INFECTION AND IMMUNITY, Apr. 1989, p. 1151–1157
0019-9567/89/041151-07$02.00/0

Vol. 57, No. 4

Brucella abortus Regulates Bovine Macrophage–T-Cell Interaction by Major Histocompatibility Complex Class II and Interleukin-1 Expression

GARY A SPLITTER* AND KAREN M. EVERLITH

Department of Veterinary Science, University of Wisconsin—Madison, 1655 Linden Drive, Madison, Wisconsin 53706

Received 24 October 1988/Accepted 29 December 1988

T-cell activation is dependent on nominal antigen associated with major histocompatibility complex (MHC) class II molecules and interleukin-1 (IL-1), both provided by antigen-presenting cells. We have studied the effects of *Brucella abortus* and recombinant bovine gamma interferon (IFN-γ) on bovine macrophage expression of MHC class II and IL-1 molecules and subsequent T-cell proliferation in response to *B. abortus*. When peripheral blood mononuclear cells were cocultured with *B. abortus* and IFN-γ, increasing amounts of IFN-γ, from 1 to 100 U/ml, down regulated T-cell proliferation. Expression of MHC class II molecules on macrophages was increased independently by IFN-γ or *B. abortus* treatment. Thus, class II molecule expression was not the cause of down regulation of T-cell proliferation as observed in other systems. However, *B. abortus*–IFN-γ-treated macrophages obtained from overnight cultures had minimal membrane IL-1 compared with macrophages treated with *B. abortus* alone. Loss of membrane IL-1 required IFN-γ and the *o*-polysaccharide of the lipopolysaccharide. IFN-γ at 1 U/ml in combination with *B. abortus* produced a 32% decrease in T-cell response, while IFN-γ at 100 U/ml added to *B. abortus*-treated cultures produced an 82% reduction in T-cell response. Membrane IL-1 levels were not altered when recombinant bovine IFN-α or the rough strain 45/20 of *B. abortus*, which lacks the *o*-polysaccharide, was used. Secreted IL-1 levels were unaffected by IFN-γ and *B. abortus* treatment. The addition of recombinant bovine IL-1β (0.001 to 0.1 ng/ml) to *B. abortus*- and IFN-γ-treated cultures failed to provide a signal necessary for T-cell proliferation. These data suggest that membrane IL-1 has a key role in T-cell activation in response to *B. abortus*. When the *o*-polysaccharide of *B. abortus* lipopolysaccharide is combined with IFN-γ at an inappropriate time during an immune response, T-cell proliferation is prevented and cannot be restored by the addition of exogenous IL-1.

T-cell proliferation requires both nominal antigen associated with major histocompatibility complex (MHC) class II molecules and de novo synthesis of interleukin-1 (IL-1) by stimulated macrophages (42). The mechanisms that induce MHC class II and IL-1 molecules are not fully understood. A number of extrinsic as well as intrinsic factors that induce or augment the expression of MHC class II (40, 41) or IL-1 (10, 23, 38) molecules have been identified. Facultative intracellular bacteria contribute to interferon (IFN) production by lymphocytes (2). IFN-γ is known to augment MHC class II molecule expression on macrophages (37). Lipopolysaccharide (LPS) (21) or a lymphokine produced by T cells (38) can induce synthesis of IL-1 by macrophages. This lymphokine is distinct from IFN-γ, tumor necrosis factor, and colony stimulating factor 1 and may be one mechanism by which T cells signal macrophages to induce IL-1 (38). Two distinct but structurally related forms of IL-1 (termed IL-1α and IL-1β) have been identified (for a review, see reference 29); both forms appear to be associated with T-cell activation and can be present in secreted or membrane-bound states (15, 27). Both secreted and membrane-associated IL-1 are biologically active and participate in T-lymphocyte activation (18).

Any process that alters macrophage expression of MHC class II molecules or IL-1 may significantly influence immune responses. LPS can suppress expression of class II molecules on macrophage surfaces (13), and it has been reported that LPS inhibits both IFN-γ induction and maintenance of class II molecule expression on macrophages (36). In the absence of IFN-γ, LPS had no significant effect on macrophage class II molecule expression. Furthermore, LPS or IL-1 activation of macrophages produces high levels of prostaglandins which can modulate macrophage functions (5, 6).

Bovine macrophages serve as important host cells for the facultative intracellular bacterium *Brucella abortus*. Host resistance to disease caused by facultative intracellular bacteria depends on appropriate interaction between specifically sensitized T cells and macrophages. Because macrophages are host cells for *B. abortus*, they might also be central in initiating both positive and negative regulation of immune responses in infected cattle. We have shown previously that bovine macrophages can present *B. abortus* in the context of MHC class II molecules to T cells (34). To examine the nature of the T-cell response to macrophage-associated *B. abortus*, we have studied MHC class II and IL-1 molecule expression by macrophages following treatment with the bacterium alone or in combination with IFN-γ.

MATERIALS AND METHODS

Animals. Guernsey, Brown Swiss, and Holstein cattle (ages 6 months to 8 years) were maintained at a University of Wisconsin—Madison farm and vaccinated 2 to 4 times with the viable attenuated smooth *B. abortus* 19 vaccine (Jensen-Salsbury Laboratory, Kansas City, Mo.). All animals were serologically negative for antibodies to *B. abortus* at the time of these studies.

