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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

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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 immuno 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.

Materials and Methods

Cells and reagents

The murine lymphoma A20 cell line (20) was maintained in DMEM medium (BioWhittaker) containing 2 mM glutamine, 50 μM 2-2-ME, 10% M 2-2-ME, 10%
heat-inactivated FCS (PAA Laboratories), 100 mM penicillin, and 100 µg/ml streptomycin. In some preliminary experiments, the dendritic cell line D2SC1/1 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 in 20 ml of DMEM containing 10% heat-inactivated FCS, 20 ng/ml GM-CSF (PeproTech) and 100 U/ml penicillin, and 100 µg/ml streptomycin. At day 2, 25 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 Experimental Research Committee of the University of Barcelo-
na (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 obtained from Calbiochem. All other chemicals were of the highest purity available and were purchased from Sigma-Aldrich. Abs against c-jun and c-fos, c-jun, and JNK-1 were obtained from Santa Cruz Biotechnology, p38 

**Reporter plasmids**

The I-\(\alpha\)B promoter (from −1960 to 65 bp) (29) was cloned directionally into the luciferase reporter plasmid pGL3-Basic (Promega), thereby ob-

aining a plasmid named pGL3-pI\(\alpha\)B. The AP-1 box from −1737 to −1734 (5′-GCAGCTGGAAA-3′) was mutagenized by standard PCR (using primers 5′-GGTTGAGTTGAGGAA-3′ and 5′-GGTTGAGTTGAGGAA-3′), which contains a SpeI restriction site. Two independent PCRs of the plasmids 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 −1456 bp or between −1483 and −1440 bp in the nucleotide Bal 31 followed by ligation of the terminals. As control of transfection, a plasmid that expresses the Renilla gene, under the control of the TK promoter, was used.

**Transient transfections and luciferase assays**

For transfection, 20 µg of plasmid and 2 µg of pRL-TK plasmid were added to 2 × 10^6 A20 cells in DMEM medium, in a volume of 400 µl in a sterile GenePulser Cup (Bio-Rad). Cells were centrifuged and the cell pellet was resuspended in 100 µl of medium. 100 µl of media were added and the cells were further incubated at 4°C for 45 min. The production of Abs against PU.1 was described previously (26). Sec-

**Nuclear extracts and EMSA analysis**

Nuclear extracts were generated as previously described (22), with modifications. Briefly, nuclear extracts were obtained 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 MgCl_2, 10 mM KCl, 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 with a pestle and the homogenate was centrifuged at 5,000 rpm for 20 min at 4°C to pellet nuclear debris. 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 MgCl_2, 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 of high-salt buffer (20 mM HEPES (pH 7.9) at 4°C, 1.5 mM MgCl_2, 25% (v/v) glycerol, 1.2 M KCl, 0.2 mM EDTA, 0.2 mM PMSF, 0.5 mM DTT). Nuclear debris 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 Dial-

**Quantitative RT-PCR analysis**

Cells were washed twice in cold PBS and total RNA was extracted with the EZ-RNA kit (Biological Industries), following the manufacturer’s instructions. For quantitative RT-PCR analysis, RNA was treated with DNase (Roche) to remove contaminating DNA (28). For RNA synthesis, 1 µg of RNA and Moloney murine leukemia virus reverse transcriptase RNase H minus, point mutant, oligo(T)_14 primer and PCR nucleotide mix were used, following the manufacturer’s instructions (Promega). The primers and probes used to amplify mouse cDNA were: 5′-I-\(\alpha\)-B: ACCACGCGCAAATGCAGAGTTC-3′ and 5′-TGTCCTACGACTGACGTTGATGAC-3′ (ac-

Continued...
synthesized by Genetek and corresponded to the a region of the I-A\(\beta\) promoter located between –1722 bp and –1740 bp from the ATG (29), corresponding to an AP-1 box (AP-1): 5’-CCACCGTGAGTGATGGAA-3’. The mutated AP-1 boxes were AP-1 mutant: 5’-CCACCGTGAGTGATGGAA-3’ and AP-1 mutant 2: 5’-CCACCGTGAGTGATGGAA-3’. The ifn-\(\gamma\)-activated site (GAS) box was the following: 5’-CATGTATG CATATTCCTGTAAGTG-3’. The italicized nucleotides are the mutations introduced in the sequence and the underlined portions of the sequence indicate the DNA-hinding 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 \(\times\) 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 8.4), 10 mM DTT. Cells were incubated at 30°C for 15 min and collected by centrifugation at 2,000 \(\times\) 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 iomycin, 1 mM orthovanadate, 10 \(\mu\)g/ml aprotinin, 1 \(\mu\)g/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 Ikasonic U200S Control (Ika Labortecnik) (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 \(\times\) g and the pellet was resuspended in dilution buffer (1% Triton X-100, 2 g/ml EDTA, 150 mM NaCl, 20 mM Tris HCl (pH 8.1) and protease inhibitors). As input control, we reserved 100 \(\mu\)l of the mixture.

