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Inhibition of Expression of Major Histocompatibility Complex Class II Molecules in Macrophages Infected with *Leishmania donovani* Occurs at the Level of Gene Transcription via a Cyclic AMP-Independent Mechanism

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Among the important pleiotropic responses to gamma interferon (IFN-γ) during the activation of macrophages (Mϕ) is the increased expression of major histocompatibility complex class II genes. In the present study, infection with *Leishmania donovani* was shown to inhibit in parallel the induction by IFN-γ of H-2 Aβ gene transcription, class II mRNA accumulation, and H-2 A^d protein expression in cells of the murine macrophage cell line $P388D_1$. Treatment of $P388D_1$ cells with either the adenylate cyclase activator cholera toxin or the protein kinase A activator N^6-2′-*O*-dibutyryl cyclic AMP (dibutyryl cAMP) similarly inhibited the induction by IFN-γ of class II protein expression, and in parallel with *Leishmania* infection, cholera toxin inhibited the induction of mRNA for the H-2 Aα and H-2 Aβ proteins. Concentrations of intracellular cAMP were significantly increased in cholera toxin-treated cells but not in leishmania-infected cells. These findings indicate that at least one mechanism by which *Leishmania* infection attenuates the activation of Mϕ by IFN-γ involves selective, transcriptional inhibition of major histocompatibility complex class II genes via a cAMP-independent mechanism.

The cytokine gamma interferon (IFN-γ) is recognized to play a central role in the coordinate activation of macrophages (Mϕ) for full functional responses (12, 19, 21). Among the important pleiotropic responses elicited by IFN-γ during the activation of Mϕ is the increased expression of major histocompatibility complex (MHC) class II genes. Data reported from several laboratories indicate that the interactions of Mϕ with a variety of stimuli, including immune complexes (20) and intracellular microorganisms (9), may decrease or eliminate the responsiveness of these cells to IFN-γ for the expression of MHC class II molecules. Findings in this laboratory that demonstrate that infection of Mϕ with the intracellular protozoan *Leishmania donovani* abrogated responses to IFN-γ for increased expression of MHC class II molecules, and that this response was associated with decreased steady-state levels of MHC class II mRNA, have also been reported (14, 15). In the present study, experiments were carried out to examine directly whether the latter finding was due to the inhibition of class II gene transcription. Furthermore, the question of whether inhibition of MHC class II expression by *L. donovani* involved a cyclic AMP (cAMP)-dependent mechanism was also examined.

Procedures for the culture and infection of $P388D_1$ cells with *L. donovani* were used as described previously (14, 15). Also as previously described, an indirect, ^{125}I-binding assay performed under saturating conditions was used to measure surface expression of MHC class II (H-2 A^d) molecules. As shown in Table 1, compared to control cultures, cells exposed to *L. donovani* for 4 h prior to the addition of IFN-γ were significantly unresponsive to cytokine treatment for the induction of the expression of MHC class II (H-2 A^d) molecules. Evidence that increased concentrations of cAMP in Mϕ resulted in the inhibition of responses to IFN-γ for the induction of the expression MHC class II molecules, presumably through the activation of protein kinase A, has been presented previously (8, 17, 18). Furthermore, previous findings from this laboratory had shown that Mϕ infected with *L. donovani* produce increased amounts of prostaglandins, which are known to activate adenylate cyclase (13). Taken together, these findings suggested the possibility that inhibition of class II expression by *L. donovani* may involve infection-induced activation of the adenylate cyclase-protein kinase A system. To examine whether inhibition of class II expression by *L. donovani* infection might be cAMP-dependent, the effects of leishmania infection were compared with the effects observed for parallel cell cultures treated with either cholera toxin (List Biologicals Laboratories, Campell, Calif.) or dibutyryl cAMP (N^6-2′-*O*-dibutyryl cAMP; Sigma Chemical Co., St. Louis, Mo.). The effects of these treatments on basal and IFN-γ-induced levels of MHC class II expression are shown in Table 1. As can be seen, exposure of cells to either cholera toxin or dibutyryl cAMP for 1 h prior to the addition of IFN-γ significantly inhibited the induction of the expression MHC class II molecules. The findings that either direct stimulation of adenylate cyclase with cholera toxin or the more distal action of the protein kinase A activator dibutyryl cAMP was each capable of inhibiting class II expression in this system were consistent with the hypothesis that inhibition of class II expression by *L. donovani* might involve activation of the adenylate cyclase-protein kinase A system. To examine whether similar mechanisms were involved in the inhibitory effects of *L. donovani* and protein kinase A activators on class II expression, the effects of infection with leishmania or treatment with cholera toxin on steady-state levels of class II mRNA in

