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Class II Transactivator Is Required for Maximal Expression of HLA-DOB in B Cells

Uma M. Nagarajan,* Jonathan Lochamy,* Xinjian Chen,† Guy W. Beresford,* Roger Nilsen,*, Peter E. Jensen,‡ and Jeremy M. Boss²*.

HLA-DO, encoded by the HLA-DOA and HLA-DOB genes, has been shown to function as a modulator of Ag presentation. DNA microarray comparisons between B cells wild-type and mutant for the master regulator of MHC class II transcription, class II transactivator (CIITA), identified HLA-DOA and HLA-DOB as being up-regulated by CIITA. Although HLA-DOA had been shown previously to be regulated by CIITA, HLA-DOB expression was suggested to be independent of CIITA. A series of assays including quantitative RT-PCR, promoter-reporter assays, chromatin immunoprecipitations, and intracellular staining were performed to corroborate the DNA microarray analysis. The combined data demonstrate that HLA-DOB levels are increased by CIITA, and that this difference has an impact on the overall level of HLA-DO expression. Additionally, unlike the classical MHC class II genes, HLA-DOB expression was present in the absence of CIITA, indicating that additional factors mediate HLA-DOB expression in B cells. The Journal of Immunology, 2002, 168: 1780–1786.

Antigen presentation by B cells and APCs is a complex process involving several steps (reviewed in Ref. 1). The process begins with the synthesis of MHC class II α- and β-chains that associate with each other and the invariant chain (Ii) trimers in the endoplasmic reticulum. This association prevents the binding of endogenously synthesized peptides to the MHC class II molecule. The class II Ii complex is transported to endosomes where the Ii is degraded by proteolytic enzymes, leaving a small portion of Ii, termed class II-associated Ii peptide bound to the peptide-binding cleft of the MHC class II molecule. When Ag-containing endosomes fuse with the lysosomes, the pH drops and the class II-associated Ii peptide is replaced by antigenic peptides with higher affinities for the MHC-binding cleft in a manner that is dependent on the activity of HLA-DM. The peptide-MHC complex is then transported to the plasma membrane.

HLA-DO, a nonclassical MHC class II-like protein encoded in the class II region of the MHC, is composed of HLA-DOα and HLA-DOβ (Refs. 2 and 3) and HLA-DOB (4, 5). HLA-DOα differs from other components of the class II Ag presentation pathway in that it is selectively expressed in B cells and a subset of thymic epithelial cells, but not other professional APCs (6–10). Its function remains poorly understood and controversial (11). HLA-DO binds tightly to HLA-DM in the endoplasmic reticulum and the complex remains associated after transport to endosomal compartments (9). Several studies have demonstrated that HLA-DO inhibits the peptide loading function of HLA-DM, although DO-DM complexes may retain activity in the highly acidic environment of the lysosome-related MHC class II compartments in B cells (12–15). However, it has also been reported that HLA-DO enhances HLA-DM activity (16). Currently, there is no information on whether HLA-DO expression is modulated in B cells. Whatever its function, HLA-DO plays a role in modulating peptide loading and Ag presentation by B cells.

The MHC class II isotypes (HLA-DR, -DQ, and -DP), HLA-DM, and Ii genes have been shown to be regulated by a common set of transcription factors that bind to a series of promoter proximal elements termed the W, X1, X2, and Y boxes (reviewed in Ref. 17). These factors include RFX, X2BP/CREB, NF-Y, and class II transactivator (CIITA). RFX is composed of the subunits RFX5, RFXAP, and RFX-B/ANK (18–21), and was shown to bind as a complex to the X1 box of the upstream enhancer element of these genes (22, 23). X2BP/CREB binds to the X2 box in a cooperative manner with RFX (23, 24). NF-Y also binds cooperatively to its sequence, the Y box, which is located downstream of the X-box region sequences (25). CIITA functions as a coactivator by interacting with the above factors once they are bound to their respective DNA elements (26–28). CIITA is expressed constitutively in Ag-presenting cells and can be induced by IFN-γ in other cell types (29). Both RFX and CIITA are required for MHC class II and HLA-DM gene expression (30, 31). Patients carrying mutations in their genes for CIITA or any one of the RFX components exhibit a SCID called the bare lymphocyte syndrome (BLS; reviewed in Ref. 32). Unlike the other MHC class II-like genes, regulation of the HLA-DO genes by CIITA has been controversial. In previous reports, IFN-γ did not induce HLA-DO in non-Ag-presenting cells, suggesting that CIITA does not play a role in its regulation (4). However, a recent report suggests that the two chains of HLA-DO, HLA-DOα and HLA-DOβ, are differentially regulated (33). HLA-DO was shown to be regulated by RFX, but not regulated by CIITA, while HLA-DOα was regulated by both factors.

