

# An Isotype-specific Activator of Major Histocompatibility Complex (MHC) Class II Genes That Is Independent of Class II Transactivator

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## Summary

Patients with one type of major histocompatibility complex class II combined immunodeficiency have mutations in a gene termed class II transactivator (CIITA), which coordinately controls the transcription of the three major human class II genes, HLA-DR, -DQ, and -DP. However, the experimentally derived B-lymphoblastoid cell line, clone 13, expresses high levels of HLA-DQ in the absence of HLA-DR and HLA-DP, despite its mapping by complementation analysis to this group. It was possible that one of the clone 13 CIITA alleles bore a mutation that allowed HLA-DQ, but not HLA-DR or -DP transcription. Alternatively, another factor, distinct from CIITA, might control HLA-DQ expression. We report here that ectopic expression of CIITA cDNAs derived by reverse transcriptase polymerase chain reaction from clone 13 do not restore expression of HLA-DQ in another CIITA-deficient cell line, RJ2.2.5. In addition, no CIITA protein is detectable in clone 13 nuclear extracts. In contrast, somatic cell fusion between clone 13 and RJ2.2.5 restored expression of the HLA-DQ haplotype encoded by the RJ2.2.5 DQB gene. Taken together, these data demonstrate the existence of an HLA-DQ isotype-specific *trans*-acting factor, which functions independently of CIITA.

The human MHC class II genes encode highly polymorphic cell surface glycoproteins, which are responsible for presenting protein antigens to CD4<sup>+</sup> T cells and thus initiating immune responses (1). In the human, the genes for three isotypes (HLA-DR, -DQ, and -DP) of MHC class II antigens are located in the HLA-D locus on chromosome 6. Each isotype is expressed on the cell surface as an  $\alpha\beta$  heterodimer. The expression of MHC class II antigens is tightly regulated and is limited to certain cells in the immune system including B cells, macrophages, dendritic cells, thymic epithelium, and activated T cells. Furthermore, surface levels of class II antigens can be both up- and downregulated by a variety of physiologic stimuli. In addition to constitutive and inducible expression on cells in the immune system, class II antigens can also be induced on otherwise class II-negative cells, such as epithelial cells, tumor cells and rheumatoid synovium, by exposure to cytokines such as IFN- $\gamma$  (2).

In general, the class II genes have been found to be regulated coordinately; that is, all three isotypes are usually expressed concomitantly, although not always to the same level.

B lymphocytes constitutively express all class II genes, and macrophages, when stimulated with IFN- $\gamma$ , show a coordinate increase in the expression of all class II genes (3). Furthermore, in most types of MHC class II combined immunodeficiency (MHC class II CID or the bare lymphocyte syndrome), a disease characterized by the complete lack of expression of class II antigens, all class II genes are coordinately silenced (4).

Our understanding of how class II genes are coordinately regulated comes in large part from an analysis of the *cis* and *trans* elements necessary for their transcription. Examination of the 5'-flanking sequences of the class II genes has revealed a set of highly conserved sequence motifs common to all the class II genes within and between species. These conserved elements, designated the W/S, X1, X2, and Y boxes, have been shown by mutational analysis in transient transfection experiments to be critical for the constitutive and inducible expression of the class II genes (2, 5).

A number of proteins that either bind directly to these *cis* elements or are necessary for their activity have been identified, both through electrophoretic mobility shift assays

(EMSA)<sup>1</sup> followed by protein purification or, more recently, by complementation/expression cloning. The latter process has taken advantage of the existence of both MHC class II CID patient- and experimentally derived mutant cell lines that do not express class II genes but can be induced to do so upon somatic cell fusion with a class II-positive cell line (6). Somatic cell fusion between the mutant cell lines has revealed at least five distinct complementation groups (I–V) and, accordingly, the existence of at least five genes critical for class II expression (7–11). Two of these genes, CIITA (class II transactivator) and RFX5, have been isolated by expression cloning from a wild-type cDNA library using cell lines from MHC class II CID complementation groups II and IV, respectively, as recipients. It should be noted that a second nomenclature for these groups is also used: groups A, B, and C (12) correspond to groups II, I, and IV, respectively. Introduction of a wild-type cDNA encoding either the CIITA or RFX5 protein into cell lines of the appropriate complementation group resulted in a coordinate reexpression of all class II genes (13, 14).

We have studied MHC class II expression in clone 13, a B-lymphoblastoid cell line (B-LCL) derived from the class II-positive Burkitt's lymphoma cell line Jijoye (15). Unlike the other CIITA-deficient cell lines in complementation group II, clone 13 shows a noncoordinate pattern of expression of class II antigens: HLA-DQ is expressed but HLA-DR and -DP are not. Transfection of wild-type CIITA into clone 13 causes the reexpression of both HLA-DR and -DP, and an increase in levels of HLA-DQ, thus confirming that CIITA is indeed defective in clone 13 as suggested by the complementation analyses (16). Recently, it has been demonstrated that the HLA-DQB, but not the DRA promoter, was active in clone 13 cells, a function that was mapped by DQ/DR chimeric constructs to the DQB X2 box (17). In this study, we have investigated whether or not the expression of HLA-DQ in clone 13 is dependent upon a potentially isotype-specific allele of CIITA whose mutation affects HLA-DR and -DP, but not -DQ expression. We demonstrate that the expression of HLA-DQ in clone 13 is due to an isotype-specific *trans*-acting factor that acts independently of CIITA.

## Materials and Methods

**Cell Lines and Transfections.** The B-LCL clone 13 (15), RJ2.2.5 (18), and Raji (ATCC CCL 86; American Type Culture Collection, Rockville, MD) were maintained in RPMI 1640 medium made 10% in heat-inactivated FCS supplemented with 10 mM Hepes, pH 7.2, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 mM 2-mercaptoethanol, and 2 mM l-glutamine (complete medium). For transfections, cells were harvested, washed once in PBS, and resuspended to a concentration of  $2 \times 10^7$  cells/ml in PBS, 10 mM Hepes, pH 7.2.  $10^7$  cells in 0.5 ml were mixed with 10 μg DNA, transferred to an 0.4-cm electroporation cuvette and

electroporated at 250 V, 960 μF using a Bio-Rad GenePulser II (Bio-Rad, Melville, NY). After electroporation, cells were washed once in complete medium and plated at a density of  $2\text{--}4 \times 10^5$  cells/ml. Drug selection was initiated 48 h after transfection by the addition of either hygromycin B (450 μg/ml) or neomycin (750 μg/ml) as appropriate. Cells were analyzed for expression of surface antigens 14 d after transfection by flow cytometry.

**PCR.** For RT-PCR, 2 μg of clone 13 RNA was reverse transcribed using SuperScript (GIBCO BRL, Gaithersburg, MD) according to the instructions of the manufacturer. A portion of this cDNA was used in PCR reactions with pairs of CIITA-specific primers (see below) to generate the clone 13 cDNA fragments. The 6.6-kb PCR fragment derived from either clone 13 or Raji genomic DNA was prepared using primers F246 and R248 and the Expand Long Template PCR System (Boehringer-Mannheim, Indianapolis, IN). All PCR fragments were subcloned into pCRII (Invitrogen, San Diego, CA) for subsequent manipulation and sequence analysis.