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TABLE 1. Effects of cholera toxin, dibutyryl cAMP, and leishmania infection on basal and IFN-γ-induced cell surface expression of MHC class II molecules (H-2 A^d) by $P388D_1$ cells[a]

Treatment group	Mean ± SEM H-2 A^d expression (cpm)
Control	113 ± 57
Cholera toxin	0
Dibutyryl cAMP	35 ± 35
L. donovani	88 ± 72
IFN-γ	4,390 ± 715[b]
Cholera toxin + IFN-γ	478 ± 274[c]
L. donovani + IFN-γ	382 ± 152[c]
Dibutyryl cAMP + IFN-γ	1,532 ± 187[c]

[a] MHC class II expression was measured in an indirect ^{125}I-binding assay as described in the text. Control, cholera toxin- or dibutyryl cAMP-treated, or leishmania-infected cells were cultured for 48 h with and without IFN-γ (125 U/ml). Cells were exposed to cholera toxin (1 μg/ml) or dibutyryl cAMP (1 mM) for 1 h or infected with *L. donovani* (multiplicity of infection, 20:1; ultimate infection rate of 75 to 80% with 6 to 8 amastigotes per cell) for 4 h prior to the addition of cytokine. Measurements for each treatment group were performed in triplicate. The results for one experiment are shown and are representative of similar results obtained for three independent experiments.

[b] Significantly different from control (untreated) cells (corrected P of <0.01) by one-way analysis of variance.

[c] Significantly different from the group treated with IFN-γ alone (corrected P of <0.01) by one-way analysis of variance.

IFN-γ-treated cells were compared. Total cellular RNA was isolated as described by Chomczynski and Saachi (5), and RNA was subjected to Northern (RNA) blot analysis and autoradiography as described previously (14). Blots were hybridized with probes generated from the following cDNA inserts: pADC3 encoding the H-2 Aα chain (2), pIA-b-1 encoding the H-2 Aβ chain (16), and pBA1b encoding bovine β-actin (6). Inserts were subcloned into Bluescript+ vector (Stratagene, La Jolla, Calif.), and orientations were determined by restriction endonuclease analysis. ^{32}P-labeled RNA probes were synthesized in vitro as follows: linearized plasmid DNA (1 μg) was mixed with 4 μl of a nucleotide mix (2.5 mM each ATP, GTP, and CTP; 0.25 mM UTP), 2 μl of 0.1 M dithiothreitol, 2 μl of bovine serum albumin (1 mg/ml, RNase-free), 4 μl of 5× transcription buffer, 2 to 4 μl of [^{32}P]UTP (800 Ci/mmole; New England Nuclear, Boston, Mass.), 4 U of RNase inhibitor (RNasin; Promega Biotec, Madison, Wis.), and 10 U of either T7 or T3 RNA polymerase (Stratagene) to a final volume of 20 μl. The mixture was incubated at 37°C for 60 min, and transcription was terminated by the addition of 2 to 3 U of RNase-free DNase I (Boehringer Mannheim, Mannheim, Germany) with continued incubation at 37°C for 15 min. Ten micrograms of carrier tRNA (Sigma) was added, and the entire mixture was diluted to 100 μl and extracted with an equal volume of phenol-chloroform-isoamyl alcohol (50:49:1). The organic phase was back extracted with 100 μl of TE (10 mM Tris-HCl, 1 mM EDTA [pH 7.5]) buffer. The aqueous phases were combined, and radiolabeled RNA was precipitated by the addition of 0.3 volume of 7.5 M ammonium acetate and 2.5 volumes of 95% ethanol. The RNA pellet was solubilized in 50 μl of TE buffer and heated at 90°C for 10 min before being mixed with the hybridization buffer. RNA for analysis was prepared from normal, leishmania-infected, or cholera toxin-treated cells at 48 h after the addition of IFN-γ. As shown in Fig. 1, in comparison to control cells, IFN-γ-induced increases of steady-state levels of MHC class II mRNA (H-2 Aα chain, panel A, and H-2 Aβ chain, panel B) in leishmania-infected cells were significantly attenuated. By scanning densitometry (computing densitometer; Molecular Dynamics, Sunnyvale, Calif.), steady-state levels of H-2 Aα and H-2 Aβ mRNA in infected, interferon-treated Mϕ were, respectively, only 20 and 45% of the levels observed for control, interferon-treated Mϕ. Similarly, treatment of cells with cholera toxin (Fig. 2) also resulted in the inhibition of class II mRNA accumulation. Densitometric analysis re-