While performing a search for novel genes regulated by CIITA, a comparison between the transcripts produced by the B cell line Raji (wild-type for CIITA) and its irradiated mutant daughter line, R12.2.5, which is mutant for CIITA, was performed. More than...
12,000 cDNAs were compared. In this analysis, HLA-DOB was consistently found to be expressed at a higher level in Raji cells when compared with R2J.2.5, suggesting a role of CIITA in its expression. Because this observation was inconsistent with the above reports, a detailed analysis of the role of CIITA in HLA-DOB expression was conducted. Quantitative RT-PCR, intracellular staining and flow cytometry, HLA-DOB promoter-reporter assays, and chromatin immunoprecipitations (ChIP) were performed to confirm the DNA microarray data. The combined data suggest that HLA-DOB is in fact regulated by CIITA, which can impact the overall level of HLA-DO expression and ultimately, Ag presentation.

Materials and Methods

Cell lines

Raji, a Burkitt’s lymphoma-derived cell line, is wild-type for CIITA and postared for MHC class II gene expression (34). The human B cell line R2J.2.5 was derived by mutagenesis from Raji cells and selected for loss of HLA class II Ag expression (35). R2J.2.5 is deficient for CIITA (26, 36).

The SJO cell line was derived from a patient with BLS and is defective for CIITA (26, 36).

DNA microarray hybridization and analysis

Test DNA microarray chips from Affymetrix (Santa Clara, CA) were used to check the cRNA for equal hybridization to 5′ and 3′ oligonucleotides of housekeeping genes before each experiment. Four independent experiments were conducted. The initial set used the Affymetrix Hu6800 (A-D) microarrays. A second set used the Affymetrix Hu133A-1 (A-D). A third set used the Affymetrix Hu133A-2 (A-D). A fourth set used the Affymetrix Hu133Plus2B (A-D) microarrays. The Hu6800 and Hu133A-1 microarrays were used for the DNA microarray hybridizations. The Hu133A-2 and Hu133Plus2B microarrays were used for the comparative analysis. The Hu6800 and Hu133A-1 microarrays were analyzed by the Microarray Suite 4.0 software and the Hu133A-2 and Hu133Plus2B microarrays were analyzed by the Microarray Suite 5.0 software. The data were published into the MICRODB software followed by comparative analysis using the DMT software (Affymetrix).

