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Lipopolysaccharide Up-Regulates MHC Class II Expression on Dendritic Cells through an AP-1 Enhancer without Affecting the Levels of CIITA

Cristina Casals, Marta Barrachina, Maria Serra, Jorge Lloberas, and Antonio Celada

The expression of MHC class II genes is strictly tissue specific. In a limited number of cells, the expression of these genes is inducible by cytokines and only in dendritic and B cells is expression constitutive. LPS blocks the cytokine-dependent induction of these genes, but enhances their expression in dendritic and the B cell line A20. We have observed that LPS increased surface expression by raising I-A protein and mRNA levels. LPS does not enhance the expression of the transactivator CIITA. In transient transfection experiments, LPS induced the expression of the I-Aβ promoter, which contains an AP-1 box located between 1722 and 1729 bp upstream of the transcriptional start site. Mutation of this box abrogated the effect of LPS. The AP-1 box still responded to LPS when we moved it to −611 bp or even when it was in the opposite direction. LPS induced a complex that bound to the AP-1 box. However, in dendritic cells, the complex comprised c-jun and c-fos while in A20 cells only c-jun. This was confirmed by chromatin immune precipitation assays and the distinct induction of c-jun and c-fos mRNAs. Therefore, our results indicate that LPS exerts a novel regulatory mechanism in the control of MHC class II gene expression. The Journal of Immunology, 2007, 178: 6307–6315.
heat-inactivated FCS (PAAR Laboratories), 100 U/ml penicillin, and 100 μg/ml streptomycin. In some preliminary experiments, the dendritic cell line D25C/1 was also used (21). The bone marrow-derived dendritic cells were generated as previously described (22), with modifications. Briefly, bone marrow cells from the femora, tibia, and humerus were flushed. At day 0, bone marrow from each mouse was seeded in 150-mm bacteriological petri dishes with 20 ml of medium containing 10% heat-inactivated FCS, 20 ng/ml GM-CSF (PeproTech) and 100 U/ml penicillin, and 100 μg/ml streptomycin. At day 3, 20 ml of fresh complete medium was added to the plate. At day 6, nonadherent cells were collected and plated in 150-mm dishes and complemented with 20 ml of fresh complete medium per dish. At day 8, half of the culture supernatant was collected, centrifuged, and the cell pellet was resuspended in 20 ml of fresh complete medium and returned to the original plate. At day 10, cells were used. Animal use was approved by the Research Committee of the University of Barcelo-
loña (no. 2523). In some experiments, dendritic cells were prepared in the presence of GM-CSF and IL-4 (PeproTech) as described previously (23). For stimulation studies, saturating amounts of LPS (50 μg/ml for A20 cells and 10 ng/ml for dendritic cells) were used (24). LPS was purchased from Sigma-Aldrich. In several experiments the results obtained with the commercial LPS were compared with purified LPS, provided by Dr. C. Galanos (Max Planck Institute, Freiburg, Germany) (25). No differences were found. Actinomycin D and dichlorobenzimidazole riboside (DRB) were purchased from Sigma-Aldrich. PD98059, SB203580, and SP600125 were obtained from Calbiochem. All other chemicals were of the highest purity available and were purchased from Sigma-Aldrich. Abs against c-fos, c-jun, and NK-1 were obtained from Santa Cruz Biotechnology, p38 (Thr180/Tyr182) was obtained from Cell Signaling Technology, anti-CIITA was used from Abcam, and β-actin was obtained from Sigma-Aldrich. The production of Abs against PU.1 was described previously (26). Cells were washed by centrifugation through an FCS cushion. Cell surfaces were stained using specific Abs and cytofluorimetric analysis. Finally, A20 cells were incubated with fluorescein-conjugated secondary Ab (FITC-labeled sheep anti-mouse IgG; Cappel) and dendritic cells were incubated at 4°C for 45 min. Cells were then washed by centrifugation through an FCS cushion. For cDNA synthesis, 1 μg of RNA and Moloney murine leukemia virus reverse transcriptase RNase H were incubated at 4°C with 1 μl of antisense oligonucleotide primer (5′-CAGGTTCTGGTTGAAA-3′) and 10 ng/ml of primer (5′-GCGTGGAGTGTTGAAA-3′), which contains a Sp6-restricted site. Two independent PCRs of the products constructed were performed. The first was made with the 5′ oligonucleotide used for the generation of the full-length promoter and a 3′ primer containing the box sequence with a mutation. The second PCR was performed with the 3′ primer used for the entire promoter cloning and a 5′ primer with the same mutation as that introduced in the first PCR. The fragments were cloned separately into the pCR2.1 vector and then restricted. Both inserts were ligated into the pGL3-Basic, thereby obtaining the full-length promoter with the mutation. The oligonucleotides used to introduce the mutations contained the same mutated nucleotides as those described for the probes used in the DNA binding assays. A similar strategy was followed to mutate the Y′, X′, and S′ boxes or to introduce the AP-1 binding box in an opposite orientation. A deletion between –1483 and –1460 bp or between –1410 and –1400 bp was obtained using the nucleases Bal 31 following by ligation of the terminals. As control of transcription, a plasmid that expresses the Renilla gene, under the control of the TK promoter, was used.