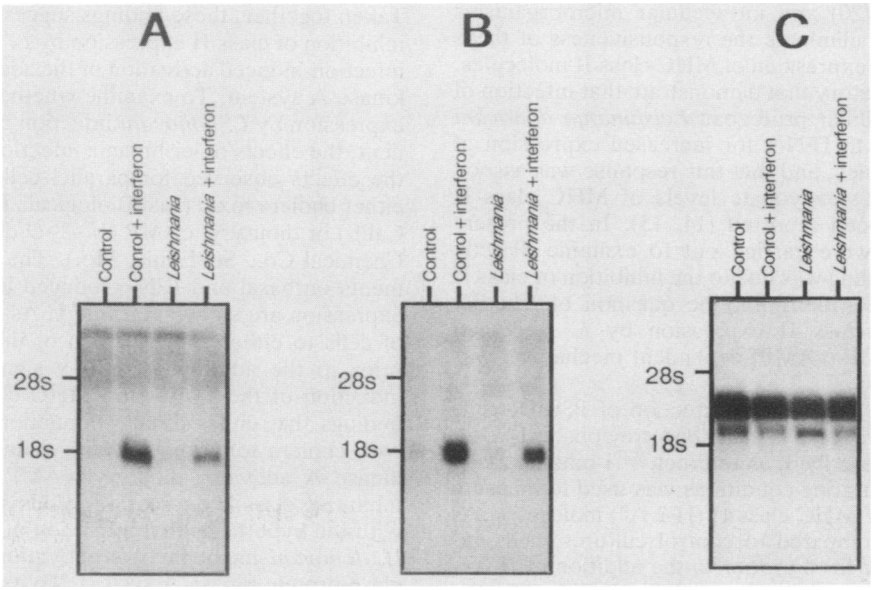

FIG. 1. Northern blot hybridization analysis of the H-2 Aα and Aβ chains and actin mRNA in $P388D_1$ cells. Total cytoplasmic RNA (10 μg) was fractionated on a 1.0% agarose–formaldehyde gel and transferred to Hybond-N. Hybridization with a ^{32}P-antisense RNA probe for the Aα chain (A), for the Aβ chain (B), and for bovine β-actin (C) exposed at −70°C for 24, 4, and 4 h, respectively. $P388D_1$ cells were cultured for 48 h as follows (lanes are labeled as indicated in parentheses): alone (control), with IFN-γ (125 U/ml) (control + interferon), infected with *L. donovani* (*Leishmania*), or infected with *L. donovani* for 4 h prior to the addition of IFN-γ (125 U/ml) (*Leishmania* + interferon).