Real-time RT-PCR

Multiple RNA preparations using RNeasy method (Qiagen) were prepared for quantitative RT-PCR analysis. RNA (2 μg) was reverse transcribed using Superscript II RT (Life Technologies), and buffers from a RT-PCR kit (Applied Biosystems, Branchburg, NJ) according to the manufacturer’s directions. A total of 1/20 of the reverse transcription reaction was used for quantitative RT-PCR in a reaction containing SYBR Green, 1X SYBR (BioWhittaker Molecular Applications, Rockland, ME), 0.04% gelatin, 0.3% Tween, 50 mM KCl and 20 mM Tris (pH 8.3), 3 mM MgCl2, 0.2 mM dNTP, and 50 nM of each primer. A two-step PCR with denaturation at 95°C for 15 s and annealing and extension at 60°C for 1 min for 40 cycles was conducted in a BIO-RAD i-cycler (Bio-Rad, Hercules, CA). An additional set of PCR-using primers for the GAPDH transcripts was conducted to provide a normalization reference in the reactions. The primer sets used were: HLA-DOB, 5′-CTTGGAGTGAGGAGCTCA and 5′-CAG CTCTTGGACCCCTATTACC; HLA-DOA, 5′-CAGCGATCGGAGCT CCAG and 5′-GGATCACCTACGGGGACAC; and GAPDH, 5′-CCATG GGGAAGGTGAAGGTCGGAGTC and 5′-GGTTGTCAGGACGGCAT TGCTGATG. The HLA-DRa primer sets have been previously described (27). The threshold cycle values for HLA-DO and HLA-DRa genes were normalized to the threshold cycle of GAPDH and converted to linear scale. All real-time RT-PCR were conducted at least three times from independent RNA preparations. The average of these experiments is presented relative to the levels in Raji cells.

Plasmid construction and transient transfection

Plasmids pHACIITA and pCIITA20.2 express wild-type and dominant-negative forms of CIITA, respectively (39, 40). pG3L-DRa was a generous gift from the lab of Dr. P. van den Elsen (Leiden University Leiden, The Netherlands) (41). A 317-bp fragment upstream of HLA-DOB gene transcription start site was PCR-amplified and inserted into Kpn/IThrhol digested pG3L to create the HLA-DOB promoter-reporter pG3L-DOB. Primers used for the PCR of the HLA-DOB promoter sequence were 5′- CCCGTACCCTGCTCTGATTCTTCC and 5′-GGGAAGGTGAAGGTCGGAGTC and GCCCCGGATAGGA. For transient transfections, 10 μg of the luciferase reporter (pGL3-DOA, pHACIITA, or pCIITA20.2) was transfected into Raji and R2J.2.5 cells by electroporation, as described previously (42). A total of 1 μg of pTK-RL (Promega, Madison, WI), which expresses a Renilla luciferase gene, was cotransfected to normalize for transfection efficiency, and pUC18 was added to all transfection to bring the DNA concentration to 50 μg. Cells were harvested 24 h after transfection, washed, and lysed by three rounds of freezing and thawing. Luciferase activity was measured using the Dual Luciferase Assay kit (Promega).

Stable transfections

Stable cell lines expressing the pHACIITA expression plasmid were created in R2J.2.5, A431, and Jurkat cells. All transfected pools were selected for 2–3 wk in G418. The R2J.2.5-CIITA transfectant pool was stained for MHC class II proteins using the pan murine anti-MHC class II following the manufacturer’s instructions. Positive cells were selected by binding to rat monoclonal antibodies (Becton Dickinson) and magnetic beads (Dynabeads, Lake Success, NY). The cells bound to beads were washed in PBS containing 5 mM EDTA and 1% BSA, and the cells were cultured in selection medium until they detached from the beads. A431 cells expressing wild-type CIITA were generated by transfecting the cells with pHACIITA using Fugene (Roche Molecular Biochemicals, Indianapolis, IN), followed by selection in medium containing 1 mg/ml G418. The cells were checked for class II expression 3 wk later by flow cytometry. The Jurkat-CIITA line was transfected by electroporation and selected on G418. The pool of cells was analyzed after 3 wk of selection. The Jurkat-CIITA lines represent pools of CIITA-expressing and -nonexpressing cells, as noted by their HLA-DR expression levels (see text and Fig. 7), whereas the majority of the transfectants of the A431 cells express HLA-DR (data not shown).

Analysis of HLA-DOB protein expression by flow cytometry

Specific (HLA-DM-PE and HLA-DR-PerCP) and isotype-matched negative-control Abs were purchased from BD PharMingen (Franklin Lakes, NJ). Unlabeled anti-HLA-DOβ mAb, a gift from Dr. H. Kropshofer (German Cancer Research Center, Heidelberg, Germany) (16), was FITC-conjugated as described in Coligan et al. (43). Intracellular staining was performed using Fix & Perm kit (Caltag Laboratories, Burlingame, CA) following the manufacturer’s instructions. Briefly, cells were fixed with 2% paraformaldehyde and blocked with PBS/1% BSA and fixed with medium A for 15 min at room temperature. Cells were then washed twice with PBS/1% BSA and incubated in medium B containing HLA-DM or HLA-DOβ Abs for 15 min at room temperature.
Cells were then washed three times and analyzed by flow cytometry in a FACSCalibur (BD PharMingen).