The following oligonucleotide primers were used in this study. Primers preceded by F are complementary to the noncoding strand of the CIITA cDNA, whereas those primers preceded by an R are complementary to the CIITA coding strand. The position number given in parentheses indicate the nucleotide position of the 5' end of the primer. Nucleotide numbering is based on Steimle et al. (13) F204, 5'-GGAAGCTGAGGGCAGGAG-3' (nt 248); R207, 5'-CCCCAGCCAATAGCTCTTG-3' (nt 1,409); F206, 5'-CTCCAACAAGCTTCCAAAAT-3' (nt 1,096); R214, 5'-CGCATCCAGTCCAGATGCT-3' (nt 3,094); F215, 5'-GATGGC-CAAAGGCTTAGTCCAAC-3' (nt 2,182); R201, 5'-CCTCTC-AGCGCCCCATTAGTGTC-3' (nt 4,447); R251, 5'-CAT-CACCCGGAGGACACCAT-3' (nt 3,346); F212, 5'-GTCCCT-GAAGGATGTGGAAGAC-3' (nt 2,884); F246, 5'-TTCCCC-AACTGGTGCGGATCCTCACGGCC-3' (nt 3,032); R248, 5'-TTGTACTGGACGTCCATCACCCGGAG GGAC-3' (nt 3,360).

**Plasmids.** The expression plasmids pCMV.CIITA, which directs the expression of wild-type CIITA, and pCMV.K<sup>b</sup>, which directs the expression of the murine MHC class I antigen K<sup>b</sup>, have been described previously (16). Both plasmids are maintained episomally and confer resistance to hygromycin in transfected cells. The plasmid pREP9.D<sup>d</sup> was constructed by excising a 1,058-bp EcoRI fragment bearing the D<sup>d</sup> cDNA from pD<sup>d</sup>V (the gift of D. Singer, NCI, NIH Bethesda, MD). After treatment with Klenow and ligation of SalI linkers, the D<sup>d</sup> cDNA was cloned into the XhoI site of pREP9 (Invitrogen). This plasmid is also maintained episomally but confers resistance to neomycin.

The clone 13-derived PCR fragments spanning the CIITA cDNA were subcloned into pCRII (Invitrogen) for sequence analysis and further subcloning. Chimeric CIITA cDNA molecules were constructed from the subcloned clone 13 fragments and from wild-type CIITA by taking advantage of the unique NotI, BsaWI, and BamHI restriction sites present in the CIITA cDNA and inserted into pCEP4 (Invitrogen). These expression constructs are designated pCMV.CIITAΔ4, pCMV.CIITAΔ93, and pCMV.CIITAΔ147 and encode mutant forms of CIITA detected in clone 13 (as described in Results) bearing the 4-, 93-, or 147-bp deletions, respectively.

**Western Blot Analysis of CIITA.** Nuclear and cytoplasmic extracts from Raji, clone 13, or RJ2.2.5 cells transfected with either pCMV.K<sup>b</sup>, pCMV.CIITA, pCMV.CIITAΔ4, pCMV.CIITAΔ93, or pCMV.CIITAΔ147 (19) were prepared as described (20). 30 μg protein was run on a 6% SDS-polyacrylamide gel and trans-

<sup>1</sup>Abbreviations used in this paper: B-LCL, B-lymphoblastoid cell line; CID, combined immunodeficiency; CIITA, class II transactivator; EMSA, electrophoretic mobility shift assays.

ferred to nitrocellulose. The filters were probed with a cocktail of murine anti-CIITA monoclonal antibodies (all IgG1) (19). Antibody probed filters were reacted with horseradish peroxidase-conjugated goat anti-mouse IgG1 (Southern Biotechnology Associates, Birmingham, AL) and were developed using a Blotting Detection Kit (Amersham Corp., Arlington Heights, IL).

**Cytogenetic Analysis.** Clone 13 was cultured in RPMI 1640 medium with 10% FCS and 1% l-glutamine. Metaphase chromosomes were prepared by standard methods and chromosome preparations were GTG-banded (21). Fluorescence in situ hybridization with a digoxigenin-dUTP-labeled whole chromosome paint for chromosome 16 (Oncor, Gaithersburg, MD) was performed. The whole chromosome paint was detected with a rhodamine-conjugated antibody to digoxigenin as described elsewhere (22). Chromosomes were identified by counterstaining with DAPI (4',6-diaminido-2-phenylindole). Hybridizations were viewed with an epifluorescence microscope equipped with a triple band-pass filter set (FITC-Texas red-DAPI; ChromaTech, Brattleboro, VT) for the detection of the whole chromosome paint. A standard single band pass filter (Zeiss) was used to view the DAPI counterstain. Cells were scored for subtle rearrangements of chromosome 16 not detectable by routine GTG banding.

**Preparation and Analysis of Cell Fusions.** For the preparation of stable fusions between RJ2.2.5/K<sup>b</sup> and clone 13/D<sup>d</sup>, 10<sup>7</sup> cells of each type were mixed in a 50-ml centrifuge tube and washed three times in serum-free medium. The cells were disaggregated by three passages through an 18-gauge needle and were washed once more in serum-free medium. Cell pellets were gently dispersed and warmed to 37°C. Fusion was initiated by the dropwise addition of 1 ml PEG 1,500 (50% wt/vol) (Boehringer-Mannheim), over the course of 1 min. 50 ml of serum-free medium was then added slowly over a 5-min period. Cells were rested at room temperature for 5 min, pelleted at 250 g for 3 min, and plated in 50 ml of complete medium. Drug selection in hygromycin and neomycin at the concentrations given above was started at 48 h after fusion.

For flow cytometric analysis of the fused cell population, the following antibodies were used: K<sup>b</sup> expression was detected by primary staining with mAb Y-3 (ATCC HB-176) followed by reaction with PE-sheep anti-mouse IgG (Sigma Chemical Co., St. Louis, MO). D<sup>d</sup> expression was detected by an anti-D<sup>d</sup>-FITC (PharMingen, San Diego, CA). Expression of DQ2 was detected by primary staining with an anti-DQ2 human monoclonal IgM (Pel-Freez Clinical Systems, Brown Deer, WI) followed by staining with a biotinylated mouse anti-human IgM mAb (PharMingen) and streptavidin-Red 670 (GIBCO BRL). Appropriate isotype control reagents were purchased from PharMingen. All antibody incubations and washes were performed at 4°C in PBS containing 0.3% BSA and 0.1% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. Cells were fixed in 1% formaldehyde before analysis on a FACScan® (Becton Dickinson, San Jose, CA) that had been calibrated with Becton Dickinson Calibrate Beads. Viable cells were selected using forward and side scatter analysis.