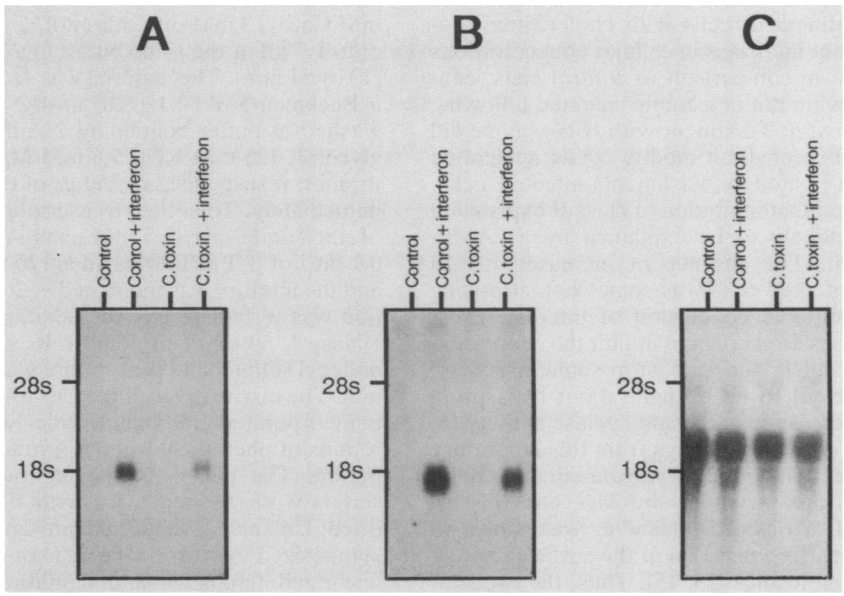

FIG. 2. Northern blot hybridization analysis of the H-2 Aα and Aβ chains and actin mRNA in P388D$_1$ cells. RNA was prepared and processed as described in the legend to Fig. 1 and was hybridized with a ^{32}P-antisense RNA probe for the Aα chain (A), for the Aβ chain (B), or for bovine β-actin (C), each exposed at −70°C for 24, 6, or 6 h, respectively. Cells were cultured for 48 h as follows (lanes are labeled as indicated in parentheses): alone (control), with IFN-γ (125 U/ml) (control + interferon), with cholera toxin (1 μg/ml) (c. toxin), or with cholera toxin (1 μg/ml) for 1 h prior to the addition of IFN-γ (125 U/ml) (c. toxin + interferon).

vealed that steady-state levels of H-2 Aα and H-2 Aβ mRNA in Mϕ treated with both cholera toxin and interferon were, respectively, only 31 and 53% of the levels observed for control, interferon-treated Mϕ. In contrast, neither infection (Fig. 1C) nor toxin treatment (Fig. 2C) significantly affected steady-state levels of β-actin mRNA.

The finding that both cholera toxin and leishmania infection had similar effects on IFN-γ-induced class II protein expression and steady-state levels of mRNA provided support for the hypothesis that inhibition of class II expression during infection might involve a cAMP-dependent mechanism. To examine this possibility further, cellular cAMP concentrations were determined for control and infected cells in the presence and absence of IFN-γ treatment. One hour prior to treatment of cells with stimuli, the phosphodiesterase inhibitor 3-isobutyl-1-methyl xanthine (Aldrich Chemical Co., Milwaukee, Wis.) (final concentration, 0.5 mM) was added to the cultures to inhibit enzymatic degradation of cAMP. Following cell treatment, monolayers were washed, and cells were recovered by being scraped into 0.5 ml of 0.5 M Tris–5 mM EDTA (pH 7.4). Cells were then sonicated at 30 W/s and centrifuged for 10 min at 1,000 × *g*, and cAMP concentrations in the supernatants were assayed (in EDTA buffer) by radioimmunoassay as previously described, with minor modifications (4). The anti-cAMP antibody was produced in rabbits by using cAMP conjugated to thyroglobulin. The validity of the assay was established by linearity of standard dilutions, loss of immunoreactivity following incubation of samples with phosphodiesterase, and comparison with a commercial kit from New England Nuclear. Chloramine-T was used to prepare ^{125}I-labeled cAMP tyrosine methyl ester (unlabeled cAMP was from Sigma). The incubation mixture consisted of 100 μl of standards or samples, 100 μl of antibody, and 100 μl of iodinated tracer (~15,000 cpm), which were combined and incubated overnight at 4°C. Free and bound fractions were separated by using goat anti-rabbit immunoglobulin G (100 μl, 1:50) in the presence of a pool of normal rabbit serum (100 μl, 1% serum). After the addition of 750 μl of polyethylene glycol 8,000 (10% [vol/vol]), the tubes were centrifuged at 2,700 × *g* at 4°C for 20 min. The supernatants were discarded, and the radioactivity in the precipitates was detected in an LKB 1275 Minigamma Counter. The lower limit of detection for cAMP was 0.2 pmol per tube. The results shown in Table 2