**ChIP**

ChIP using CIITA and RFX5 Abs were conducted as described previously (24, 27). Primer pairs used to amplify HLA-DOB promoter were: 5′-AT TGGAAGCTCCTCAGATTGACAACA and 5′-TCTGCAAGGCAACA ATGTTGAGTTGTA. PCR products were amplified for 35 cycles and analyzed by agarose gel electrophoresis.

**Results**

DNA microarray analyses identify HLA-DOB and HLA-DOA as genes that are up-regulated in Raji cells compared with RJ2.2.5 cells

DNA microarrays were used to identify new genes that were differentially regulated between wild-type B cells and those deficient in the master regulator of MHC class II expression, CIITA. The cell lines chosen for this analysis were the two best-matched cell lines, Raji and RJ2.2.5. Raji cells are a B lymphoblastoid cell line derived from a patient with Burkitt lymphoma (34). RJ2.2.5 cells were derived directly from Raji cells by mutagenesis and selection for the loss of MHC class II genes (35). RJ2.2.5 cells are completely negative for MHC class II and HLA-DM expression due to the complete deletion of one CIITA allele and a large internal deletion of the other allele (26, 36). Four separate RNA samples were prepared from each of these cell lines. Initially, the HuFL6800 DNA microarray series was available and was used in one set of analyses. Following this initial screen, the U95A microarrays (Affymetrix) became available and were used instead due to their higher density and greater complexity of transcripts represented. Biotinylated cRNA prepared from RNA derived from both cell types were used for hybridization to the gene chips and the results were analyzed as described in Materials and Methods. HLA-DRA, -DRB, -DQA, -DMA and -DMB, and the li genes were found to be expressed at high levels in Raji and absent or low (background hybridization) in RJ2.2.5. These data and their complete analysis will be presented separately. Thus, all the MHC class II genes regulated by CIITA were identified.

Consistent with previous analyses, HLA-DOA showed a 2- to 10-fold increase in Raji cells compared with RJ2.2.5 cells on the U95A chips (Table I). However, due to previous reports (33), it was surprising to find that HLA-DOB transcripts were consistently 1.8- to 2.6-fold higher in Raji cells compared with RJ2.2.5 cells (Table I). This result suggested that CIITA may regulate HLA-DOB, and warranted further investigation.

**HLA-DOβ protein expression is very low in CIITA- and RFX5-deficient cells**

As a first step to study the expression of HLA-DO protein, the level of HLA-DOβ protein was assayed in RJ2.2.5 cells. Intracellular staining of HLA-DOβ and HLA-DM was performed in Raji, RJ2.2.5, and the RFX5-deficient, BLS-derived cell line SJO. A previous analysis found no HLA-DOA or HLA-DOB transcription in the absence of RFX5 (33). The stained cells were analyzed by flow cytometry. In comparison to Raji cells, RJ2.2.5 showed very low levels of HLA-DOβ (Fig. 1). SJO cells also displayed very low levels of both HLA-DM and HLA-DOβ (Fig. 1). These data suggest that both CIITA and RFX are required for the expression of the heterodimer HLA-DO. However, this interpretation does not exclude the possibility that the HLA-DOβ protein is unstable in the absence of its heteromeric partner, HLA-DOα, which is also absent in both mutant cell lines.