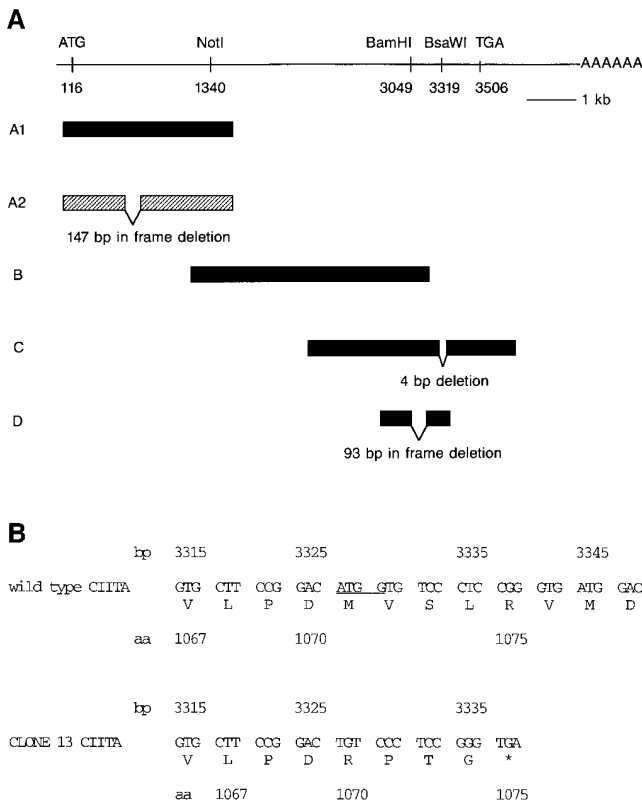
For RT-PCR analysis of HLA-DQ expression in the fusions and in the individual fusion partners, total RNA was isolated (23). After DNase I treatment, 2 µg of RNA was reverse transcribed using SuperScript (GIBCO BRL) according to the manufacturer's instructions. A portion of this first-strand cDNA was used in PCR reactions containing either DQw2,3,4 group-specific primers or DQw1 group-specific primers (23a). Aliquots of the PCR reactions were then digested with the appropriate restriction endonucleases to determine the precise allelic forms of DQ present.

## Results

**Identification of Mutant CIITA mRNA in Clone 13.** Stable transfection of clone 13 with a wild-type copy of a CIITA cDNA carried in an episomally maintained expression vector, pCMV-CIITA, has been shown to restore expression of HLA-DR and HLA-DP and increase expression of HLA-DQ consistent with previous complementation analyses (16). Therefore, CIITA is at least partially defective in clone 13. However, it was not possible to determine from these data whether HLA-DQ expression in clone 13 was due to a mutation in CIITA that preserved its ability to transactivate HLA-DQ, but not HLA-DR or -DP, or was secondary to the presence in clone 13 of a CIITA-independent, isotype-specific *trans*-acting factor. To address this issue, we determined the nature of the mutation(s) present in the clone 13 CIITA genes.

Northern blot analysis of poly(A)<sup>+</sup> RNA revealed the presence of apparently full-length CIITA transcripts in clone 13, although at levels somewhat lower than those seen in class II-positive B-LCL (data not shown). Based on this finding, we used RT-PCR to generate fragments spanning the entire CIITA cDNA. Initially, three pairs of oligonucleotide primers, F204/R207, F206/R218, and F215/R201, were used to produce three overlapping PCR fragments from which a full-length clone 13 CIITA cDNA could be reconstructed. These PCR fragments are shown schematically in Fig. 1 A. The primer pair (F204, R207) used to generate the 5'-most fragment (fragment A) of the CIITA cDNA yielded two PCR products: one apparently full-length (fragment A1), and a second fragment ~150 bp shorter (fragment A2). The internal fragment (fragment B) was generated with primers (F206, R214) and the 3'-most fragment (fragment C) was generated with primers (F215, R201). Both of these fragments appeared to be full length. Sequence analysis of the subcloned PCR fragments revealed that fragments A1 and B corresponded to the wild-type CIITA sequence, whereas fragment A2 harbored a 147-bp in-frame deletion of nucleotides 597-743 that corresponded exactly to a previously described splice variant of CIITA called CIITA-10 found in Raji cells (24). However, multiple independently derived isolates of fragment C revealed a 4-bp deletion beginning at position 3,326 (Fig. 1 B). This frame-shift mutation introduces a premature stop codon into the transcript, which would result in a protein of 1,074 amino acids as opposed to the 1,130-amino acid wild-type CIITA protein. In addition, by using primers F251 and R212 we were able to detect an additional fragment (fragment D), which contained a second mutation, a 93-bp in-frame deletion of nucleotides 3,085-3,177 (Fig. 1 A).

**Confirmation of the Mutations in Genomic DNA.** To determine whether the mutant cDNAs detected by RT-PCR in clone 13 corresponded to genomic mutations, we generated a 6.6-kb PCR fragment from clone 13 genomic DNA, which spans the regions in the cDNA containing both the 93- and 4-bp deletions using primers F246 and R248. A similar 6.6-kb fragment was generated from Raji genomic DNA to serve as a control for wild-type CIITA. These



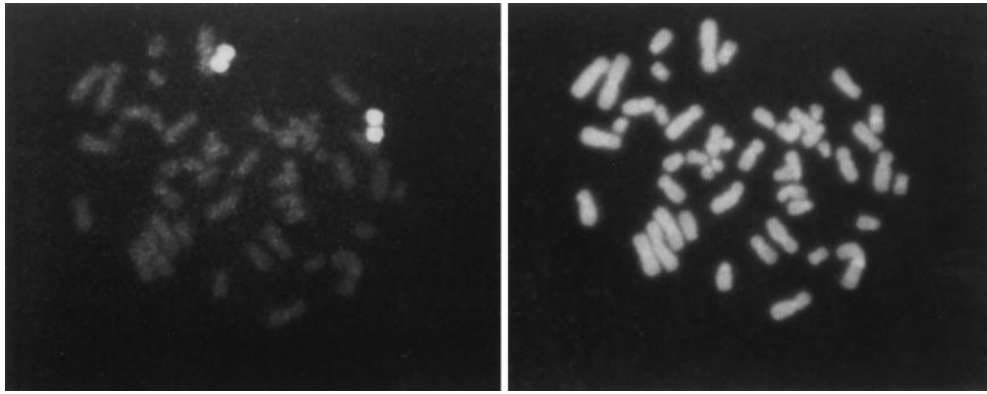
**Figure 1.** Schematic representation of the CIITA cDNA fragments generated by RT-PCR from clone 13. (A) The primers used to generate fragments A1, A2, B, C, and D are described in Results. Fragments A1 and B appear to correspond to wild-type CIITA, whereas fragments C and D represent previously undetected deletions in the CIITA cDNA. Fragment A2 carries a deletion which has previously been detected in Raji (24). (B) Sequence of the 4-bp mutation detected in clone 13 cDNA and genomic DNA. The four nucleotides underlined in the wild type sequence are deleted in clone 13.

fragments were subcloned and multiple independent isolates were sequenced in the regions of both mutations. We reasoned that the 4-bp deletion detected in the clone 13 cDNA was likely to result from a parallel 4-bp deletion in the genomic DNA and, indeed, 10 out of 10 independent genomic isolates sequenced harbored this deletion (Fig. 1 B). All Raji isolates analyzed showed wild-type sequence.