T-lymphocyte enrichment. Peripheral blood mononuclear (PBM) cells were isolated by Ficoll-Hypaque as previously

* Corresponding author.

described (34) 6 months to 4 years following vaccination. Nonadherent cells were obtained from 60×10^6 PBM cells cultured overnight in 75-cm^2 flasks containing RPMI 1640 medium supplemented with 100 U of penicillin and 100 μg of streptomycin per ml, 2 mM L-glutamine, 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 5×10^{-5} M 2-mercaptoethanol, and 10% fetal bovine serum. Nonadherent cells were washed, and contaminating macrophages were removed by passage through a Sephadex G-10 column (11). Further purification was accomplished by treating the nonadherent cells with the anti-class II molecule monoclonal antibody (MAb) H4 (One Lambda, Los Angeles, Calif.) (19) and anti-bovine light chain MAb DAS 9 (the gift, as was ascites fluid, from D. Goldsby) (35) followed by complement. MAb H4 was dialyzed to remove NaN_3 and then was used at a 1:10 dilution. DAS 9 ascites fluid was used undiluted.

APC. PBM cells were added to microdilution plates at 2×10^6 cells per well. Acetone-killed *B. abortus* smooth strain 1119 or rough strain 45/20 (gifts of D. T. Berman) (24, 26) were added at the optimal concentration for T-cell proliferation of 500 μg [dry weight] per ml (34). Recombinant bovine IFN-γ or IFN-α (Genentech, Inc., South San Francisco, Calif.) was added simultaneously with the bacteria to selected cultures at the concentrations indicated below. After overnight culture, nonadherent cells were removed, and adherent cells were washed three times with phosphate-buffered saline (PBS), irradiated (2,500 rad), and used as antigen-presenting cells (APC). By flow cytometry (EPICS; Coulter Electronics, Inc., Hialeah, Fla.), more than 80% of the adherent cells were present in a monocyte gate, and more than 80% of the adherent cells stained for esterase. These adherent cells were termed macrophages and were used as APC. Certain macrophage cultures were maintained for 7 days prior to use. Alternatively, macrophages were obtained by culturing PBM cells overnight on glass, and after removal of nonadherent cells by treatment with EDTA, the cells were counted and added at selected concentrations to microdilution wells.

APC fixation. After stimulation of APC by overnight exposure to antigen or medium, they were washed and fixed with 0.5 or 1% (wt/vol) paraformaldehyde for 15 min at room temperature. Following fixation, the cells were washed three times with PBS, and fresh medium was added. The fixed cells were incubated for 24 h at 37°C to remove residual paraformaldehyde (16) and were washed prior to use in either proliferative or membrane IL-1 determinations (17).

T-cell proliferation. The ability of APC to stimulate 4×10^5 autologous enriched T cells was assessed in a 6-day proliferative assay with [^{3}H]thymidine (6.7 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.) added during the final 16 h of incubation. In selected experiments, recombinant bovine IL-1β (Immunex, Seattle, Wash.) was added at 0.1, 0.01, or 0.001 ng/ml to cultures containing enriched bovine T cells or D10.G4.1 murine cells.

IL-1 assay. The IL-1-sensitive murine T-cell clone D10.G4.1 (ATCC TIB 224) was maintained as described elsewhere (12) by using C3H/HeJ (*H-2^k*) spleen cells and 25 U of recombinant human IL-2 (Biogen, Boston, Mass.) per ml. To measure secreted IL-1, supernatants from unstimulated and stimulated APC were collected. Dilutions of these supernatant fluids were added to triplicate microdilution wells in 100-μl portions, followed by 100 μl of 2×10^4 D10.G4.1 cells and 2.5 μg of concanavalin A (Sigma Chemical Co., St. Louis, Mo.) per ml. Supernatant from *Escherichia coli* LPS-stimulated murine spleen cell cultures served as a positive IL-1 control. Also, D10.G4.1 cells were added directly to irradiated or paraformaldehyde-fixed APC as a method of determining total IL-1 versus membrane-bound IL-1, respectively. [^{3}H]thymidine was added during the final 8 h of the 72-h incubation. Following culture, the cells were harvested and isotope incorporation was determined in a scintillation counter.

MHC class II molecule expression assays. PBM cells were treated with recombinant bovine IFN-γ, *B. abortus*, or IFN-γ and *B. abortus* overnight in glass petri dishes. Following removal of nonadherent cells, an enzyme-linked immunosorbent assay (ELISA) was used to detect MHC class II molecule expression on adherent cells. Round-bottom microdilution plates were coated with 3% gelatin for 2 h at 37°C and washed three times prior to use. Glass-adherent cells (2×10^6) detached by 0.35% EDTA with 0.6% glucose in PBS were incubated in the microdilution plates for 1 h with 10% heat-inactivated normal rabbit serum to block Fc receptors. After incubation, the cells were pelleted and suspended in PBS containing 1% bovine serum albumin. MAb H4 was diluted 1:300 in PBS containing 1% bovine serum albumin and added in 100-μl portions to wells. Plates were incubated for 1 h on ice and washed three times with PBS, and cells were assayed for class II molecules by ELISA as previously described (33). MAb 262404, specific for a bovine herpesvirus 1 glycoprotein, was used as an isotypic control (22).

Alternatively, class II molecule expression was detected by flow cytometry by using MAb H4. Glass-adherent cells (2×10^6), obtained as described above, were incubated on ice for 1 h with a 1:300 dilution of MAb H4 and then washed with PBS. Fluorescein-conjugated rabbit anti-mouse immunoglobulin G (heavy and light chains) (Jackson Immunoresearch Laboratories, Inc., Avondale, Pa.) was added to cell suspensions, and they were incubated for an additional hour. After three washes, the cells were examined by flow cytometry. The rabbit anti-mouse IgG was used at an optimal saturating concentration (data not shown).

