated cultures. Thus, it is likely that *E. papillata* sporozoites were destroyed intracellularly, because few live extracellular sporozoites were observed at 24 to 96 h ASI.

On the basis of the findings in this study, we speculate that a dormant stage equivalent to the hypnozoite (11, 12) may survive within the bovine host in the form of a resistant, slowly developing sporozoite or a new intracellular morphological form. Such a dormant parasite form could be responsible for the occurrence of winter coccidiosis in yearling calves which are certain to be infected with, and partially immune to, several species of *Eimeria*. Within a few days to 2 to 3 weeks after yearling calves have been stressed, many of these animals become severely infected with *E. bovis* and *Eimeria zuernii* and exhibit typical symptoms of coccidiosis

(13; personal observations). Because in the present study intracellular and motile extracellular sporozoites of *E. bovis* were observed at 240 h ASI of ConAS-treated BM, it is likely that these parasites were still viable and capable of developing into meronts. Preliminary studies have shown that parasites from ConAS-treated BM will develop to first-generation meronts when transferred to untreated BM cultures (unpublished data). If such a mechanism is responsible for outbreaks of winter coccidiosis, it is likely that the parasite may be arrested at several stages in its development (i.e., sporozoite, merozoite, or gametocyte).

ACKNOWLEDGMENTS

We thank G. M. Callis and K. F. Knoblock for technical assistance.
This work was supported by grants 26000-972 and 26000-974 from the U.S. Department of Agriculture.