The 93-bp deletion mutant cDNA could arise from three possible mechanisms: (a) a genomic deletion of the same 93 bp, (b) a genomic mutation in a splice donor or splice acceptor site that would result in exon skipping, as is the case in the mutant CIITA present in the CIITA-deficient cell line BLS-2 (13) or (c) a splice variant similar to those detected in Raji (24). Sequence analysis of six independent isolates of the 6.6-kb genomic PCR fragment from clone 13 showed that the 93-bp region was indeed present in the genome and that it constitutes a single exon. Furthermore, the splice donor and acceptor sites flanking this exon are completely wild type (i.e., identical to Raji). Therefore, it is likely that the 93-bp mutant cDNA in clone 13 represents a splice variant.

**Cytogenetic Analysis of Clone 13.** Because we were only able to detect the presence of the 4-bp mutation in PCR fragments derived from genomic DNA, we wished to determine whether clone 13 bore a single copy of chromosome 16 or whether clone 13 was, in fact, diploid for chromosome 16. We also examined chromosome 6, which encodes the MHC locus. Therefore, we performed GTG banding on chromosome spreads from clone 13. This analysis suggested that both chromosome 16 (the location of the CIITA gene) and chromosome 6 (the location of the MHC) were intact. The chromosomal abnormalities of seven karyotyped cell from clone 13 were the t(8;14) translocation typical of EBV-transformed Burkitt's lymphoma cell lines and trisomy 13 and 21, i.e., 48, XY, t(8;14) (q24.1; q32.3), +13, +21 [7]. The significance of these trisomies is presently unclear and may represent clonal evolution. Analysis of chromosome 16 in clone 13 by whole chromosome painting revealed that in 49 of 50 cells examined, chromosome 16 was present in two copies per cell and in one cell three copies of chromosome 16 were present. This latter single cell finding is defined as pseudomosaicism and is thought to represent a technical artifact, but low level mosaicism for trisomy 16 cannot be excluded. Fig. 2 shows an analysis of a typical clone 13 cell by whole chromosome painting for chromosome 16 and by DAPI counterstaining. Furthermore, there was no evidence for translocation of part(s) of chromosome 16 to any other chromosome, although small deletion would not be detected. Thus, it is likely that clone 13 does carry two copies of the structural gene for CIITA.

**Clone 13 Does Not Express CIITA Protein.** To examine further whether the HLA-DQ expression present in clone 13 was due to CIITA, we performed immunoblotting analysis on nuclear extracts from clone 13 cells transfected with either pCMV.K<sup>b</sup>, pCMV.CIITA, pCMV.CIITAΔ4, pCMV.CIITAΔ93, and pCMV.CIITAΔ147. Wild-type CIITA has a calculated molecular mass of  $1.24 \times 10^5$  kD, whereas the mutant CIITAs have a calculated molecular mass of between  $1.18$  and  $1.21 \times 10^5$  kD. Endogenous CIITA was readily detected in untransfected Raji cells (data not shown) or in Raji cells transfected with the control plasmid pCMV.K<sup>b</sup> (Fig. 3, lane 1). Transfection of Raji with pCMV.CIITA resulted in higher levels of wild-type CIITA (Fig. 3, lane 2). In clone 13, endogenous CIITA could not be detected in cells transfected with the control plasmid pCMV.K<sup>b</sup> (Fig. 3, lane 3) but transfection with pCMV.CIITA resulted in the overexpression of CIITA (lane 4). Transfection of clone 13 with either pCMV.CIITAΔ4 or pCMV.CIITAΔ147 resulted in the overexpression of these truncated mutant forms of CIITA (Fig. 3, lanes 5 and 7, respectively), whereas transfection with pCMV.CIITAΔ93 did not result in the production of detectable protein. Similar results were obtained when nuclear extracts prepared from RJ2.2.5 cells transfected with the same panel of plasmids were examined (data not shown). Thus, despite the fact that clone 13 transcribes the CIITA gene, we find no evidence of endogenous CIITA protein in clone 13. It is possible that (a) the CIITA mRNA in clone 13 is not trans-



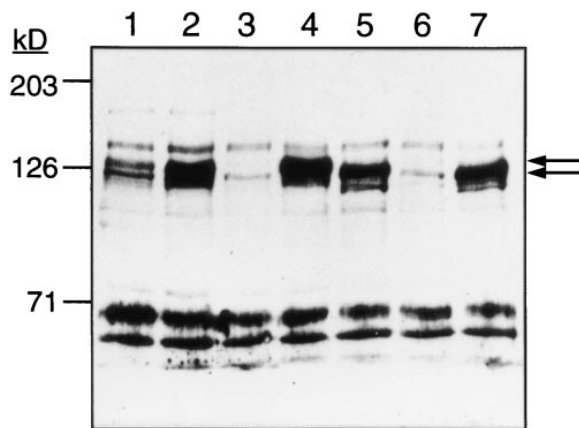
**Figure 2.** Chromosome painting for chromosome 16. Cells were painted for chromosome 16 as described in Materials and Methods. The image on the left shows a cell painted for chromosome 16. Two copies of chromosome 16 are evident in this cell. No translocations, insertions, or other rearrangements of chromosome 16 are observed. The image on the right is the corresponding DAPI image (for chromosome identification) of the image on the left.

lated, (b) that the mutant CIITA protein is not stable, or (c) that it is present but in levels below the limits of detection. The fact that a truncated mutant CIITA can be overexpressed from the plasmid pCMV.CIITA $\Delta$ 4 suggests that the third possibility is most likely.

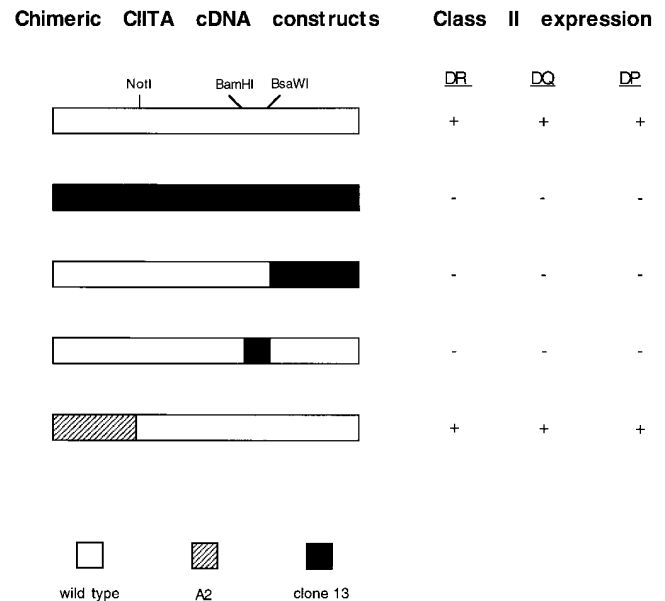
**Mutant CIITAs from Clone 13 Do Not Activate HLA-DQ Expression.** In the event that CIITA protein was present in clone 13 but was present at levels too low to detect, we constructed chimeric CIITA cDNAs bearing the clone 13 CIITA mutations in the context of a wild-type CIITA cDNA. These chimeric cDNAs were cloned into pCEP4, an episomally maintained expression vector as described in Materials and Methods. All expression constructs were stably introduced into RJ2.2.5, a CIITA-deficient cell line that maps to MHC class II CID complementation group II and fails to express all class II antigens. We reasoned that if CIITA were directly responsible for the pattern of HLA

class II expression in clone 13 (HLA-DR<sup>-</sup>, -DQ<sup>+</sup>, -DP<sup>-</sup>), that CIITA might also function similarly in another CIITA-deficient cell line.