RESULTS

Activation of MHC class II molecules by IFN-γ and *B. abortus*. On the basis of the ELISA, recombinant bovine IFN-γ increased class II molecule expression on macrophages. As little as 1 U of IFN-γ per ml produced a fivefold increase in class II molecules detected (Fig. 1). Similarly, a fourfold increase in class II molecule expression on macrophages was observed when PBM cells were treated with *B. abortus* (Fig. 1). Simultaneous addition of IFN-γ and *B. abortus* to PBM cells did not increase class II molecule expression on macrophages higher than either treatment alone, suggesting that *B. abortus* and IFN-γ at the levels tested do not have an additive effect. Therefore, class II molecule expression can be increased independently by the addition of recombinant bovine IFN-γ or *B. abortus* to the cultures.

Detection of bovine IL-1 by using the murine D10.G4.1 cell line. Since IL-1 is not produced constitutively and extracellular stimuli induce its synthesis (14), is was possible that *B. abortus* could induce production of both membrane and secreted forms of IL-1. An increasing concentration of *B. abortus* cultured with bovine macrophages for 24 h induced membrane IL-1 synthesis in a dose-dependent manner (Fig. 2). To examine the effect of adherent cell numbers, selected numbers of APC were cultured in microdilution wells with 500 μg of *B. abortus* per ml to allow production of both

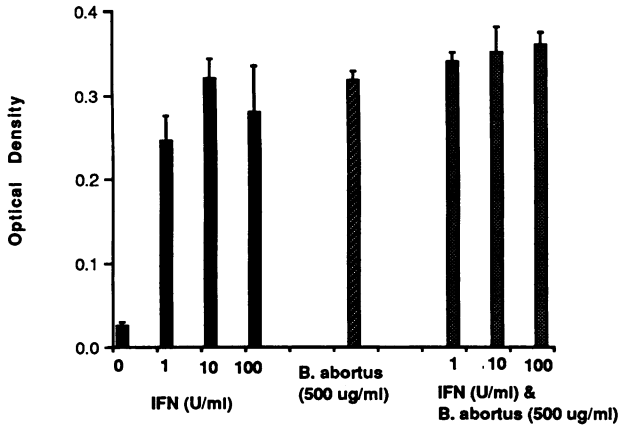

FIG. 1. Detection by ELISA of MHC class II molecule expression. PBM cells were treated overnight with recombinant bovine IFN-γ (■), *B. abortus* (500 μg/ml, ▨), or recombinant bovine IFN-γ and *B. abortus* (▩). Nonadherent cells were removed, and a MAb to class II molecules (H4) was added to adherent cells. The ELISA was performed as described in Materials and Methods and is representative of data obtained from seven animals. Isotype control values were subtracted from treatment values. Error bars represent standard deviations. Data are representative of four experiments.

membrane and secreted IL-1. In parallel experiments, cells were fixed with 1% paraformaldehyde to evaluate membrane IL-1 induction in the absence of secreted IL-1. The detection limit of our system required 10^3 or more APC for measurement of total or membrane IL-1 with the murine cell line G10.D4.1 (Fig. 3). *B. abortus* stimulated the production of total IL-1 from irradiated macrophages (Fig. 3A) and membrane IL-1 from paraformaldehyde-treated macrophages (Fig. 3B). Less IL-1 was detected from the paraformaldehyde-treated macrophages than from the nonfixed cells at concentrations of 10^3 cells per well.

In contrast, adherent cells present in the PBM cell population cultured with *B. abortus* and IFN-γ showed a decrease in membrane IL-1 expression (Fig. 4). Cultures treated overnight with *B. abortus* alone produced increased amounts

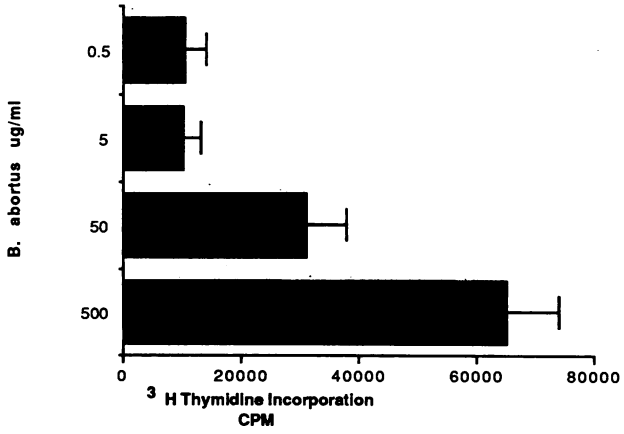

FIG. 2. Proliferation of D10.G4.1 cells to paraformaldehyde-fixed bovine macrophages treated with *B. abortus*. Adherent bovine cells were treated for 24 h with the indicated concentrations of bacteria, washed, and fixed with 0.05% paraformaldehyde. D10.G4.1 and 2.5 μg of concanavalin A per ml were added to the fixed cells, and proliferation of D10.G4.1 was measured after 24 h.

of total IL-1 in comparison with control cultures. This is similar to the findings shown in Fig. 3, which illustrates that macrophages treated with *B. abortus* alone produced both secreted and membrane IL-1. Cultures treated with *B. abortus* and IFN-γ produced secreted IL-1, but the levels of membrane IL-1 were reduced more than fivefold compared with total IL-1 levels or membrane IL-1 levels in cultures treated with *B. abortus* only (Fig. 4).