LITERATURE CITED

1. **Baker, P. E., A. Hagemo, K. F. Knoblock, and J. P. Dubey.** 1983. *Toxoplasma gondii*: microassay to differentiate *Toxoplasma* inhibiting factor and interleukin 2. Exp. Parasitol. **55**:320–330.
2. **Baker, P. E., and K. F. Knoblock.** 1982. Bovine costimulator. I. Production kinetics, partial purification and quantification in serum-free Iscove's medium. Vet. Immunol. Immunopathol. **3**:365–379.
3. **Byrne, G. I., and C. L. Faubion.** 1982. Lymphokine-mediated microbiastatic mechanisms restrict *Chlamydia psittaci* growth in macrophages. J. Immunol. **128**:469–474.
4. **Chinchilla, M., and J. K. Frenkel.** 1978. Mediation of immunity to intracellular infection (*Toxoplasma* and *Besnoitia*) within somatic cells. Infect. Immun. **19**:999–1012.
5. **Chinchilla, M., and J. K. Frenkel.** 1984. Specific mediation of cellular immunity to *Toxoplasma gondii* in somatic cells in mice. Infect. Immun. **46**:862–866.
6. **Hammond, D. M., F. L. Andersen, and M. C. Miner.** 1963. The site of the immune reaction against *Eimeria bovis* in calves. J. Parasitol. **49**:415–424.
7. **Hammond, D. M., F. L. Andersen, and M. L. Miner.** 1963. The occurrence of a second generation in the life cycle of *Eimeria bovis* in calves. J. Parasitol. **49**:428–434.
8. **Hammond, D. M., G. W. Bowman, L. R. Davis, and B. T. Simms.** 1946. The endogenous phase of the life cycle of *Eimeria bovis*. J. Parasitol. **32**:409–427.
9. **Hammond, D. M., J. V. Ernst, and M. L. Miner.** 1966. The development of first-generation schizonts of *Eimeria bovis*. J. Protozool. **13**:559–564.
10. **Hoover, D. L., and C. A. Nacy.** 1984. Macrophage activation to kill *Leishmania tropica*: defective intracellular killing of amastigotes by macrophages elicited with sterile inflammatory agents. J. Immunol. **132**:1487–1493.
11. **Krotoski, W. A., D. M. Krotoski, P. C. C. Garnham, R. S. Bray, R. Killick-Kendrick, C. C. Draper, G. A. T. Targett, and M. W. Guy.** 1980. Relapses in primate malaria: discovery of two populations of exoerythrocytic stages. Preliminary note. Br. Med. J. **1**:153–154.
12. **Markus, M. B.** 1978. Terminology for invasive stages of protozoa of the subphylum Apicomplexa (Sporozoa). S. Afr. J. Sci. **74**:105–106.
13. **Marquardt, W. C.** 1976. Some problems of host and parasite interactions in the coccidia. J. Protozool. **23**:287–290.
14. **Meltzer, M. S., W. R. Benjamin, and J. J. Farrar.** 1982. Macrophage activation for tumor cytotoxicity: induction of macrophage tumoricidal activity by lymphokines from EL-4, a continuous T cell line. J. Immunol. **129**:2802–2807.
15. **Miller-Edge, M., and G. A. Splitter.** 1984. Bovine interleukin 2 (IL-2) production and activity on bovine and murine cell lines. Vet. Immunol. Immunopathol. **7**:119–130.
16. **Nacy, C. A., A. H. Fortier, M. G. Pappas, and R. R. Henry.** 1983. Susceptibility of inbred mice to *Leishmania tropica* infection: correlation of susceptibility with in vitro defective macrophage microbicidal activities. Cell. Immunol. **77**:298–307.
17. **Nacy, C. A., and M. S. Meltzer.** 1979. Macrophages in resistance to rickettsial infection: macrophage activation in vitro for killing of *Rickettsia tsutsugamuski*. J. Immunol. **123**:2544–2549.
18. **Nacy, C. A., M. S. Meltzer, E. J. Leonard, and D. J. Wyler.** 1981. Intracellular replication and lymphokine-induced destruction of *Leishmania tropica* in C3H/HeN mouse macrophages. J. Immunol. **127**:2381–2386.
19. **Nogueira, N., and Z. A. Cohn.** 1978. *Trypanosoma cruzi*: induction of macrophage microbicidal activity. J. Exp. Med. **148**:288–300.
20. **Oster, C. N., and C. A. Nacy.** 1984. Macrophage activation to kill *Leishmania tropica*: kinetics of macrophage response to lymphokines that induce antimicrobial activities against amastigotes. J. Immunol. **132**:1494–1500.
21. **Pfefferkorn, E. R., and P. M. Guyre.** 1984. Inhibition of growth of *Toxoplasma gondii* in cultured fibroblasts by human recombinant gamma interferon. Infect. Immun. **44**:211–216.
22. **Rose, M. E.** 1982. Host immune responses, p. 329–371. *In* P. L. Long (ed.), The biology of the coccidia. University Park Press, Baltimore.
23. **Schreiber, R. D., J. L. Pace, S. W. Russell, A. Altman, and D. H. Katz.** 1983. Macrophage activating factor produced by a T cell hybridoma: physicochemical and biosynthetic resemblance to γ-interferon. J. Immunol. **131**:826–830.
24. **Shirahata, T., and K. Shimizu.** 1980. Production and properties of immune interferon from spleen cell cultures of toxoplasma infected mice. Microbiol. Immunol. **24**:1109–1120.
25. **Shirahata, T., and K. Shimizu.** 1982. Growth inhibition of *Toxoplasma gondii* in cell cultures treated with murine type II interferon. Jpn. J. Vet. Sci. **44**:865–871.
26. **Sibert, G. J., and C. A. Speer.** 1981. Fine structure of nuclear division and microgametogony of *Eimeria nieschulzi* Dieben, 1924. Z. Parasitenkd. **66**:179–189.
27. **Speer, C. A.** 1983. The coccidia, p. 1–64. *In* J. B. Jensen (ed.), In vitro cultivation of protozoan parasites. CRC Press, Inc., Boca Raton, Fla.
28. **Turco, J., H. A. Thompson, and H. H. Winkler.** 1984. Interferon-γ inhibits growth of *Coxiella burnetii* in mouse fibroblasts. Infect. Immun. **45**:781–783.
29. **Usinger, W. R., W. G. Smith, and G. A. Splitter.** 1981. Bovine T cells do not require auxiliary cells for response to selected mitogens. Vet. Immunol. Immunopathol. **2**:381–391.
30. **Zar, J. H.** 1974. Biostatistical analysis, p. 139–142, 151–155. Prentice-Hall, Inc., Englewood Cliffs, N.J.