The various chimeric CIITA cDNAs used for these transfection studies and the results of flow cytometric analysis of RJ2.2.5 cells transfected with either pCMV.CIITA, or with the chimeric constructs are shown in Fig. 4. As shown previously, transfection of wild-type CIITA into RJ2.2.5 caused reexpression of HLA-DR, -DQ, and -DP. Notably, chimeric CIITA cDNAs bearing either the 4-bp or the 93-bp deletion were unable to recapitulate the pattern of HLA-D antigen expression seen in clone 13. Interestingly, a CIITA cDNA bearing the 147-bp deletion previously identified as a splice variant was fully active, despite the fact that the deletion region maps to a portion of the



**Figure 3.** Lack of expression of CIITA protein in clone 13. Raji (lanes 1–2) and clone 13 cells (lanes 3–7) were stably transfected with either pCMV.K<sup>b</sup> (lanes 1 and 3), pCMV.CIITA (lanes 2 and 4), pCMV.CIITA $\Delta$ 4 (lane 5), pCMV.CIITA $\Delta$ 93 (lane 6), or pCMV.CIITA $\Delta$ 147 (lane 7). Nuclear extracts were prepared and analyzed by Western blot using a cocktail of three murine anti-CIITA mAbs. CIITA is not detected in clone 13 transfected with pCMV.K<sup>b</sup>, but is readily detected in Raji cells transfected with pCMV.K<sup>b</sup>. The upper arrow indicates the position of wild-type CIITA, while the lower arrow indicates the position of the truncated CIITA proteins encoded by pCMV.CIITA $\Delta$ 4 and pCMV.CIITA $\Delta$ 147.

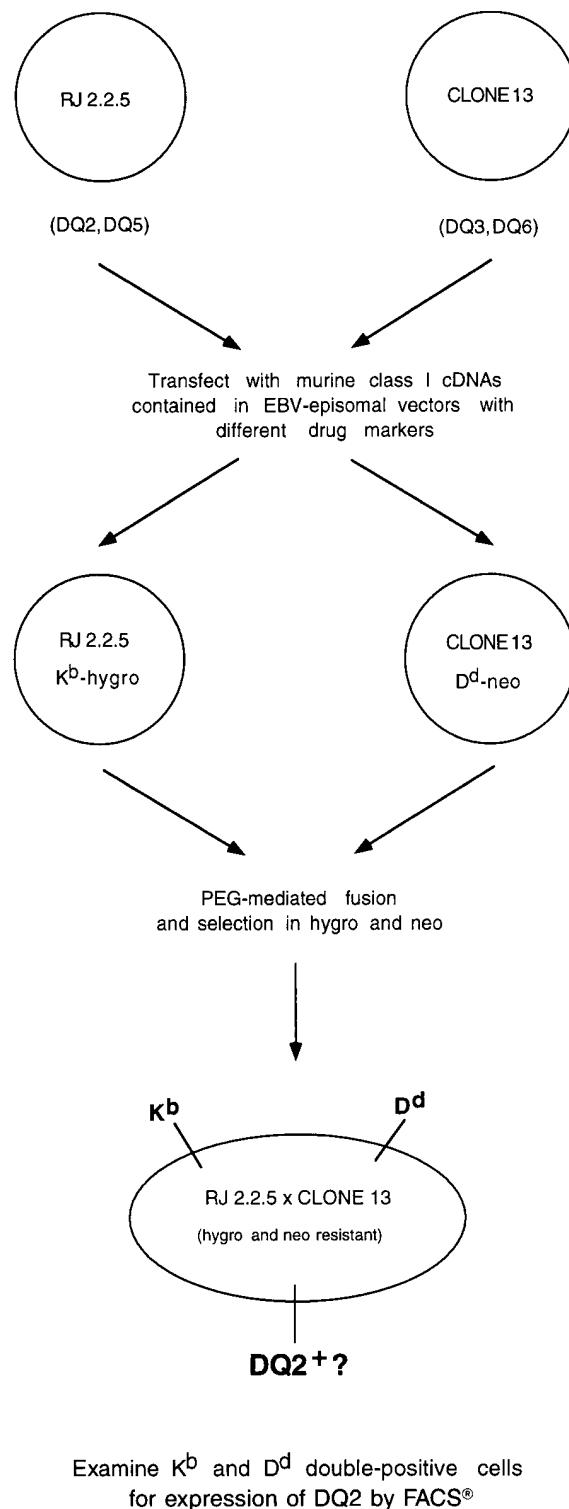


**Figure 4.** MHC class II expression directed by chimeric CIITA cDNAs transfected into RJ 2.2.5 cells. Expression plasmids bearing chimeric CIITA cDNA derived from the indicated sources were stably transfected into RJ2.2.5 cells and analyzed for the expression of HLA-DR, -DP, and -DQ after selection in hygromycin. Surface expression of HLA antigens was detected by flow cytometry.

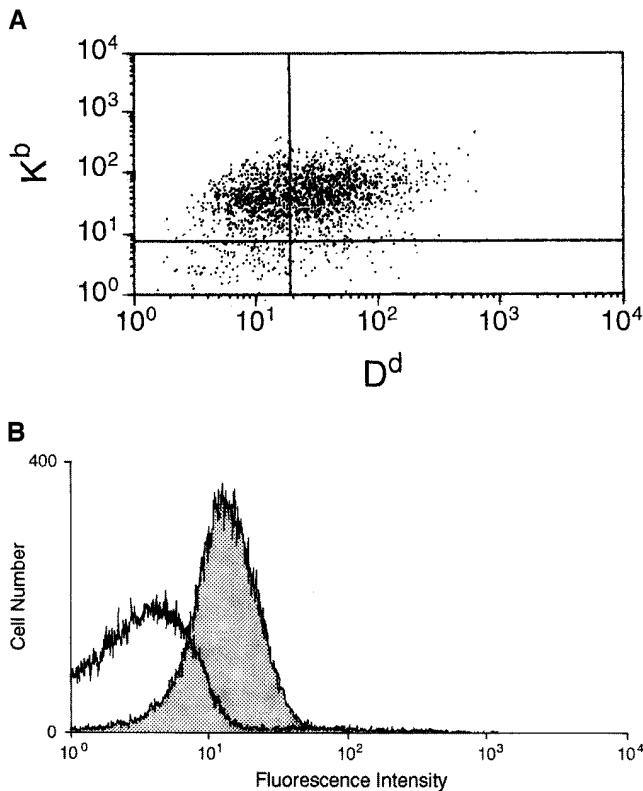
proline-serine-threonine (PST)-rich domain that has been shown to be necessary for optimal transactivation by CIITA (16). Thus, the mutations in CIITA in the clone 13 alleles rendered them completely unable to transactivate all three HLA-D genes. These data suggested that the expression of HLA-DQ in clone 13 may be regulated by a novel *trans*-acting factor different from and independent of CIITA.