T-cell proliferation in response to *B. abortus*- or *B. abortus*–IFN-γ-treated macrophages. Because *B. abortus* induced both MHC class II molecule expression and IL-1 synthesis, it was important to correlate these observations with the ability of autologous T-cells to proliferate in response to macrophage-associated *B. abortus*. Macrophages within a PBM cell population were treated with *B. abortus*, recombinant IFN-γ, or both. The ability of the treated macrophages expressing either total IL-1 (irradiated macrophages) or membrane IL-1 (paraformaldehyde-fixed macrophages) to induce proliferation of antigen-specific T cells was determined. Macrophages treated with *B. abortus* induced T-cell proliferation (Fig. 5). Likewise, macrophages treated with *B. abortus* and fixed with paraformaldehyde were still capable of inducing T-cell proliferation. However, cultures treated with *B. abortus* and IFN-γ had a 62% reduction in PBM proliferation compared with cultures treated only with *B. abortus*. Similarly, cultures treated with *B. abortus* and IFN-γ and then by paraformaldehyde fixation of the macrophages had a 57% reduction in proliferation of PBM cells. MHC class II molecule expression on macrophages treated in parallel culture and analyzed by flow cytometry with MAb H4 had 63 and 76% staining cells for irradiated and fixed macrophages, respectively (data not shown). When IFN-γ was added to macrophages concurrently with *B. abortus*, no marked reduction in class II expression was observed on unfixed (69% staining cells) or paraformaldehyde-fixed (52% staining cells) macrophages (data not shown). Down regulation of T-cell proliferation appeared dependent on the initial PBM cell population and macrophage state because down regulation was not observed when overnight-cultured macrophages only or 7-day-old macrophages were treated with *B. abortus* and IFN-γ (data not shown).

Determination of T-cell suppression due to IFN-γ concentration and the strain of *B. abortus*. To determine if down regulation of T-cell proliferation by IFN-γ and *B. abortus* was associated with the LPS portion of the bacteria, as has been suggested for *E. coli* (36), *B. abortus* smooth strain 1119, which possesses the outer *o*-polysaccharide of LPS, was compared with rough strain 45/20, which lacks the outer *o*-polysaccharide (24, 26). Increasing concentrations of recombinant bovine IFN-γ were added to PBM cell cultures containing *B. abortus* 1119. Following overnight incubation, nonadherent cells were removed, and enriched T cells were added. Table 1 shows a concordant decrease of proliferation as IFN concentrations increased. IFN-γ at 1 U/ml caused a 32% inhibition, and IFN-γ at 100 U/ml caused an 82% inhibition of T-cell proliferation. In contrast, no marked inhibition of proliferation was observed with similar concentrations of strain 45/20 and IFN-γ. Also, cotreatment with recombinant bovine IFN-α, ranging from 1 to 100 U/ml, and *B. abortus* 1119 failed to inhibit T-cell proliferation (data not shown).

Effect of adding IL-1 to cultures treated with *B. abortus* and IFN-γ. Because membrane but not total IL-1 levels were reduced (Fig. 4) and a reduction of T-cell proliferation was observed with both unfixed and paraformaldehyde-fixed macrophages (Fig. 5), the possibility that the bovine IL-1

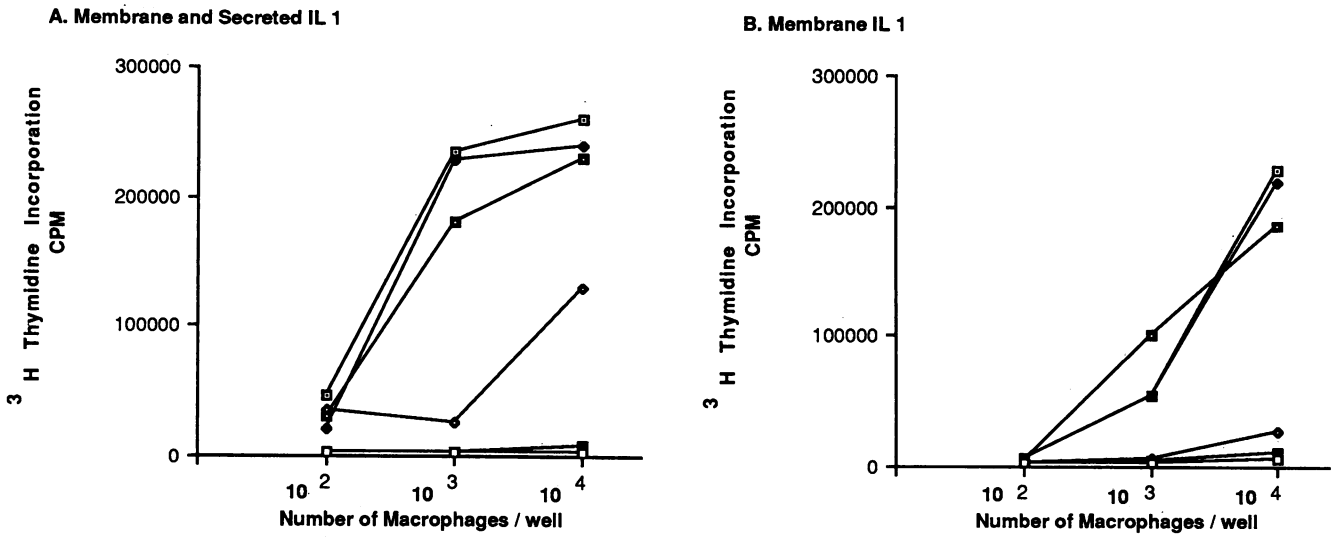

FIG. 3. Proliferation of D10.G4.1 cells to measure membrane and secreted IL-1. Glass-enriched macrophages of three animals were cultured with *B. abortus* plus 710 (⊡), 766 (◆), or 549 (◘), or medium only, i.e., 710 (◇), 766 (■), or 549 (□), overnight. Macrophages were irradiated as a source of total (secreted and membrane) IL-1 (A) or paraformaldehyde fixed as a source of membrane IL-1 (B). D10.G4.1 cells were added to macrophages as described in Materials and Methods. Standard deviations were less than 5%.

produced was not biologically active on bovine cells was considered. To determine if exogenous IL-1 could prevent suppression, recombinant bovine IL-1β was added to *B. abortus*–IFN-γ-treated cultures at the time of T-cell addition. The addition of IL-1 at selected doses failed to alter the suppressed T-cell state (Fig. 6).