**Reporter plasmids**

The I-ββ promoter (from –1960 to 65 bp) (29) was cloned directionally into the luciferase reporter plasmid pGL3-Basic (Promega), thereby obtaining a plasmid named pGL3-ββA. The AP-1 box from –1737 to –1717 (5′-CCGGCCGCGCCTG) was mutated by replacement with 5′-CCGGTGTTGTTGAAA-3′, which contains a Sp6-restricted site. Two independent PCRs of the products constructed were performed. The first was made with the 5′ oligonucleotide used for the generation of the full-length promoter and a 3′ primer containing the box sequence with a mutation. The second PCR was performed with the 3′ primer used for the entire promoter cloning and a 5′ primer with the same mutation as that introduced in the first PCR. The fragments were cloned separately into the pCR2.1 vector and then restricted. Both inserts were ligated into the pGL3-Basic, thereby obtaining the full-length promoter with the mutation. The oligonucleotides used to introduce the mutations contained the same mutated nucleotides as those described for the probes used in the DNA binding assays. A similar strategy was followed to mutate the Y′, X′, and S′ boxes or to introduce the AP-1 binding box in an opposite orientation. A deletion between –1483 and –1460 bp or between –1410 and –1400 bp was obtained using the nucleases Bal 31 following by ligation of the terminals. As control of transcription, a plasmid that expresses the Renilla gene, under the control of the TK promoter, was used.

**Nuclear extracts and EMSA analysis**

Nuclear extracts were prepared from A20 and dendritic cells as previously described (29), with some modifications. Briefly, cell pellets were washed twice in cold PBS buffer, and then resuspended in five volumes of hypotonic buffer (10 mM HEPES (pH 7.9) at 4°C, 1.5 mM MgCl2, 10 mM KCl, 5 mM MgCl2, 25% (v/v) glycerol, 20 mM KCl, 0.2 mM EDTA, 0.2 mM PMSF, 0.5 mM DTT) and centrifuged at 1500 rpm for 5 min at 4°C. The pellet was resuspended in three volumes of hypotonic buffer and allowed to stand on ice for 10 min. The lysates were homogenized in a potter and the homogenate was centrifuged at 5000 rpm for 20 min at 4°C to pellet crude nuclei. The nuclear pellet was resuspended in 1/2 vol of low-salt buffer (20 mM HEPES (pH 7.9) at 4°C, 1.5 mM MgCl2, 25% (v/v) glycerol, 20 mM KCl, 0.2 mM EDTA, 0.2 mM PMSF, 0.5 mM DTT), followed by the addition of 1/2 vol high-salt buffer (20 mM HEPES (pH 7.9) at 4°C, 1.5 mM MgCl2, 25% (v/v) glycerol, 1.2 M KCl, 0.2 mM EDTA, 0.2 mM PMSF, 0.5 mM DTT). Crude nuclei were extracted at 4°C for 30 min with continuous stirring, followed by centrifugation at 14,000 rpm for 30 min. Supernatants were dialyzed with the PlusOne Mini Dialysis kit (Amer sham Biosciences) in buffer (20 mM HEPES (pH 7.9), 20% (v/v) glycerol, 100 mM KCl, 0.2 mM EDTA, 0.2 mM PMSF, 0.5 mM DTT) at 4°C. The extracts were cleared by centrifugation at 14,000 rpm for 20 min and the supernatant was collected in aliquots and stored at –80°C until use. Protein concentrations in the nuclear extracts were determined using the Bradford protein assay.