**Real-time RT-PCR shows differences in the level of mRNA for HLA-DOB in Raji and RJ2.2.5 cells**

Table I. Fold change in transcript levels of DOA and DOB in Raji vs RJ2.2.5

<table>
<thead>
<tr>
<th>DNA Microarray Gene Chip</th>
<th>HuFL6800 U95A</th>
<th>U95A</th>
<th>U95A</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DOA</td>
<td>Not represented</td>
<td>13.9</td>
<td>2</td>
</tr>
<tr>
<td>HLA-DOB</td>
<td>2.6</td>
<td>1.8</td>
<td>2.1</td>
</tr>
</tbody>
</table>

* Each microarray represents a separate RNA isolation comparison between Raji and RJ2.2.5.

Previous studies comparing RJ2.2.5 and Raji cell lines for HLA-DOB expression used Northern blots (4, 33). While Northern blots have been a long-standing reference for RNA levels, quantitative comparisons to a reference RNA is difficult, such that small changes could be missed. To verify the DNA microarray data, quantitative RT-PCR using real-time instrumentation was used. Additionally, a pool of RJ2.2.5 cells stably transfected with a CIITA expression vector (termed RJ2.2.5-CIITA cells) and selected for HLA-DR surface expression were generated and analyzed to determine whether CIITA could have a direct effect on expression of the HLA-DO genes. RJ2.2.5-CIITA cells expressed about one-tenth the level of surface HLA-DR as Raji cells (data not shown). Using the reverse transcription reaction from RNA prepared from these three cell lines, quantitative PCR were performed for HLA-DOB, HLA-DOA, HLA-DRA, and GAPDH transcripts. The results were normalized to the levels of GAPDH. Significant levels of HLA-DOB transcripts were detected in RJ2.2.5 cells, but this level is 5- to 6-fold less than in Raji (Fig. 2). HLA-DOA transcripts were also detected in RJ2.2.5 cells, although they were close to background. In contrast, HLA-DRA expression was absent in RJ2.2.5 cells. The analysis of the RJ2.2.5-CIITA cell line showed a 3-fold increase in the level of HLA-DOB mRNA over that observed in RJ2.2.5 cells. Although not fully reverted to their levels in Raji cells, both HLA-DOA and HLA-DRA mRNAs were also substantially increased over their RJ2.2.5 levels. The level of HLA-DRA in this stable cell line, though high, was still 10-fold less than in Raji cells. This level of HLA-DRA mRNA is in agreement with the level of HLA-DR expressed on the surface of this cell line (data not shown), and may reflect the overall level of CIITA expressed in the transfected cells.

Real-time RT-PCR using RNA from SJO indicates complete absence of HLA-DOB transcripts (data not shown). These findings indicate that mRNA transcripts for HLA-DOB are present in RJ2.2.5 (CIITA-/-) cells, but are up-regulated in cells that contain CIITA. Because these results were in disagreement with previously published Northern blot analysis (4, 33), several stocks of RJ2.2.5 cells were analyzed with similar results.

**FIGURE 1.** HLA-DOβ protein is not expressed in CIITA- and RFX5-deficient cell lines. Raji, RJ2.2.5 (CIITA-/-), and SJO (RFX5-/-) cell lines were stained for intracellular HLA-DOβ and HLA-DM using specific mAbs. The cells were analyzed by flow cytometry.
CIITA plays a direct role in the induction of the pGL3-DOB reporter

Analysis of 5' upstream sequence of the HLA-DOB promoter reveals a potential X-Y box-like region. This sequence is very similar to other class II elements (Fig. 3). However, it has been shown previously that a 250-bp fragment of HLA-DOB promoter was not sufficient to confer activation of a reporter gene (44). To test whether this sequence was functionally active in a more sensitive luciferase reporter assay, a 317-bp fragment spanning the WXY box of the HLA-DOB promoter was cloned upstream of a luciferase reporter gene, and the resulting construct termed pGL3-DOB. pGL3-DOB was transiently transfected into both Raji and RJ2.2.5 cells, and the expression of the reporter assayed. The relative activity of the reporter was 12-fold decreased in RJ2.2.5 in comparison to Raji (Fig. 4). The pGL3-DRA reporter, containing the luciferase gene cloned downstream to HLA-DRA promoter, was used as a positive control. The pGL3-DRA reporter expressed 50-fold higher in Raji cells in comparison to RJ2.2.5 cells.