TABLE 2. Effects of cholera toxin and infection with *L. donovani* on cAMP levels in P388D$_1$ cells[a]

Treatment group	Time of treatment (h)	Mean ± SEM cAMP level (pmol/10^6 cells)
Control	1	13.5 ± 3.5
	4	17.1 ± 1.1
	24	13.9 ± 1.8
Cholera toxin	1	272.3 ± 87.6[b]
	4	486.8 ± 82.1[c]
	24	180 ± 57.5
L. donovani	1	15.1 ± 1.7
	4	20.8 ± 11.9
	24	17.3 ± 5.7
IFN-γ	24	18.9 ± 1.5
Cholera toxin + IFN-γ	24	179.7 ± 55.4
L. donovani + IFN-γ	24	17.4 ± 1.7

[a] Cells were either untreated, exposed to cholera toxin (1 μg/ml), or infected with *L. donovani* (at a parasite to cell ratio of 20:1; the ultimate infection rate was 83 to 89% with 5 to 10 amastigotes per cell). At the indicated times, cells were processed and intracellular cAMP content was measured by using a rabbit antibody as described in the text. For the groups that were treated with IFN-γ, cytokine was present for the final 16 h of incubation at a concentration of 200 U/ml. The results shown were obtained from three independent experiments. In each individual experiment, two replicate values were obtained for each condition.

[b] Significantly different from the corresponding value for the control group (corrected P of <0.05) by one-way analysis of variance.

[c] Significantly different from the corresponding value for the control group (corrected P of <0.01) by one-way analysis of variance.

demonstrate that treatment of cells with cholera toxin resulted in time-dependent increases in cellular concentrations of cAMP. In contrast, in comparison to control cells, concentrations of cAMP were not detectably elevated following infection with *L. donovani*. Treatment with IFN-γ alone did not affect cAMP levels, nor did it modify cyclic nucleotide levels in either toxin-treated or leishmania-infected cells. These findings indicated that inhibition of class II expression by *L. donovani* is unlikely to be explained by a cAMP-dependent mechanism. The absence of increased cAMP levels in leishmania-infected cells was somewhat surprising and suggested an additional conclusion of interest. Prostaglandins of the E series are known to inhibit the expression of MHC class II molecules, and at least in some instances, this inhibition is believed to be brought about by a prostaglandin-mediated increase in adenylate cyclase activity (8, 17, 18). Furthermore, previous findings from this laboratory demonstrated that infection with *L. donovani* stimulated the synthesis of E series prostaglandins by Mϕ, and this increased synthesis of E series prostaglandins was shown to account, at least in part, for inhibition of the surface expression of MHC class II molecules (13, 15). Thus, the expectation was that net adenylate cyclase activity would be increased in leishmania-infected macrophages. Normal levels of cAMP in leishmania-infected cells, as demonstrated in the present study, indicate that inhibition of class II expression induced by *L. donovani* occurs via a cAMP-independent mechanism and suggests that prostaglandin-mediated suppression of class II expression may also occur in the absence of activation of the cAMP-protein kinase A pathway. An alternative interpretation, and one that is consistent with previous results from this laboratory (15), is that inhibition of class II expression during leishmania infection may occur, at least to some extent, via a prostaglandin- and cAMP-independent mechanism. In this case, it would be anticipated that cyclooxygenase inhibitors would only partially reverse this defect.