*An HLA-DQ-specific Transactivator Is Present in Clone 13.* To provide direct evidence for an HLA-DQ isotype-specific transacting factor in clone 13, we designed a somatic cell fusion experiment between clone 13 and another CIITA-deficient cell line, RJ2.2.5, taking advantage of the fact that these two cell lines have different DQB haplotypes as determined by DNA-based tissue typing. Clone 13 is DQB1\*0602(DQ6) and DQB1\*0301(DQ3), whereas RJ2.2.5 is DQB1\*0501(DQ5) and DQB1\*0201(DQ2). We reasoned that if clone 13 has a factor capable of specifically activating the HLA-DQ genes, it should be able to reactivate expression of DQ in another CIITA-deficient cell line from group II CID upon fusion of these two cells. This strategy is outlined in Fig. 5. In brief, RJ2.2.5 cells stably transfected with pCMV.K<sup>b</sup>, an episomally maintained vector that expresses K<sup>b</sup>, a murine class I molecule, and confers hygromycin resistance were fused to clone 13 cells transfected with pREP9.D<sup>d</sup>, a second episomally maintained vector, which directs the expression of D<sup>d</sup>, another murine class I antigen, and confers neomycin resistance. Both RJ2.2.5/K<sup>b</sup> and clone 13/D<sup>d</sup> retain the pattern of class II expression as seen in RJ2.2.5 and clone 13, respectively (data not shown). The fusion was then selected in both hygromycin and neomycin to generate a population of stable RJ2.2.5/K<sup>b</sup> by clone 13/D<sup>d</sup> fusion products. This population was analyzed by three-color flow cytometry by staining for the expression of K<sup>b</sup>, D<sup>d</sup>, and DQ2. Because each fusion partner expresses a different murine class I antigen, the fused cells can be detected as staining doubly for K<sup>b</sup> and D<sup>d</sup>. These double-positive cells can then be specifically examined for the expression of DQ2, one of the allelic forms of DQ encoded by RJ2.2.5. The results of such a triple-color analysis performed on a population of stably fused cells is presented in Fig. 6. Fig. 6 A shows a large number of cells (52%) that stain for both K<sup>b</sup> (PE) and D<sup>d</sup> (FITC). When the K<sup>b</sup>D<sup>d</sup> double-positive cells (Fig. 6 A, upper right hand quadrant) which were also stained for DQ2 were compared with the K<sup>b</sup>D<sup>d</sup> double-positive cells stained with an isotype-matched control antibody a clear shift in fluorescence intensity was observed (Fig. 6 B). No expression of either HLA-DR or HLA-DP could be detected in the fused cell population, demonstrating the isotype specificity of this *trans*-acting factor (data not shown).

In addition to the surface expression of DQ2 in the stable cell fusions, we were also able to detect HLA-DQ2 mRNA in the fusions by RT-PCR. RNA prepared from two separate somatic cell fusion experiments, from the individual fusion partners, RJ2.2.5 and clone 13, and from Raji (the class II-positive cell line from which RJ2.2.5 was derived), was DNase I treated and reverse transcribed. The resulting first-strand cDNA was used as a template in



**Figure 5.** Does clone 13 express a DQ-specific transactivator? Schema of the protocol used to generate stable fusions between RJ2.2.5/K<sup>b</sup> and clone 13/D<sup>d</sup>. RJ2.2.5 cells stably transfected with pCMV.K<sup>b</sup> and clone 13 cells stably transfected with pREP9.D<sup>d</sup> were fused and grown in both hygromycin and neomycin to select for fused cells. After selection, the fused cell population was analyzed for expression of DQ2 by FACS<sup>®</sup> and by RT-PCR.



**Figure 6.** Stable fusions of RJ 2.2.5/K<sup>b</sup> and clone 13/D<sup>d</sup> express DQ2 on the surface. (A) RJ2.2.5/K<sup>b</sup> × clone 13/D<sup>d</sup> fused cell populations were triply stained with antibodies for K<sup>b</sup>, D<sup>d</sup>, and with either a mAb (IgM) specific for DQ2 or with a control IgM and analyzed for the expression of K<sup>b</sup> and D<sup>d</sup>. (B) Cells that were positive for both of these markers were further analyzed for the presence of DQ2. The unshaded peak represents K<sup>b</sup>+D<sup>d</sup>+ cells stained with an isotype control antibody, while the shaded peak represents those K<sup>b</sup>+D<sup>d</sup>+ cells stained with a reagent specific for DQ2.

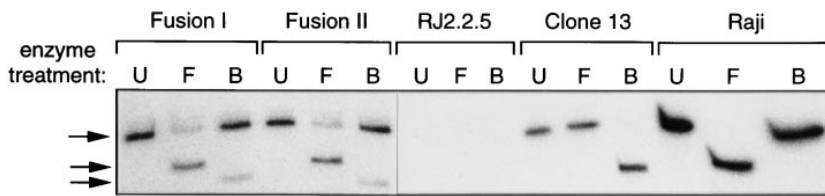
PCR reactions with group-specific primers that amplify a region from exon 2 of the HLA-DQB1 gene (23a). One set of primers (GH28NL and QB204) generates a 237 bp product specific for the DQw2,3,4 alleles, whereas the second set (GH28NL and QB202) amplifies a 237-bp fragment specific for the DQw1 allele. DQ alleles can be further distinguished by taking advantage of restriction site polymorphisms that occur among the individual DQ alleles. Again, we chose to look at the expression of DQ2 in the fused populations because RT-PCR experiments showed that RJ2.2.5 was slightly leaky for the transcription of DQB\*0501 (data not shown) but did not express any DQB\*0201. In populations of stable fusions between RJ2.2.5/K<sup>b</sup> and clone 13/D<sup>d</sup>, the DQw2,3,4 group-specific primers would be expected to amplify both a product from the DQB\*0301 gene expressed by clone 13 and a product from the DQB\*0201 gene of RJ2.2.5 if it is reexpressed. These two products can be distinguished on the basis of their sensitivity to digestion by the restriction endonucleases FokI and BglI: the DQB\*0201 product is cut by FokI but not by BglI, whereas the DQB\*0301 product is cut by BglI but not by FokI. Fig. 7 shows the results of such an analysis. The

RT-PCR products generated by group-specific primers for DQw2,3,4 from the various cell populations were either left uncut or digested with FokI or BglI. Transcripts derived from both the DQB\*0201 and the DQB\*0301 alleles were detected in all three populations of stably fused cells whereas clone 13 alone, as expected, transcribed only the DQB\*0301 allele. No transcription of the DQB\*0201 gene was detected in RJ2.2.5 in contrast with its presence in the class II-positive parent, Raji.