EMSA s were performed as described previously (29). Briefly, binding reactions were prepared with 10 μg of nuclear extracts and 20,000 cpm 32P-labeled probe in the presence of 2 μg of poly(dI·dC), in a final volume of 15 μl containing 1× binding buffer (12 mM HEPES (pH 7.9), 60 mM KCl, 5 mM MgCl2, 0.12 mM EDTA, 0.3 mM PMSF, 0.3 mM DTT, 12% glycerol). An 8 μl protein sample containing 5–10 μg total protein was incubated with the probe for 15 min at 4°C. The radiolabeled probe was then added and incubated for an additional 15 min at room temperature. Samples were loaded onto 8% acryl-
amide gel containing 5% glycerol and 0.25% TBE, and electrophoresed at 4°C. Band-shift gels were dried and bands were visualized using the Phos-
imager (Molecular Dynamics). For supershift experiments, following band-shift reaction, Abs were added to a radiolabeled probe, incubated for 10 min. For competition experiments, 100-fold excess of unlabeled probes were included in the binding reaction. Oligonucleotides used as probes in the assay were 5′-labeled using T4 polynucleotide kinase (USB). All probes were
synthesized by Genotek and corresponded to the a region of the I-Aβ promoter located between −1722 bp and −1740 bp from the ATG (29), corresponding to an AP-1 box (AP-1): 5′-CCACCGTGAGTCATGGGA-3′. The mutated AP-1 boxes were AP-1 mutant 1: 5′-CCACCGTGAGTTGTTGAA-3′ and AP-1 mutant 2: 5′-CCACCGACTAGTCGAGA-3′. The IFN-γ-activated site (GAS) box was the following: 5′-CATGTTATG CATATCCGTTAAGTG-3′. The italicized nucleotides in the sequence and the underlined portions of the sequence denote the DNA-binding boxes. The oligonucleotides used as probes in the EMSA assays contained the same mutated nucleotides as those used to introduce the mutations in the reporter plasmids for luciferase assays.

**Chromatin immunoprecipitation assay**

Approximately 2 × 10^7 of dendritic or A20 cells were grown on 15-cm^2 dishes and cross-linked by addition of formaldehyde (to 1% final concentration) to attached cells (31). Cross-linking was performed at room temperature for 20 min. Cells were washed twice in PBS and collected with a scraper or by centrifugation into 3 ml of 0.1 M Tris-HCl (pH 9.4), 10 mM DTT. Cells were incubated at 30°C for 15 min and collected by centrifugation at 2,000 × g for 5 min at 4°C. The resulting pellet was resuspended sequentially in cold PBS, in buffer I containing 10 mM HEPEs (pH 6.5), 0.25% Triton X-100, 10 mM EDTA, 0.5 mM EGTA, and protease inhibitors (1 mM PMSF, 1 mM iomonicyn, 1 mM orthovanadate, 10 μg/ml aprotinin, 1 μg/ml leupeptin), and buffer II in 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 10 mM HEPEs (pH 6.5), and protease inhibitors and centrifuged at each step. Cells were resuspended in lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl (pH 8.1) and protease inhibitors) and sonicated on ice using the lcasion U200s Control (Ika Labortechnik) (15 pulses of 10 s, 30% cycle and 30% amplitude). The size of the fragments obtained (between 200 and 1,200 bp) was confirmed by electrophoresis. The fragments were centrifuged 10 min at 16,000 × g and the pellet was resuspended in dilution buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris HCl (pH 8.1) and protease inhibitors). As input control, we reserved 100 μl of the mixture.