DISCUSSION

The present data demonstrate that when IFN-γ and *B. abortus* were cocultured with PBM cells, there was a concomitant decrease in macrophage membrane IL-1 expression and T-cell proliferation. No alteration in total IL-1 was observed. MHC class II molecules on the APC were not responsible for decreased T-cell proliferation, because both recombinant bovine IFN-γ and *B. abortus* independently and in combination increased class II expression on macrophages. T cells from immunized animals could proliferate when *B. abortus* was presented by macrophages without the addition of recombinant IFN-γ. Both total and membrane IL-1 were present when macrophages were treated with *B. abortus*.

Demonstration of the decrease in membrane IL-1 and T-cell proliferation was dependent on the simultaneous addition of IFN-γ and *B. abortus* to whole PBM cells prior to removing nonadherent cells. A second factor, apparently necessary for the decrease in T-cell proliferation, was the presence of the *o*-polysaccharide of *B. abortus*. Smooth *B. abortus* 1119 possesses the outer *o*-polysaccharide of the LPS and when combined with recombinant IFN-γ produced a down regulation of the T-cell response. Rough strain 45/20 does not possess the outer *o*-polysaccharide (24, 26) and when combined with IFN-γ did not alter T-cell proliferation. The data are compatible with the hypothesis that IFN-γ and the outer *o*-polysaccharide of *B. abortus* can down regulate T-cell proliferation, perhaps by decreasing the expression of

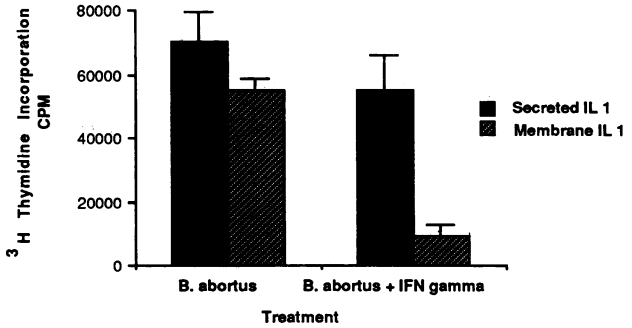

FIG. 4. Proliferation of D10.G4.1 cells to measure membrane versus secreted IL-1 expression following macrophage treatment. Adherent cells present in 2×10^6 PBM cells were treated overnight with *B. abortus* or *B. abortus* and IFN-γ. Following overnight treatments, supernatants were removed as the source of secreted IL-1 and the adherent cells were fixed with 1% paraformaldehyde as the source of membrane IL-1. D10.G4.1 cells were cultured with supernatants (■) or fixed cells (▩) to assay for IL-1. Data are representative of eight experiments.

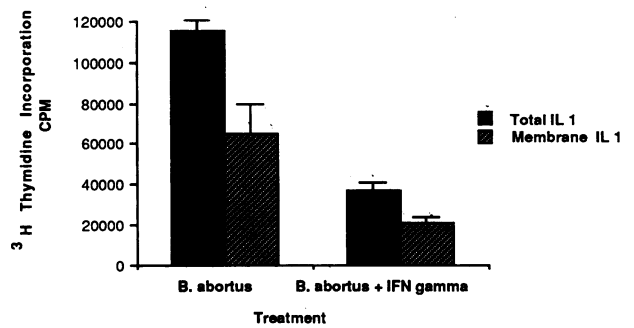

FIG. 5. Proliferation of T cells added to unfixed (■) or paraformaldehyde-fixed (▩) macrophages previously treated with *B. abortus* or IFN-γ and *B. abortus*. PBM cells were cultured overnight with IFN-γ, *B. abortus*, *B. abortus* and IFN-γ, or medium alone, and then nonadherent cells were removed. Enriched T cells were added, and proliferation to the antigen-pulsed macrophages was determined after a 6-day culture. Response of cultures treated with medium or IFN-γ alone was less than 10,000 cpm. Data are representative of eight experiments.

TABLE 1. T-cell proliferation[a] to *B. abortus* in the presence of IFN-γ

IFN-γ concn (U/ml)	Proliferation in medium only	Smooth strain 1119[b]		Rough strain 45/20[c]	
		Proliferation	% Inhibition[d]	Proliferation	% Inhibition
0	15,550 ± 3,407	110,543 ± 2,529	ND	81,641 ± 3,380	ND
1	13,348 ± 5,255	75,917 ± 1,970	31.8	70,788 ± 1,304	8.5
10	13,477 ± 180	37,184 ± 5,871	74.1	72,909 ± 2,023	5.4
100	12,909 ± 1,063	29,289 ± 7,729	82.1	63,992 ± 1,366	18.6

[a] Mean counts per minute ± standard deviation of triplicate values from one animal.
[b] Strain 1119 possesses the outer *o*-polysaccharide.
[c] Strain 45/20 lacks the outer *o*-polysaccharide. ND, Not done.
[d] % Inhibition = [1 − (counts per minute with IFN treatment/counts per minute without IFN treatment)] × 100.