The results of Northern analyses presented above demonstrated that both infection with *L. donovani* and activation of the adenylate cyclase-protein kinase A system inhibited IFN-γ-induced accumulation of class II mRNA in $P388D_1$ cells. Although the finding that steady-state levels of actin mRNA were not influenced by infection excluded a nonspecific, generalized effect on mRNA stability, it remained possible that decreased steady-state levels of class II mRNA in leishmania-infected, IFN-γ-treated cells could have resulted from selective effects of infection on the stability of MHC class II message. Alternatively, decreased class II mRNA levels in infected cells could have been related to direct effects on MHC class gene transcription rates. The latter mechanism would be consistent with previously published data indicating that the induction by IFN-γ of the expression of MHC class II molecules requires transcriptional activation of class II genes (1, 3). To examine whether leishmania infection influenced class II gene transcription, nuclear run-on assays were performed to measure transcription rates for Aβ mRNA. For nuclear run-on assays, transcriptionally active nuclei were isolated by sucrose density gradient centrifugation. Typically, 17×10^6 to 25×10^6 cells were used for each assay. Cells were pelleted, washed in cold phosphate-buffered saline, and resuspended in 1.7 ml of ice-cold lysis buffer (10 mM Tris-HCl [pH 7.5], 2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.3 M sucrose, 3 mM dithiothreitol). Cells were lysed by adding 17 μl of 20% Nonidet P-40 and incubated on ice for 10 min. The lysate was mixed with an equal volume of cushion buffer (10 mM Tris-HCl [pH 7.5], 2 mM $MgCl_2$, 3 mM $CaCl_2$, 3 mM dithiothreitol, 2 mM sucrose) and layered onto 1.7 ml of the same buffer in a Beckman centrifuge tube (13 by 51 mm). The material was sedimented at 15,000 rpm in a Beckman SW 50.1 rotor at 4°C for 45 min. Nuclei were washed in buffer containing 25 mM Tris-HCl [pH 8], 25% glycerol, 125 mM KCl, 5.6 mM $MgCl_2$, and 2.5 mM dithiothreitol; resuspended in 160 μl of the same buffer; and used immediately. To initiate transcription, 60 U of RNasin, 40 μl of nucleotide mix (2.5 mM each ATP, GTP, and CTP) and 0.1 mCi of [^{32}P]UTP was added to the suspension of nuclei, and the mixture was incubated at 26°C for 30 min. Transcription was terminated by the addition of 40 U of RNase-free DNase I, 50 μg of proteinase K, and 20 μl of 20% sodium dodecyl sulfate, and the mixture was incubated at 37°C for 15 min. The mixture was diluted 10-fold with TE buffer, and the unincorporated radionucleotide was eliminated by two rounds of phenol-chloroform extraction and ethanol precipitation. The pellets containing radiolabeled nuclear transcripts were washed twice with 70% ethanol and vacuum-dried. Unlabeled, sense and antisense RNA transcripts were synthesized in vitro for nuclear run-on assays using 5 μg of linearized templates and conditions similar to those described for RNA probe synthesis, except that the nucleotide mixture contained 2.5 mM UTP and carrier RNA was not included. The RNA precipitates were solubilized in TE buffer and quantified by measuring A_{260}. Slot blots were prepared by immobilizing aliquots of nonradioactive sense and antisense RNA for class II Aβ chain and bovine β-actin onto Hybond-N membranes by using a slot blot apparatus (Bio-Rad, Mississauga, Ontario, Canada) according to the manufacturer's recommendations. The amount of ^{32}P-labeled nuclear RNA added was normalized according to the trichloroacetic acid-precipitable counts to ensure that all the membranes had equivalent counts available for hybridization. Typically, about 2×10^6 cpm (as determined by trichloroacetic acid precipitation) per membrane (6 by 9 cm) was used at a concentration of 0.5×10^6 cpm/ml of hybridization buffer. Membranes were hybridized for 18 h at 65°C, and the conditions for washing were identical to those described for RNA blot hybridization. Initial run-on transcription assays were performed by using nuclei from the A20 B cell line since this cell line constitutively expresses MHC class II antigens. These studies served to establish that the nonradioactive mRNA immobilized on the Hybond-N membranes was in excess during the hybridization and that nuclear class II gene transcripts extended in vitro retained strand specificity, as evidenced by hybridization to nonradioactive antisense mRNA only. These studies demonstrated (data not shown) that strand-specific detection of the Aβ nuclear transcript took place and that 0.8 μg of nonradioactive antisense mRNA was sufficient for binding of a wide range of input radiolabeled transcripts. For β-actin, 1.6 μg of nonradioactive mRNA was found to be adequate to ensure that an excess was available for hybridization with ^{32}P-labeled nuclear transcripts. Shown in Fig. 3 are representative results (for one of three independent experiments) of a slot blot hybridization analysis in which excess unlabeled sense and antisense mRNA for both H-2 Aβ and bovine β-actin was bound to Hybond-N membranes and hybridized with [^{32}P]UTP-labeled nuclear RNA transcripts derived from either normal or leishmania-infected $P388D_1$ cells. Compared with noninduced control cells, noninfected IFN-γ-treated (for 24 h before isolation of nuclei) cells showed a significant increase in Aβ mRNA transcription. This response was evidenced by the increase in signal intensity in the slot in which nonradioactive antisense RNA