Chromatin was precleared with protein A-Sepharose at 50%, 20 μg of salmon sperm DNA, 2.6 μg of unspecific IgGs, and 6.6 μg of preimmune serum at 4°C overnight. Spin and collected supernatant of precleared chromatin was incubated with 2 μg of each Ab at 4°C 18 h. After that, protein A-Sepharose at 50% was added, and the mixture was incubated at 4°C overnight. The beads were collected and washed sequentially for 10 min at 4°C with TSE 1 (150 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1)), TSE II (500 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1)), and buffer III (0.25 LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl (pH 8.1)). The beads were washed with Tris-EDTA buffer and eluted three times with 0.1 M NaHCO3, 1% SDS. Cross-links were reversed by incubating samples at 65°C overnight, and DNA of samples was purified with a GFX purification kit (Amersham Biosciences), eluted with 100 μl of the mixture.

**JNK activity assay**

JNK activity was measured as described previously (32). Briefly, cells were lysed and immunoprecipitated with protein A-Sepharose and anti-JNK1 Ab. After several washes, the reaction was performed with 1 μg of GST-c-Jun (1–169) (MBL) as JNK substrate, 20 μM ATP, and 1 μCi γ32P-ATP. SDS-PAGE electrophoresis was performed and exposed to Agfa x-ray films.

**Western blot analysis**

Total cytoplasmic extracts were made by lysing cells as described previously (33). SDS-PAGE was performed and transferred to nitrocellulose membranes (Hybond-C; Amersham Biosciences). After blocking the extracts, they were incubated with primary and secondary Abs and detection was done using an EZ-ECL kit (Biological Industries). Extracts were then exposed to x-ray films (Agfa). β-actin was used as a loading control. Analysis of maximal expression was determined with a Molecular Analyst System (Bio-Rad).

**Statistical analysis**

To calculate the statistical differences between the control and treated samples, we used the Student’s paired t test. Values of p < 0.05 or lower were interpreted as significant.
constitutively express MHC II molecules on the cell surface. Upon LPS stimulation of dendritic cells for 24 h, surface expression of these molecules increased progressively up to twice basal levels (Fig. 1A), decreasing progressively after 48 h. Similar results were obtained when dendritic cells were obtained in the presence of GM-CSF or GM-CSF and IL-4. This increase was not related to differentiation because the levels of CD11c, a marker for dendritic cells (34), was not modified (Fig. 1B). The mature B cell line, A20, is devoid of macrophage characteristics (18) and constitutively expresses MHC II I-A molecules on the cell surface. Upon LPS stimulation for 24 h, surface expression of these molecules in this cell line increased progressively up to 100% over basal conditions (Fig. 1, C and D).

The steady-state levels of I-Aβ mRNA were measured in dendritic and in A20 cells at a series of times of treatment with LPS. The determination of β-actin, whose mRNA level does not change in response to treatment with LPS, was used as a control for each time point. Similar results were obtained when we used as control the L14 (mitochondrial RNA) or the 18s rRNA. The increase in surface expression of I-A after LPS treatment was preceded by an increase in the steady-state levels of β-chain mRNA, as determined by real-time PCR (Fig. 2, A and B). However, distinct kinetics for the increase in mRNA were observed. For dendritic cells, mRNA levels augmented between 1 and 3 h, while for A20 cells, the increases started at 3 h. These observations suggest that distinct mechanisms occur depending on the cell type. A similar increase in I-Aα mRNA was observed after addition of LPS in dendritic and in A20 cells (data not shown).

The requirement of CIITA has been extensively documented in constitutive and inducible MHC II genes (35). For this reason, here we examined the involvement of this transactivator in LPS-induced up-regulation of I-A. Real-time PCR was used to measure the changes in CIITA mRNA. The increase in I-Aβ was not preceded by an increase in CIITA in either dendritic (Fig. 2C) or A20 cells (Fig. 2D). At early time points after LPS treatment (5, 10, 15, and 20 min), no increased levels of mRNA for I-Aβ or CIITA were detected. The levels of CIITA protein during the 3 h following LPS treatment were unchanged (data not shown). In the B cell line RJJ2.2.5, which is defective in CIITA (36), no expression of MHC II molecules was found after treatment with LPS (data not shown). These results indicate that although CIITA was necessary, LPS up-regulated I-Aβ by a mechanism, probably an enhancer, without increasing this transactivator.