membrane IL-1 on the macrophage. This down-regulating effect appeared dependent on the initial PBM cell population and macrophage state because down regulation was not observed when overnight macrophages only or 7-day-old macrophages were used. We have not yet determined the initiator of this effect, but others have been able to demonstrate that T cells can produce a lymphokine that initiates IL-1 synthesis (38, 39). Therefore, it is possible that appropriate stimuli or the lack thereof might induce T cells to effect a down regulation of macrophage membrane IL-1. Interestingly, an early description of IL-1 induction indicated that T-cell-induced IL-1 produced an additional component not present in LPS-induced IL-1 (23). This component might represent an altered, biologically inactive IL-1 or a T-cell-derived product, as was speculated by the authors (23). In support of a T-cell-derived product, IFN-γ can maintain the accessory function of APC for the induction of suppressor T cells (28). Presence of lymphocytes in the macrophage population when cultured with IFN and *B. abortus* might contribute to the simultaneous increase in the ability to induce suppressor T cells, as shown by others (28). This hypothesis would require additional experiments that are hampered by a lack of MAbs characterizing suppressor cells or a murine MHC I-J-like equivalent molecule in cattle.

IL-1 can be induced by both the polysaccharide and lipid A components of *E. coli* and *Salmonella minnesota* (21). The endotoxic activities of LPS reside exclusively in lipid A and not in the polysaccharide portion of the molecule (21). Therefore, the polysaccharide has the potential for IL-1 immunostimulatory activity. Also, polysaccharide appears to be the major component of *B. abortus* participating in the down regulation of IL-1, since strain 45/20 could not produce the down regulatory effect (Table 1). The present study indicates that the polysaccharide of *B. abortus* LPS effects both positive and negative immunostimulatory activity, depending on the interaction with other molecules or lymphokines.

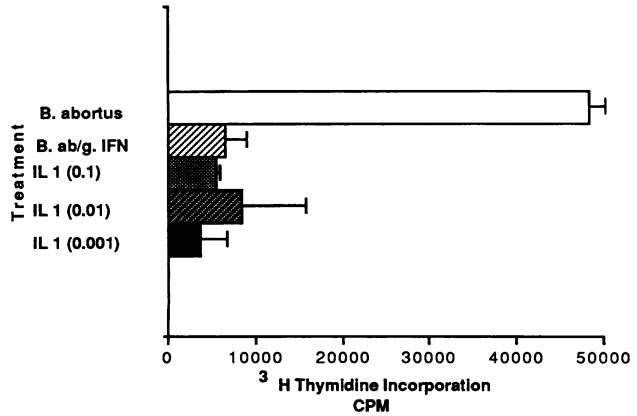

FIG. 6. Effect of recombinant bovine IL-1β on T-cell proliferation when added to *B. abortus*–IFN-γ-treated cultures. Cultures were as described in Materials and Methods. Data are from one experiment. Dose-dependent biologic activity of recombinant IL-1 was demonstrated when the activity was assayed on D10.G4.1 cells.

Recently, it has been possible to demonstrate both positive (4) and negative (36) effects of LPS on lymphokine production and T-cell responses. LPS has been reported to augment MHC class II molecule expression (43), and similar results were obtained in the present study with *B. abortus*. In contrast, LPS reportedly inhibits both IFN-γ induction of macrophage MHC class II molecule expression and IFN maintenance of these molecules in a dose-dependent manner (36). These findings are different from ours because MHC class II molecule expression was not decreased with simultaneous IFN-γ and bacterial treatment in the present study. This difference could be reflected in known differences in the LPS of *E. coli* and *B. abortus* (25) or in other antigens present in the *Brucella* organism. However, our findings indicate a decrease in membrane IL-1 expression on the surface of macrophages, which was caused by the outer *o*-polysaccharide. Down modulation of cytokine receptors by LPS has been reported by others (8) and is similar to the decrease in membrane IL-1 observed in the present study.

Membrane IL-1 probably represents a product derived from the 31-kilodalton IL-1α and not the β precursor (1). Others have shown that only IL-1α- and not IL-1β-specific antibody stains the surface of LPS-activated monocytes (9). The importance of membrane IL-1 amounts in lymphocyte activation has been demonstrated with murine T-cell lines and APC expressing various levels of membrane IL-1 (15). Therefore, the membrane IL-1 form is of functional importance for a T-cell response, and factors that would alter membrane IL-1 would affect T-cell proliferation.

These present experiments suggest a novel mechanism to regulate T-cell proliferation not described previously. Components required are the outer *o*-polysaccharide of *B. abortus* LPS, IFN-γ, and possibly a peripheral blood cell population(s). These components lead to a decline in membrane IL-1 on the macrophage surface and concomitant down regulation of T-cell proliferation. Interaction of lymphokine and LPS allow a distinction between events necessary at the macrophage level for antigen presentation and events that interfere with antigen recognition. *E. coli* LPS in combination with IL-1 can induce prostaglandin E_2 release from macrophages, as shown by others (7). Addition of IFN-γ to this system can inhibit IL-1 from producing prostaglandin E_2 and establishing antagonistic roles for IL-1 and IFN. LPS can serve as a second signal for activation of macrophages where prostaglandin E_2 is not produced (7). Previously,

others have not demonstrated whether IFN could alter expression of IL-1 on macrophage surfaces. However, the findings in the present study would extend the observation of previous workers (7) to suggest that IFN can down regulate membrane IL-1 in the presence of LPS. Whether LPS from other gram-negative bacteria can produce this effect or whether it is restricted to *B. abortus* remains to be determined. IL-1 inhibitors have been reported by others but appear to be different from either IFN or LPS (3, 20, 32), and viral infections have been noted to produce IL-1 inhibitory factors directly from macrophages (30, 31). These descriptions have indicated an effect on secreted IL-1, but little evidence indicating an alteration in membrane but not secreted IL-1 has been reported. The interaction of *B. abortus* containing the outer *o*-polysaccharide and IFN-γ to down regulate membrane IL-1 appears unusual. Clearly, more work will be required to determine how host cytokines interact with foreign antigens and cells of the immune system to down regulate molecules that participate in immune responses.