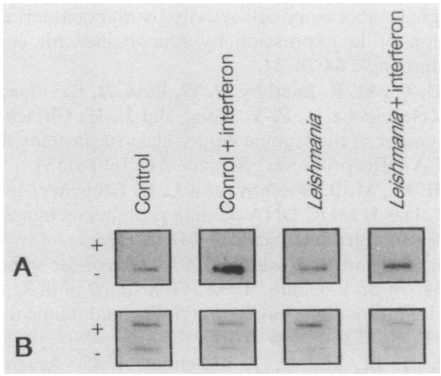

FIG. 3. Slot blot hybridization of nonradioactive sense (+) and nonradioactive antisense (−) mRNA with [^{32}P]UTP-labeled nuclear mRNA transcripts prepared in vitro by using transcriptionally active nuclei as described in the text. Each slot contained 0.8 μg of nonradioactive Aβ RNA (A) or 1.6 μg of cold, bovine β-actin RNA (B). Cells were cultured for 24 h as follows (lanes are labeled as indicated in parentheses): alone (control), with IFN-γ (125 U/ml) (control + interferon), infected with *L. donovani* (*Leishmania*), infected with *L. donovani* for 4 h prior to the addition of IFN-γ (125 U/ml) (*Leishmania* + interferon).

was bound (Fig. 3A, compare the first and second lanes labeled −). The specificity of this result was confirmed by the absence of a corresponding increase in signal intensity in the slot with nonradioactive sense Aβ mRNA (Fig. 3A, compare the first and second lanes labeled +). In contrast, when nuclei from IFN-γ-treated infected cells were used to generate labeled mRNA transcripts, the increase in signal intensity relative to noninduced, infected cell was significantly attenuated (Fig. 3A, compare the third and fourth lanes labeled −). By scanning densitometry, compared with the response observed for IFN-γ-treated control cells, the level of IFN-γ-induced H-2 Aβ gene transcription in leishmania-infected cells was reduced by 70%. These findings indicated that IFN-γ-induced transcription of the MHC class II Aβ gene was significantly inhibited in infected cells. Transcription rates for bovine β-actin in both normal and infected cells were also measured to control for the possibility that infection resulted in a global decrease in gene transcription. As can be seen in Fig. 3B, IFN-γ did not significantly influence the rates of actin gene transcription. Moreover, in contrast to the case of Aβ gene transcription, there were no significant differences in the signal intensities obtained by using ^{32}P-labeled nuclear mRNA actin transcripts from either normal or infected cells. Thus, infection with *L. donovani* did not have a generalized suppressive effect on cellular gene transcription. In multiple experiments, hybridization signals of equivalent intensities were observed in slots containing either sense or antisense mRNA for bovine β-actin mRNA. This effect would not have been detected if hybridization membranes had been loaded with the usual plasmid DNA, as both nonradioactive sense and nonradioactive antisense strands would have been present in each slot. Although an explanation for the failure to observe a clear increase in signal intensity in slots containing nonradioactive antisense compared with nonradioactive sense actin mRNA cannot be offered with certainty, this result may be due to in vitro bidirectional extension of sense and antisense RNA transcripts of the actin gene. Nevertheless, the results of this experiment demonstrated that attenuation of responses to IFN-γ for increased expression of MHC class mRNA and protein in Mϕ infected with *L. donovani* was related to (infection-induced) inhibition of class II gene transcription.