To determine whether the increase in mRNA levels is due to production of mRNA or inhibition of its degradation, we measured the rate of mRNA degradation. A20 cells were treated with DRB (37) at a concentration sufficient to block all further RNA synthesis, as determined by [3H]UTP incorporation (18). RNA was isolated from aliquots of cells at 1, 3, and 6 h after the addition of DRB, which allowed us to estimate the half-life of I-Aβ mRNA. Under these conditions, the mRNA was stable (Fig. 3A). After 6 h of DRB treatment, no modifications in the half-life of I-Aβ mRNA were detected. Identical results were obtained when we blocked transcription by combined treatment with DRB and actinomycin D (Fig. 3B). As a control, we determined the half-life of c-myc mRNA. The mRNA of this proto-oncogene is unstable and shows a half-life of <1 h (38). When we extrapolated the amounts of c-myc RNA, we obtained a half-life of around 30 min (Fig. 3, A and B). Similar stability of I-Aβ mRNA was found in dendritic cells and the stability was similar to that shown by I-Aα mRNA. These results demonstrate that augmented mRNA levels are not due to an increase in the half-life of RNA but to an increase in transcriptional rate.

**LPS induces the transcription of I-Aβ through an AP-1 box**

Having observed the capacity of LPS to induce the expression of I-Aβ and I-Aα and established the lack of involvement of CIITA in

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**FIGURE 3.** I-Aβ mRNA is stable. A20 cells were treated with DRB (20 μg/ml) or DRB and actinomycin D (5 μg/ml). I-Aβ mRNA was then measured by quantitative RT-PCR after the times indicated. Cell viability was >95% for all culture conditions. As a control, we also determined the half-life of c-myc. The figure shows one representative result of three independent experiments.

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**FIGURE 4.** The increase in I-Aβ promoter expression induced by LPS is dependent of an AP-1 box. A20 cells were transiently transfected with the following reporter plasmids: A, empty vector; B, I-Aβ promoter –1969 to +65 bp; C, same as B but with a mutated AP-1 box; D, same as B, but with mutated Y′, X′, and S′ boxes; E, same as B, with a deletion from −1483 to −136 bp; F, same as B, but with the AP-1 box in the opposite orientation; G, same as B, with a deletion from −1479 and −40 bp, H, The S, X, and Y sequences of the SXY module were mutated. Each measurement was performed in triplicate and the mean values and the SD are shown. The figure is representative of four independent experiments. In B and D–F, a significant difference was found when we compared each point with the corresponding control and four distinct assays (p < 0.01).
the signal transduction pathway, we next analyzed the functional activity of the I-\(\alpha\beta\) promoter to delineate the critical sequence elements for this induction. The TRANSFAC database was used to examine the promoter sequence for possible regulatory areas in which the transcription factor binds (39). This analysis showed that the promoter region contains various putative sites for the binding of transcription factors that may be involved in LPS signal transduction. One of these was an AP-1 box located between 1722 and 1729 bp upstream of the transcriptional start site (29). To examine the role of this putative area, we determined the effect of LPS on the promoter linked to the luciferase gene, which was used as the reporter. Using this assay, when we transfected A20 cells, the control (without the promoter) showed basal luciferase activity. Each construction was cotransfected with the Renilla expression vector to correct for differences in transfection efficiency. The construct containing the 2025 bp of the promoter showed basal transcriptional activity; however, under the effect of LPS, this expression increased 2-fold (Fig. 4). When the AP-1 box was mutated, LPS-dependent inducibility was lost. The AP-1 box is near the conserved elements located upstream (Y‘X’S’ module) between −1722 and −1730 bp from the ATG (29). Because this area is involved in the transcription of class II genes (40), we mutated these boxes. In this construction, basal activity was increased but addition of LPS led to a 4-fold increase in induction. We found that the upstream boxes repress the promoter activity by modifying the chromatin structure (manuscript in preparation). When we reduced the distance between the boxes located proximally or upstream to 368 bp (Fig. 4) or to 23 bp, LPS continued to increase the induction of the luciferase gene. This effect was also observed when the orientation of the AP-1 box was modified (Fig. 4). However, when we deleted the proximal area of the promoter, basal induction and susceptibility to LPS were lost. Finally, as has been shown previously, the mutation of the proximal boxes abolished the expression (41). Similar results were observed in dendritic cells. These data indicate that the constitutive induction of I-\(\alpha\beta\) in B and dendritic cells is upregulated by LPS through an AP-1 region, which acts as an enhancer because it was independent of distance or orientation.