ACKNOWLEDGMENTS

This work was supported by U.S. Department of Agriculture grants 86-CRCR-1-2210 and 58-6125-6-1 and by grants from Genentech, Inc., and the College of Agricultural and Life Sciences, University of Wisconsin.

LITERATURE CITED

1. **Bakouche, O., D. C. Brown, and L. B. Lachman.** 1987. Subcellular localization of human monocyte interleukin 1: evidence for an inactive precursor molecule and a possible mechanism for IL 1 release. J. Immunol. **138**:4249–4262.
2. **Beller, D. I., and K. Ho.** 1982. Regulation of macrophage populations. V. Evaluation of the control of macrophage Ia expression in vitro. J. Immunol. **129**:971–976.
3. **Berman, M. A., C. I. Sandborg, B. S. Calabia, B. S. Andrews, and G. J. Friou.** 1986. Studies of an interleukin-1 inhibitor: characterization and clinical significance. Clin. Exp. Immunol. **64**:136–145.
4. **Blanchard, D. K., J. Y. Djeu, T. W. Klein, H. Friedman, and W. E. Stewart II.** 1986. Interferon-γ induction by lipopolysaccharide: dependence on interleukin 2 and macrophages. J. Immunol. **136**:963–970.
5. **Bonney, R. J., and J. L. Humes.** 1984. Physiological and pharmacological regulation of prostaglandin and leukotriene production by macrophages. J. Leukocyte Biol. **35**:1–10.
6. **Boraschi, D., and A. Tagliabue.** 1984. Multiple modulation of macrophage functions by lymphokines: different effects of interferon and macrophage activating factor, p. 71. *In* E. Pick (ed.), Lymphokines, vol. 9. Academic Press, Inc., New York.
7. **Browning, J. L., and A. Ribolini.** 1987. Interferon blocks interleukin 1-induced prostaglandin release from human peripheral monocytes. J. Immunol. **138**:2857–2863.
8. **Chen, B. D.-M., H.-S. Lin, and S. Hsu.** 1983. Lipopolysaccharide inhibits the binding of colony-stimulating factor (CSF-1) to murine peritoneal exudate macrophages. J. Immunol. **130**:2256–2260.
9. **Conlon, P. J., K. H. Grabstein, A. Alpert, K. S. Pricket, T. P. Hopp, and S. Gillis.** 1987. Localization of human mononuclear cell interleukin 1. J. Immunol. **139**:98–102.
10. **Economou, J. S., and H. S. Shin.** 1978. Lymphocyte-activating factor. I. Generation and physicochemical characterization. J. Immunol. **121**:1446–1452.
11. **Jerrells, T. R., J. H. Dean, G. L. Richardson, and R. B. Huberman.** 1980. Depletion of monocytes from human peripheral blood mononuclear leukocytes: comparison of the Sephadex G10 column method with other commonly used techniques. J. Immunol. Methods **32**:11–29.
12. **Kaye, J., S. Gillis, S. B. Mizel, E. M. Shevach, T. R. Malek, C. A. Dinarello, L. B. Lachman, and C. A. Janeway.** 1984. Growth of a cloned helper T cell line induced by a monoclonal antibody specific for the antigen receptor: interleukin 1 is required for the expression of receptors for interleukin 2. J. Immunol. **133**:1339–1345.
13. **Koerner, T. J., T. A. Hamilton, and D. O. Adams.** 1987. Suppressed expression of surface Ia on macrophages by lipopolysaccharide: evidence for regulation at the level of accumulation of mRNA. J. Immunol. **139**:239–243.
14. **Kurt-Jones, E., H. W. Virgin IV, and E. R. Unanue.** 1986. In vivo and in vitro expression of macrophage membrane interleukin 1 in response to soluble and particulate stimuli. J. Immunol. **137**:10–14.
15. **Kurt-Jones, E. A., D. I. Beller, S. B. Mizel, and E. R. Unanue.** 1985. Identification of a membrane-associated interleukin 1 in macrophages. Proc. Natl. Acad. Sci. USA **82**:1204–1208.
16. **Kurt-Jones, E. A., J.-M. Kiely, and E. R. Unanue.** 1985. Conditions required for expression of membrane IL 1 on B cells. J. Immunol. **135**:1548–1550.
17. **Kurt-Jones, E. A., H. W. Virgin IV, and E. R. Unanue.** 1985. Relationship of macrophage Ia and membrane IL 1 expression to antigen presentation. J. Immunol. **135**:3652–3654.
18. **Le, J., D. Weinstein, U. Gubler, and J. Vilcek.** 1987. Induction of membrane-associated interleukin 1 by tumor necrosis factor in human fibroblasts. J. Immunol. **138**:2137–2142.
19. **Lewin, H. A., C. C. Calvert, and D. Bernoco.** 1985. Cross-reactivity of a monoclonal antibody with bovine, equine, ovine, and porcine peripheral blood B lymphocytes. Am. J. Vet. Res. **46**:785–788.
20. **Liao, Z., A. Haimovitz, Y. Chen, J. Chan, and D. L. Rosenstreich.** 1985. Characterization of a human interleukin 1 inhibitor. J. Immunol. **134**:3882–3886.
21. **Loppnow, H., L. Brade, H. Brade, E. T. Rietschel, S. Kusumoto, T. Shiba, and H.-D. Flad.** 1986. Induction of human interleukin 1 by bacterial and synthetic lipid A. Eur. J. Immunol. **16**:1263–1267.
22. **Marshall, R. L., L. L. Rodriguez, and G. J. Letchworth III.** 1986. Characterization of envelope proteins of infectious bovine rhinotracheitis virus (bovine herpesvirus 1) by biochemical and immunological methods. J. Virol. **57**:745–753.
23. **Mizel, S. B., J. J. Oppenheim, and D. L. Rosenstreich.** 1978. Characterization of lymphocyte-activating factor (LAF) produced by a macrophage cell line, P388D_1. II. Biochemical characterization of LAF induced by activated T cells and LPS. J. Immunol. **120**:1504–1508.
24. **Moreno, E., L. M. Jones, and D. T. Berman.** 1984. Immunochemical characterization of rough *Brucella* lipopolysaccharides. Infect. Immun. **43**:779–782.
25. **Moreno, E., R. S. Kurtz, and D. T. Berman.** 1984. Induction of immune and adjuvant immunoglobulin G responses in mice by *Brucella* lipopolysaccharide. Infect. Immun. **46**:74–80.
26. **Moreno, E., M. W. Pitt, L. M. Jones, G. G. Schurig, and D. T. Berman.** 1979. Purification and characterization of smooth and rough lipopolysaccharides from *Brucella abortus*. J. Bacteriol. **138**:361–369.
27. **Nagelkerken, L. M., and P. J. C. van Breda Vriesman.** 1986. Membrane-associated IL 1-like activity on rat dendritic cells. J. Immunol. **136**:2164–2170.
28. **Noma, T., and M. E. Dorf.** 1985. Modulation of suppressor T cell induction with γ-interferon. J. Immunol. **135**:3655–3660.
29. **Oppenheim, J. J., E. J. Kovacs, K. Matsushima, and S. K. Durum.** 1986. There is more than one interleukin 1. Immunol. Today **7**:45–56.
30. **Roberts, N. J., A. H. Prill, and T. N. Mann.** 1986. Interleukin 1 and interleukin 1 inhibitor production by human macrophages exposed to influenza virus or respiratory syncytial virus. J. Exp. Med. **163**:511–519.
31. **Rodgers, B. C., D. M. Scott, J. Mundin, and J. G. Sissons.** 1985. Monocyte-derived inhibitor of interleukin 1 induced by human cytomegalovirus. J. Virol. **55**:527–532.
32. **Scala, G., Y. D. Kuang, R. E. Hall, A. V. Muchmore, and J. J. Oppenheim.** 1984. Accessory cell function of human B cells. I. Production of both interleukin 1-like activity and an interleukin 1 inhibitory protein by an EBV-transformed human cell line. J.