The results from the above experiment suggest that the HLA-DOB promoter may be able to use CIITA for its activity. To address this possibility and prove that the expression of HLA-DOB was not due to other genes that could be mutated in RJ2.2.5, RJ2.2.5 cells were cotransfected with pGL3-DOB reporter and CIITA or with a mutant of CIITA that is transcriptionally inactive and dominant-negative, CIITA20.2 (45). The pGL3-DOR reporter was induced 20-fold in the presence of CIITA (Fig. 5). The dominant-negative CIITA20.2 mutant did not have any effect on the pGL3-DOB promoter activity, suggesting that CIITA is functioning as a coactivator in this system. An HLA-DRA promoter reporter was assayed in parallel and serves as a control for the system. Its response to CIITA and CIITA20.2 were as previously published (45).

CIITA binds to HLA-DOB promoter in vivo

To obtain evidence that CIITA interacts directly with the HLA-DOB promoter in vivo, a ChIP assay was conducted in Raji cells using an Ab directed against CIITA. Through the cross-linking of live cells with formaldehyde, ChIP assays provide direct evidence for the association of a factor with a given DNA sequence in vivo (46, 47). Immunoprecipitation of CIITA- and RFX5-containing chromatin using formaldehyde-fixed Raji cell lysates, followed by PCR, revealed specific bands for the HLA-DOB promoter for both Abs (Fig. 6). Thus, both RFX5 and CIITA can be found at the HLA-DOB promoter in vivo and demonstrate that CIITA can or does play a role in the regulation of the HLA-DOB gene.

CIITA does not increase HLA-DOB levels in non-B cells

The above results indicate that CIITA can bind to the HLA-DOB promoter and increase its expression in B cells. To show that this interaction results in the expression of HLA-DOβ protein, RJ2.2.5-CIITA cells were analyzed for their level of HLA-DOβ expression by intracellular staining and flow cytometry (Fig. 7A). The results showed increased levels of both HLA-DM and DOβ in the...
Clearly, the Jurkat-CIITA transformant pool showed signs of HLA-DM expression, but no HLA-DOB (Fig. 7B). HLA-DM and HLA-DOB in A431-CIITA cells showed high levels of expression, but no HLA-DOB (Fig. 7B). Similarly, the Jurkat-CIITA transformant pool showed significant HLA-DR expression, but no HLA-DOβ expression (Fig. 7C). These data demonstrate that CIITA augments HLA-DOB expression only in B cells. These findings suggest the presence of an additional tissue-specific factor for HLA-DOB expression in B cells.

Discussion
This work began with the repeated finding of increases in HLA-DOB mRNA levels in Raji cells when compared with its CIITA-deficient daughter line RJ2.2.5 by DNA microarray analysis. Because previous reports suggested that HLA-DOB was not regulated by CIITA (33), a number of assays were used to assess different aspects of HLA-DOB regulation. Using quantitative RT-PCR, a 5- to 6-fold increase in HLA-DOB transcripts was observed in Raji cells (CIITA+) compared with RJ2.2.5 cells (CIITA−). Transfection of CIITA into RJ2.2.5 cells showed that expression of the HLA-DOB was responsive to CIITA expression. Moreover, the HLA-DOB X-Y regulatory sequences respond directly to wild-type, but not to a mutant form of CIITA. Lastly, CIITA and RFX can be found associated with these sequences in vivo when analyzed by ChIP. Together, these data support the conclusion that CIITA directly influences HLA-DOB expression.