**Distinct protein complexes in dendritic and A20 cells bind to the AP-1 box in the I-\(\alpha\beta\) promoter**

To examine the proteins that bind to the areas of interest in the I-\(\alpha\beta\) promoter, gel electrophoresis-DNA binding assays were performed. Using nuclear extracts prepared from dendritic or A20 cells and a probe covering the AP-1 box, we observed a retarded band (Fig. 5). In both cell types after treatment with LPS, nuclear extracts showed increased intensity. The specificity of the binding was determined by using a probe with two mutated nucleotides. No retarded bands were detected. To further characterize the specificity of the interaction between the AP-1 box and the transcription factors, competition experiments were performed (Fig. 5). As expected, the band produced by the LPS-treated extracts was removed when we used a 100-fold excess of the same unlabeled oligonucleotide. No competition was detected when we used a 100-fold excess of a oligonucleotide containing the mutated AP-1 box or the GAS box. These observations indicate that this band corresponds to an AP-1 complex in dendritic and A20 cells.

Surprisingly, when the nuclear extracts from dendritic and A20 cells were run in the same gel, the shift showed distinct mobility (Fig. 6A). The band obtained with nuclear extracts of dendritic cells was more retarded than the shift observed with extracts of A20 cells is up-regulated by LPS through an AP-1 region, which acts as an enhancer because it was independent of distance or orientation.

**Distinct protein complexes in dendritic and A20 cells bind to the AP-1 box in the I-\(\alpha\beta\) promoter**

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A20 cells, suggesting that the protein complex bound to the probe differs with cell type. Next, we attempted to identify the AP-1 proteins that form the AP-1 complex. For this purpose, we used Abs against c-fos and c-jun. These two Abs, but not the nonspecific IgGs used as control, retarded the shift of nuclear extracts of dendritic cells and decreased the intensity of the original band (Fig. 6B). This finding suggests that the induced protein complex that binds to the AP-1 sequence is formed by c-fos and c-jun. When we used nuclear extracts from A20 cells in the same assay, only the Ab against c-jun induced a supershift, indicating that the complex involved in DNA binding was a homodimer of c-jun (Fig. 6C). A dimer of c-jun has a molecular mass of 71.88 kDa while c-jun and c-fos heterodimers have a molecular mass of 76.78 kDa, which may explain the mobility in the gel shift.

To corroborate these data, we performed chromatin immunoprecipitation experiments using Abs against c-jun and c-fos. The DNA fragments were amplified and the amounts were determined by quantitative PCR. In dendritic cells, the AP-1 box was precipitated by Abs against both c-jun and c-fos, while in A20 cells precipitation occurred only by Abs against the former (Fig. 6D). No DNA was amplified when we used Abs against the transcription factor PU.1 or when we used a distinct position of the promoter that does not contain the AP-1 box (results not shown).

LPS increases expression of MHC class II

To determine the expression of the genes corresponding to the proteins that bound to the AP-1 box, quantitative RT-PCR was performed using RNA from dendritic cells obtained at a range of times after treatment with LPS. Expression of c-fos and c-jun increased, peaking at 30 min and returning to basal levels at 3 h (Fig. 7A). Activating transcription factor-2 was not induced. Similar results were obtained with A20 cells. As expected, JNK activity was induced by LPS in both dendritic and A20 cells (Fig. 7B). However, the other MAPK, p38, was phosphorylated in the former but not in the latter (Fig. 7C). These results may explain the differences observed in the components that bound to the AP-1 box of the I-Aβ promoter.