DNA regions that are believed to be important *cis*-acting elements that regulate the transcription of class II genes have been identified. Two different conserved motifs, referred to as X and Y boxes, have been located within the 5′ flanking region of MHC class II genes in murine, rat, and human cells (7, 10, 11). These DNA regions are believed to be important *cis*-acting elements that regulate the transcription of class II genes. Furthermore, proteins have been identified (and the corresponding genes have been cloned) that bind to the Y box (YB-1) (7) and the X box (10, 11). These proteins are believed to be involved in the transcriptional regulation of class II genes. With respect to YB-1, levels of mRNA for this protein have been shown to correlate inversely with steady-state levels of MHC class II mRNA (7), and this finding has led to the suggestion that YB-1 may negatively regulate the expression of class II genes. On the other hand, for certain X-box-binding proteins, available evidence suggests that they are expressed constitutively, indicating that they may be necessary but not sufficient to support class II gene transcription (10, 11). The finding that infection with *L. donovani* inhibited class II gene transcription suggested the possibility that this effect may have involved the induction, by infection, of either increased YB-1 expression or altered expression of the X-box-binding protein. To examine the potential role of YB-1 in regulating class II gene transcription in infected P388D$_1$ cells, YB-1 mRNA levels were measured by RNA blot analysis. The YB-1 cDNA (7) was a generous gift from B. D. Schwartz of the Washington University School of Medicine, St. Louis, Mo. The insert was isolated from a λgt11 phage preparation and subcloned into Bluescript+, and an antisense RNA probe was synthesized as described above. Although a single hybridization signal was observed when RNA from P388D$_1$ cells was hybridized with radiolabeled human YB-1 antisense RNA, no differences in steady-state levels of YB-1 mRNA between normal or infected cells in either the basal or IFN-γ-induced state were detected (data not shown). The absence of an inverse correlation between YB-1 mRNA and class II Aα and Aβ mRNA levels in infected cells makes induction of increased YB-1 expression an unlikely explanation for decreased class II gene transcription in leishmania-infected cells. Northern blot analyses performed by L. Glimcher (Harvard University) using probes for either murine (mXBP) or human (hXBP) X box proteins also did not reveal changes in mRNA levels related to infection (data not shown). These findings suggest that inhibition of MHC class II gene transcription by *L. donovani* infection in P388D$_1$ cells is also unlikely to be related to direct effects on steady-state levels of XBP mRNA.

One of the important properties of IFN-γ is its ability to enhance the expression of MHC class II molecules on Mϕ during cell activation (12, 21). Results of previous studies from this laboratory demonstrated that macrophages infected with *L. donovani* had defective responses to IFN-γ for the induction of the expression of MHC class II molecules, and this finding was associated with decreased steady-state levels of class II mRNA (14, 15). The latter result could have been related either to the selective induction of a MHC class II-specific RNase activity or, alternatively, to a direct effect on transcription rates for class II genes. The results of the present study provide direct evidence that infection of Mϕ with *L. donovani* interferes with IFN-γ-induced cell activation by attenuating MHC class II gene transcription.

The data, furthermore, indicate that while inhibition of class II gene expression by both *L. donovani* and activators of the adenylate cyclase-protein kinase A system involve pretranslational mechanisms, attenuation of class II gene transcription by *L. donovani* infection is cAMP-independent.

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