## Discussion

Here, we have analyzed the molecular basis for the unusual pattern of expression of MHC class II antigens in a mutant B-lymphoblastoid cell line, clone 13. Clone 13 has previously been shown by complementation analysis to belong to group II of the five known groups of MHC class II CID. Unlike the other cell lines in this complementation group, which fail to transcribe all class II genes, clone 13 does express substantial levels of HLA-DQ in the absence of HLA-DR and -DP expression. It has recently been shown that the gene defective in complementation group II cells is CIITA, and ectopic expression of CIITA in these cells is both necessary and sufficient to restore expression of all three HLA-D isotypes. Ectopic expression of CIITA in clone 13 similarly restores HLA-DR and -DP and increases HLA-DQ expression.

One possible explanation for the clone 13 phenotype was that one allele of CIITA in clone 13 harbored a mutation that abrogated HLA-DR and -DP but not HLA-DQ transcription. We have detected a 4-bp genomic deletion in at least one allele of CIITA in clone 13; however, this gene is functionally inert when introduced into CIITA-deficient cell lines. Because we repeatedly isolated only this mutation from genomic DNA, it is possible that both alleles of the CIITA gene in clone 13 bear this 4-bp mutation. However, it is also possible that the 4-bp deletion represents only one allelic form of CIITA in clone 13 and that the other allele of the CIITA gene is deleted as is the case in RJ2.2.5. It may be that the 93 bp deletion mutant represents the second allele of the CIITA gene in clone 13 despite the presence of wild-type splice donor and acceptor site. The defects in CIITA found in other group II cell lines have been identified and include both in-frame deletions due to splice donor site mutations and mutations, which introduce premature termination codons as well as large genomic deletions (12, 25). Like the mutations in clone 13, these defects are most often located in the 3' portion of the CIITA cDNA. In any case, it is clear that there is no CIITA protein detectable by Western blot analysis in clone 13 and, furthermore, that overexpression of the clone 13 cDNAs in a CIITA-deficient cell line such as RJ2.2.5 or in clone 13 itself, does not lead to the expression of HLA-DQ. Instead, stable cell fusions between clone 13 and RJ2.2.5, restored expression of the RJ2.2.5 HLA-DQ genes, providing strong evidence that clone 13 possesses an HLA-DQ-specific transactivator. Based on the data pre-



**Figure 7.** DQ2 expression in fusions detected by RT-PCR. RNA from the indicated cell population was reverse transcribed and used as a template for PCR using DQw2,3,4 group-specific primers. Portions of the reactions were left uncut (U) or were digested with either FokI (F) or with BglII (B). Digestion of the PCR product by FokI is indicative of transcripts derived from the DQB1\*0201 allele (specific to RJ2.2.5), whereas digestion by BglII is indicative of transcripts derived from the DQB1\*0301 allele (specific to clone 13).

sented above, this transactivator is not CIITA and, further, functions in the absence of CIITA.

It is likely that this HLA-DQ transactivator mediates its effects through the DQB gene X2 element. Ono and Song (17) have shown that reporter constructs bearing the DQB promoter are active in clone 13 while similar DRA promoter constructs are not. Replacement of the DRA X2 site with that from DQB renders it transcriptionally active in clone 13. Conversely, replacement of the DQB X2 box with that from DRA abolishes transcription. These results suggest that the X2 elements of DRA and DQB might bind different factors in clone 13. Further, the factor in clone 13 that transactivates DQ is not present in other CIITA-deficient cell lines.

Isotype-selective binding of factors to class II promoters has been demonstrated previously. A family of X1 box binding proteins, RFX1-5, has been cloned, one of which, RFX5, is composed of two subunits and comprises the nucleoprotein complex called RFX (26). Recently, the 75-kD subunit of RFX5 has been identified as the gene defective in group IV MHC class II CID (14). Indeed, the introduction of a wild-type RFX5 cDNA into group IV cells not only restores class II expression but also results in the reappearance of the RFX binding complex in nuclear extracts prepared from the transfected cells. Whereas the purified RFX complex consisting of both the 75- and 36-kD subunits shows moderate binding affinity to some class II X1 boxes (DRA and DPA), its binding to others is very weak or nonexistent. Cooperative binding between NF-Y, a heterodimeric complex that binds all class II promoter Y boxes, and RFX5, however, increases the stability of complex formation between each of these factors and its binding site, thus increasing the apparent affinity of RFX5 for those X1 boxes that it cannot bind on its own.

Several isotype-selective X2 box binding proteins have also been identified either by molecular cloning or by biochemical means. Among these are hXBP-1 (27), ATF-2 or mXBP (28, 29), c-jun (30), and X2BP (31). We think it unlikely that any of these factors is responsible for the DQ expression in clone 13. The transcription factor hXBP-1, a basic region leucine-zipper protein, binds to the X2 site in the DRA and DPB, but not DQB genes. Consistent with its isotype-selective binding, transfection of antisense hXBP-1 into the class II-positive B-LCL Raji decreased HLA-DR and -DP but not HLA-DQ expression (32). This pattern of binding and transactivation is the opposite of the clone 13

phenotype. ATF-2 (mXBP) was also originally isolated by its ability to bind to the X2 site, but mice bearing a germline mutation in this gene have normal levels of class II, suggesting that ATF-2 is not an essential element of the class II transcription machinery (33). X2BP was originally identified as an X2-specific DNA binding activity in nuclear extracts prepared from Raji cells. Unlike other X2 box binding complexes, it can bind to the X2 boxes of both the  $\alpha$  and  $\beta$  chain genes of the same isotype (HLA-DR), but, like the others, individually, it does not bind well to the X2 boxes of other isotypes such as DQA, DQB, or DPA. X2BP, which is found at high levels in class II-positive B cells, can bind cooperatively to the X2 box in the presence of RFX (34) and can form stable higher order complexes with RFX and NF-Y on a DRA promoter probe in EMSA (26). These observations strongly suggest that X2BP is a functionally important X2 box factor. Nevertheless, its isotype binding profile does not match well to the clone 13 phenotype. Neither the individual binding profiles of these X2 box binding proteins, nor their ability to bind all class II promoters in a higher order nucleoprotein complex, fits the phenotype of the clone 13 HLA-DQ transactivator. Thus, it is likely that the clone 13 DQ transactivator protein has not yet been isolated. A schematic diagram of the major factors relevant to class II transcription and the *cis*-acting elements through which they are thought to act can be found in Mach et al. (12).

The ability of the clone 13 HLA-DQ transactivator to function in the absence of CIITA is intriguing. Though apparently not a DNA-binding protein itself (26), CIITA has been shown to be both necessary and sufficient for the coordinate expression of class II genes in both constitutive and inducible systems. Indeed, there has been a good correlation to date between the expression of CIITA and the expression of MHC class II. In all cell lines that map to MHC class II CID complementation group II, the introduction of a wild-type copy of CIITA causes the coordinate reexpression of all three HLA isotypes (13, 16). It has also been shown that CIITA is responsible for the induction of class II genes by IFN- $\gamma$  in cultured fibroblasts (35, 36). Additionally, the lack of class II antigen expression in plasmacytes and in activated murine T cells can be reversed by the introduction of a functional copy of the CIITA gene (37, 38). However, there are two examples of class II expression in the absence of CIITA. Class II is expressed on rare interdigitating reticular thymic cells in CIITA-defi-



cient mice (39). Furthermore, the cell line RJ2.2.5 is somewhat leaky for the expression of HLA-DR (Douhan III, J., and L.H. Glimcher, unpublished observation), despite the lack of a functional CIITA (13). Thus, the CIITA-independent expression of HLA-DQ in clone 13 is not unprecedented.