Exp. Med. **159**:1637–1652.
33. **Splitter, G., J. Burkholder, K. O'Reilly, and A. Janzer.** 1987. Anti-BoLA-w8 monoclonal antibody: production of a tissue typing reagent after blocking monomorphic sites on bovine mononuclear cells. Tissue Antigens **30**:122–127.
34. **Splitter, G. A., and K. M. Everlith.** 1986. Collaboration of bovine T lymphocytes and macrophages in T-lymphocyte response to *Brucella abortus*. Infect. Immun. **51**:776–783.
35. **Srikumaran, S., A. J. Guidry, and R. A. Goldsby.** 1983. Production and characterization of monoclonal bovine immunoglobulins G_1, G_2, and M from bovine × murine hybridomas. Vet. Immunol. Immunopathol. **5**:323–342.
36. **Steeg, P. S., H. M. Johnson, and J. J. Oppenheim.** 1982. Regulation of murine macrophage Ia antigen expression by an immune interferon-like lymphokine: inhibitory effect of endotoxin. J. Immunol. **129**:2402–2406.
37. **Steeg, P. S., R. N. Moore, H. M. Johnson, and J. J. Oppenheim.** 1982. Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. J. Exp. Med. **156**:1780–1793.
38. **Takacs, L., J. A. Berzofsky, J. York-Jolley, T. Akahoshi, E. Blasi, and S. K. Durum.** 1987. IL 1 induction by murine T cell clones: detection of an IL 1-inducing lymphokine. J. Immunol. **138**:2124–2131.
39. **Unanue, E. R., and P. M. Allen.** 1987. The basis for the immunoregulatory role of macrophages and other accessory cells. Science **236**:551–557.
40. **Unanue, E. R., D. I. Beller, C. Y. Lu, and P. M. Allen.** 1984. Antigen presentation: comments on its regulation and mechanism. J. Immunol. **132**:1–5.
41. **Wentworth, P. A., and H. K. Ziegler.** 1987. Induction of macrophage Ia expression by *Listeria monocytogenes* in congenitally athymic nude mice. J. Immunol. **138**:3167–3173.
42. **Windle, J. J., H. S. Shin, and J. F. Morrow.** 1984. Induction of interleukin 1 messenger RNA and translation in oocytes. J. Immunol. **132**:1317–1322.
43. **Ziegler, H. K., L. K. Staffileno, and P. Wentworth.** 1984. Modulation of macrophage Ia-expression by lipopolysaccharide. I. Induction of Ia expression in vivo. J. Immunol. **133**: 1825–1835.