To corroborate the role of JNK and p38 in the LPS-induced enhancement of MHC class II expression, we determined the levels of I-Aβ mRNA in dendritic cells treated with drugs such as PD 098059, SB 203580, and SP 600125, which have been extensively used as selective inhibitors of the activation of MEK/ERK, p38, and all JNK isoforms, respectively (42–44). These compounds inhibited MAPKs in the cellular models tested but did not show toxic effects (45). The inhibition of MEK/ERK did not modify the LPS-dependent enhancement of MHC II mRNA or protein expression in dendritic cells (Fig. 8). However, the inhibition of p38 or JNK blocked the increase produced by LPS (Fig. 8). All these data support the role of AP-1-binding proteins and their activation by the corresponding MAPK pathway in the induction of MHC II proteins by LPS.

Discussion

After stimulation of the pathogen-associated molecular pattern receptors of dendritic cells, RNA expression of MHC II initially increases but then decreases (46–49). The reduction is due to a decreased expression of CIITA, the master regulator of MHC II transcription. The arrest in CIITA mRNA expression is produced by transcriptional inactivation mediated by a histone deacetylation.
over a large domain spanning the regulatory region of MHC2TA (48, 49). Recently, it has been shown that after 24 h of treatment, LPS mediated repression of CIITA expression (23). The apparent contradiction with the data presented here is due to the different kinetics of activation and deactivation, as well as the distinct components of the MAPK used in each case. Induction of AP-1 is very fast (around 30 min) and the MAPKs involved are p38 and JNK. In the case of repression, the effect is mediated by ERK and p38, which requires longer to decrease histone acetylation and to repress CIITA transcription (23). However, the mechanism of the initial transient increase has not been determined (48). Here, we have shown that LPS does not induce the expression of the trans-activator CIITA in dendritic or A20 cells, which corroborates previous observations (17, 50). Although the levels of CIITA protein were not modified after 3 h of LPS treatment, we cannot exclude posttranslational modifications of CIITA that affect MHC II transcription. However, this possibility is unlikely because the mutation of the AP-1 box in the I-Aβ promoter abolished the LPS effect. An increase in MHC II Ag expression has been reported in B cells after exposure to CpG DNA because mRNA was stabilized (50). These data differ to those presented here. Under our experimental conditions, I-Aβ mRNA was stable in B and dendritic cells, as also shown in macrophages (11, 18). In addition, A + U-rich elements, which contain repeats of AUUUA sequences as well as other sequences, are absent in I-Aβ or I-Aα mRNA. These sequences bind a protein necessary for cleavage by the exonuclease (51), thereby producing the degradation of RNA (52). One difference between the two reports is that Kuchty et al. (50) used CpG DNA, which interacts with TLR9, to achieve B cell activation (53) while we used LPS, which signals through TLR4 (54).

If the half-life of mRNA was not affected, we propose that the increase in mRNA observed was a consequence of augmented transcription. The presence of CIITA is required not only for MHC II induction, but also the degree of CIITA expression correlates with the levels of MHC II (35). The observation that LPS does not induce CIITA overexpression indicates that LPS works through the induction of an enhancer mechanism. In fact, this mechanism involves the induction of an AP-1 complex that binds to a box upstream of the conserved sequences Y′-S′. When we compared the promoters of several MHC II, we detected an AP-1 box located in a similar region as in I-Aβ (Fig. 9), suggesting that the same mechanism is used by other MHC II in response to LPS.

The AP-1 box is near the conserved elements located upstream (Y′X′S′ module), an area that has been involved in vivo in the transcription of class II genes (40). This distal region functions as a type of regulatory element known as locus control region (55). It has been shown that RFX and CIITA bind to the upstream Y′-S′ module in vivo. Binding of RFX and CIITA correlated with spreading of histone acetylation from the promoter to as far as 16 kb upstream. This long-range chromatin remodeling was associated with the generation of extragenic transcripts initiating bidirectionally near the upstream Y′-S′ enhancer (39). Although the important role of the distal region in our experiments using reporter gene constructs transfected transiently, we find no differences if we delete the Y′-S′ module. Also, in vivo studies using chromatin immunoprecipitation shows that the AP-1 box was occupied after LPS treatment.