Chromatin was precleared with protein A-Sepharose at 50%, 20 \(\mu\)g of salmon sperm DNA, 2.6 \(\mu\)g of unspecific IgGs, and 6.6 \(\mu\)g of preimmune serum at 4°C overnight. Spun and collected supernatant of precleared chromatin was incubated with 2 \(\mu\)g 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 I (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 NaHCO\(_3\), 1% SDS. Cross-links were reversed by incubation at 65°C overnight, and DNA of samples was purified with a GFX purification kit (Amersham Biosciences), eluted with 30 \(\mu\)l of H\(_2\)O and assayed by quantitative PCR using the following primers: 5’-CAGAGGACAGGGGTGG-3’ and 5’-CCCGCCCTACCGAGTTTG-3’.

**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 \(\mu\)g of GST-c-jun (1–169) (MBL) as JNK substrate, 20 \(\mu\)M ATP, and 1 \(\mu\)Ci \(^{32}\)P-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). \(\beta\)-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 cell 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 RJ2.2.5, 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 to 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.

**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-\(\alpha\B\) 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 reporter. Using this assay, when we transfected A20 cells, the control (without the promoter) showed basal luciferase activity. Each reporter. Using this assay, when we transfected A20 cells, the con-

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

To examine the proteins that bind to the areas of interest in the I-\(\alpha\B\) 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 specific-

FIGURE 5. LPS induces the binding of a protein complex to the AP-1 box. Nuclear extracts were prepared from dendritic (A) or A20 cells (B) incubated with LPS (10 ng/ml for dendritic and 50 mg/ml for A20 cells) for the times indicated. The sequences of the oligonucleotides are indicated in the figure. Competition experiments were made by adding 100-fold excess of the cold oligonucleotides indicated to the nuclear extracts before addition of the radiolabeled AP-1 oligonucleotide. These experiments were performed three times and the results of one representative experiment are shown.

FIGURE 6. Dendritic and A20 cells produce distinct AP-1 complexes. Cells were incubated with LPS (10 ng/ml for dendritic and 50 mg/ml for A20) for 30 min and nuclear extracts were then obtained. A, Binding of nuclear extracts from LPS-treated dendritic and A20 cells to the AP-1 box. Anti-c-fos, anti-c-jun, or control IgG (2 \(\mu\)g) were added to extracts of dendritic (B) or A20 cells (C) before the binding assay was performed. D, Chromatin immunoprecipitation was conducted using the Abs indicated. The amplified fragment was then determined using quantitative RT-PCR. The figures are representative of four independent experiments. In D, a significant difference was found when we compared anti-c-jun or anti-c-fos for dendritic cells or anti-c-jun for A20 cells at each point with the corresponding control and four distinct assays (\(p < 0.01\)).
involved in DNA binding was a homodimer of c-jun. Ab against c-jun used nuclear extracts from A20 cells in the same assay, only the c-jun may explain the mobility in the gel shift. DNA fragments were amplified and the amounts were determined c-fos heterodimers have a molecular mass of 76.78 kDa, which 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 induces the expression of c-jun and c-fos mRNAs and JNK activation**

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-αβ promoter.

To corroborate the role of JNK and p38 in the LPS-induced enhancement of MHC II expression, we determined the levels of I-αβ 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 transactivator 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-βRI 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-βRI 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-α/β or I-α/α 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 Kuchtey et al. (50) used CpG DNA, which interacts with TLR9, to achieve B cell activation (53) whereas 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-α/β (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 transcribed 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-κB activation in TLR-triggered expression of class II molecules in B cells has been reported (17). However, we found an NF-κB box in the promoter of I-αRI 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-κB box is located, still responded to the enhancing effect of LPS. In a study

by Lee et al. (17), the removal of the NF-κB 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 the 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.
Upon maturation with inducers such as LPS, there is an increase in the synthesis of MHC II initially but it then 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
The authors have no financial conflict of interest.

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