This finding was both expected and unexpected. It was expected because the heterodimeric partner of the HLA-DOβ protein, HLA-DOα, was shown by others to be regulated by both RFX and CIITA (33). Moreover, an analysis of the upstream region of the HLA-DOB gene revealed the presence of an X-Y box regulatory sequence. X and Y box regulatory sequences are found 5′ to all MHC class II genes and serve as the direct binding sites for RFX, X2BP/CREB, and NF-Y. CIITA is known to interact with the DNA-bound factors (26–28), suggesting that CIITA should regulate HLA-DOB. The current finding was unexpected due to previous reports in which Northern blots showed little if any difference between the levels of HLA-DOB in RJ2.2.5 cells when compared with Raji cells (4, 33). However, these Northern blots were not intended to be absolutely quantitative, as a dilution series of the RNA samples was not performed, nor were the blots quantitated by other means. Thus, the relative amounts of the HLA-DOB transcripts, when compared with the loading control, could not be quantitatively determined. Complicating the issue is the fact that HLA-DOB transcripts are present in RJ2.2.5 cells. Additionally, the induction of CIITA by IFN-γ in professional and non-professional APCs (non-B cells) did not lead to an induction of HLA-DO (4), suggesting that the HLA-DO genes were regulated differently.

Because HLA-DOB mRNA is expressed in RJ2.2.5 cells in the absence of CIITA, this suggests that other factors and mechanisms play a role in controlling its expression. This notion is consistent with the findings that CIITA is required for the induction of HLA-DOB, but not for HLA-DR expression. The current work was not designed to address this issue, but it is possible that CIITA and RFX may work together to induce HLA-DOB expression in B cells.
with all the previous data and the observation that HLA-DO expression is limited to B cells and not to professional APCs. The analysis of intracellular HLA-DOβ protein levels in Raji and RJ2.2.5 showed a clear difference. This difference could be caused by several mechanisms. The first is that while HLA-DOβ mRNA is present in RJ2.2.5 cells, HLA-DOα mRNA levels are very low. If the encoded proteins are expressed at the mRNA-represented levels, then it is very possible that HLA-DOβ proteins are degraded in the absence of their HLA-DOα partner. The role of CIITA here would be to increase expression of both HLA-DOα and HLA-DOβ mRNAs such that maximal expression of HLA-DO could occur. Second, because HLA-DO transport to lysosomal vesicles and to the peptide loading compartment, the MIC is dependent on its association with HLA-DM (9). It is possible that HLA-DOβ does not accumulate in cells unless HLA-DM is present. This would be the case in RJ2.2.5, where HLA-DM is absent.

The HLA-DO genes are unusual in that their expression is mostly restricted to B cells and to thymic epithelial cells. This includes the possibility that HLA-DOα is controlled by factors that are specific to B cells and thymic epithelial cells, and that such factors are not found in other APC types. This suggestion would provide an explanation for the lack of HLA-DOβ in non-B cells exposed to IFN-γ, and also for the absence of HLA-DOβ expression in non-APCs in the presence of transfected CIITA, as shown here. It has been proposed by Cresswell and colleagues (12) that nonprofessional APCs cannot express HLA-DO in the presence of IFN-γ so that they can achieve their maximal Ag-processing capacity. Furthermore, the differential regulation of HLA-DM and HLA-DOα levels by CIITA was suggested as a mechanism for APC and non-APCs to avoid HLA-DO inhibitory activity (48). However, this is more likely to be attributed to the tissue-specific factor controlling HLA-DO expression than to CIITA. Although CIITA can augment HLA-DO expression in B cells, it cannot overcome the need for the additional tissue-specific factor in non-APCs. Thus, in B cells, a tissue-specific factor and CIITA together drive HLA-DOβ and HLA-DOα expression, thereby regulating the function of HLA-DM. The B cell is a unique Ag-presenting cell, as it presents Ag only once in its lifetime. Hence, the necessity to tightly control Ag processing and presentation may reside with HLA-DOβ and HLA-DOα expression.

The role of CIITA in HLA-DOβ expression may also be considered similar to CIITA’s role in MHC class I expression. MHC class I expression has been shown to be induced by, but not dependent on, CIITA for its expression (49, 50). The HLA-DO genes appear to have evolved more recently from class II MHC genes (reviewed in Refs. 11 and 51). It is likely that the HLA-DOβ gene has diverged further with additional elements in its promoter to allow B cell/thymus-specific expression.

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References