A direct role of NF-kB activation in TLR-triggered expression of class II molecules in B cells has been reported (17). However, we found an NF-kB box in the promoter of I-Aβ at −1097 bp, which we believe does not play a major role in the induction of class II expression by LPS. In fact, the construct that contained a deletion between −1391 and −389 bp, where the NF-kB box is located, still responded to the enhancing effect of LPS. In a study by Lee et al. (17), the removal of the NF-kB box from the promoter reduced but did not totally abrogate the effect of the CpG-DNA or LPS on MHC II expression.

Analysis of the proteins that formed the complex that bound to the AP-1 box showed a difference between dendritic cells and the A20 B cell line. In the latter, the AP-1 transcription complex comprised c-jun and c-fos while in the former only c-jun was present. These widely distributed transcription factors play a crucial role in the subsequent regulation of expression of genes involved in DNA repair, cell proliferation, cell cycle arrest, death by apoptosis, and tissue and extracellular matrix remodelling (56). In addition to being regulated at the transcriptional level, the activities of jun and fos are also controlled by phosphorylation as a result of the activation of intracellular signaling cascades. Macrophage response to LPS involves the phosphorylation of three members of the MAPK family, which includes the ERK-1/2 and the stress-activated protein kinases, p38, and JNK (57). Once activated, JNK translocates to the nucleus, where it phosphorylates c-jun and thereby enhances c-jun transcriptional activity (58). Concerning c-fos, it is phosphorylated by ERK2 (59, 60) or by p38 (61), depending on the activator. Under our experimental conditions, after LPS treatment, p38 was phosphorylated in dendritic but not in A20 cells. This may be related to the different origin of these cells, as previously reported (62), and may explain the distinct nature of the AP-1 complexes. The amounts of LPS used to activate dendritic (10 ng/ml) and A20 cells (50 μg/ml) differed because these cells use different members of the TLR family to bind LPS (63). Therefore, the signal transduction initiated after receptor engagement may differ, thereby explaining that p38, in some cases, is phosphorylated but not in others. This difference between dendritic and A20 cells may explain the distinct time courses for the cell surface expression and mRNA of MHC II. We cannot exclude that the different mechanisms described here reflect that dendritic are non-transformed cells, while A20 cells are a cell line.

Dendritic cells are present in an immature state in peripheral tissues dedicated to capturing Ags. Upon receiving an activatory signal associated with pathogens or inflammation, dendritic cells migrate to the local lymph node, mature, and present to T cells the Ags captured in the periphery. Dendritic cells are highly adept at providing T cells with a memory of past encounters with Ags in the form of MHC II-peptide complexes. This ability is conferred by a number of developmental changes that affect the Ag-presentation machinery during the so-called maturation process (64). Dendritic cells increase several fold their surface expression of MHC II molecules. This increase is accompanied with a dramatic change in localization of MHC II molecules, which are abundant in endosomal structures in immature cells but are located mostly on the plasma membrane in mature cells. The major mechanism responsible for the accumulation of MHC II molecules at the cell surface is the down-regulation of MHC II internalization and degradation. This decrease ensures that the complexes generated during maturation will remain on the cell surface for extended periods, enabling mature cells to provide antigenic memory.

FIGURE 9. Localization of AP-1 boxes in MHC II genes.
Upon maturation with inducers such as LPS, there is an increase in the synthesis of MHC II initially but then it decreases (46–49). Here, we showed that LPS exerts a novel regulatory mechanism in the control of MHC II gene expression inducing the activation of c-Jun and c-Fos that through an AP-1 box enhances the CIITA-mediated transcription of MHC II molecules.

In summary, we have found that, in cells which constitutively express MHC II molecules, LPS increases their expression through an AP-1 box that acts as an enhancer. Our results point to a novel regulatory mechanism of MHC II expression by LPS.

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Disclosures

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References