What type of mechanism or factor(s) might account for the CIITA-independent expression of HLA-DQ in clone 13? It is possible that a DQ-specific factor that is normally regulated by CIITA has lost its dependence on CIITA as a result of a dominant-acting mutation. Alternatively, a factor not normally involved in the regulation of CIITA might have acquired the ability to do so as the result of a mutation. Finally, with the exception of clone 13, the experimentally derived class II-negative cell lines in group II were generated by mutagenesis followed by immunoselection for lack of class II expression (18, 40). Clone 13, however, was derived as a subclone of P3J-HR-1 cells, a mutant B cell line in turn derived from Jijoye, a class II-positive Burkitt's lymphoma. The P3J-HR-1 cell line is a very high producer of viral capsid antigen, whereas the subclone clone 13 was identified initially for its ability to produce large amounts of virus in response to treatment with TPA (41). It is possible that expression of HLA-DQ in clone 13 is related to the transforming EBV present in these cells. Although transformation of human B lymphocytes with EBV does not usu-

ally alter their MHC class II phenotype (B lymphocytes from MHC class II-deficient patients remain class II-negative after transformation), it is known that EBV transformation often causes reexpression of class I antigens in B cells derived from BLS type I patients (9).

There are several examples of cell lines, both normal and malignant, in which noncoordinate expression of the class II genes has been observed. In human dermal fibroblasts, treatment with IFN- $\gamma$  induces the expression of HLA-DR and -DP but not HLA-DQ (42). Malignant B cells have been described that fail to express one or more specific class II isotypes (43, 44). Furthermore, in human eosinophils, HLA-DR but not HLA-DQ is induced in response to GM-CSF (45). Whether any of these examples of noncoordinate regulation of the class II genes involves a CIITA-independent pathway like that in clone 13 is currently unknown.

Despite the highly conserved nature of the class II proximal promoters and the identification of factors such as CIITA and RFX5, which are known to influence the coordinate expression of the class II genes, it is becoming increasingly clear that the regulation of expression of individual class II genes may involve gene-specific *cis* elements and *trans*-acting factors, which in some cases may result in noncoordinate expression of class II genes.

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## References

1. Schwartz, R.H. 1986. Immune response (I<sub>r</sub>) genes of the murine major histocompatibility complex. *Adv. Immunol.* 38: 31-201.
2. Glimcher, L.H., and C.J. Kara. 1992. Sequences and factors: a guide to MHC class-II transcription. *Annu. Rev. Immunol.* 10: 13-49.
3. Paulnock-King, D., K.C. Sizer, Y.R. Freund, P.P. Jones, and J.R. Parnes. 1985. Coordinate induction of Ia alpha, beta, and Ii mRNA in a macrophage cell line. *J. Immunol.* 135: 632-636.
4. de Preval, C., M.R. Hadam, and B. Mach. 1988. Regulation of genes for HLA class II antigens in cell lines from patients with severe combined immunodeficiency. *N. Engl. J. Med.* 318:1295-1300.
5. Benoist, C., and D. Mathis. 1990. Regulation of major histocompatibility complex class-II genes: X, Y and other letters of the alphabet. *Annu. Rev. Immunol.* 8:681-715.
6. Accolla, R.S., G. Carra, and J. Guardiola. 1985. Reactivation by a trans-acting factor of human major histocompatibility complex Ia gene expression in interspecies hybrids between an Ia-negative human B-cell variant and an Ia-positive mouse B-cell lymphoma. *Proc. Natl. Acad. Sci. USA.* 82:5145-5149.
7. Yang, Z., R.S. Accolla, D. Pious, B.J. Zegers, and J.L. Strominger. 1988. Two distinct genetic loci regulating class II gene expression are defective in human mutant and patient cell lines. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1965-1972.
8. Hume, C.R., and J.S. Lee. 1989. Congenital immunodeficiencies associated with absence of HLA class II antigens on lymphocytes result from distinct mutations in trans-acting factors. *Hum. Immunol.* 26:288-309.
9. Benichou, B., and J.L. Strominger. 1991. Class II-antigen-negative patient and mutant B-cell lines represent at least three,

- and probably four, distinct genetic defects defined by complementation analysis. *Proc. Natl. Acad. Sci. USA.* 88:4285–4288.
10. Peijnenburg, A., B. Godthelp, A. van Boxel-Dezaire, and P.J. van den Elsen. 1995. Definition of a novel complementation group in MHC class II deficiency. *Immunogenetics.* 41:287–294.
  11. Douhan, J., J.A. Brown, Z.L. Gleit, C.S. David, and L.H. Glimcher. 1996. Regulation of the E-beta gene in vivo: lessons from E(beta)(D) transgenic mice. *Int. Immunol.* 8:255–265.
  12. Mach, B., V. Steimle, E. Martinez-Soria, and W. Reith. 1996. Regulation of MHC class II genes: lessons from a disease. *Annu. Rev. Immunol.* 14:301–331.
  13. Steimle, V., L.A. Otten, M. Zufferey, and B. Mach. 1993. Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). *Cell.* 75:135–146.
  14. Steimle, V., B. Durand, E. Barras, M. Zufferey, M.R. Hadam, B. Mach, and W. Reith. 1995. A novel DNA-binding regulatory factor is mutated in primary MHC class II deficiency (bare lymphocyte syndrome). *Genes Dev.* 9:1021–1032.
  15. Ono, S.J., V. Bazil, M. Sugawara, and J.L. Strominger. 1991. An isotype-specific *trans*-acting factor is defective in a mutant B cell line that expresses HLA-DQ, but not -DR or -DP. *J. Exp. Med.* 173:629–637.
  16. Zhou, H., and L.H. Glimcher. 1995. Human MHC class II gene transcription directed by the carboxyl terminus of CIITA, one of the defective genes in type II MHC combined immune deficiency. *Immunity.* 2:545–553.
  17. Ono, S.J., and Z. Song. 1995. Mapping of the interaction site of the defective transcription factor in the class II major histocompatibility complex mutant cell line clone-13 to the divergent X2-box. *J. Biol. Chem.* 270:6396–6402.
  18. Accolla, R.S. 1983. Human B cell variants immunoselected against a single Ia antigen subset have lost expression of several Ia antigen subsets. *J. Exp. Med.* 157:1053–1058.
  19. Zhou, H., H.S. Su, X. Zhang, J. Douhan III, and L.H. Glimcher. 1997. CIITA-dependent and independent class II MHC expression revealed by a dominant negative mutant. *J. Immunol.* In press.
  20. Dignam, J.D., R.M. Lebovitz, and R.G. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* 11:1475–1489.
  21. Seabright, M. 1971. A rapid banding technique for human chromosomes. *Lancet.* 2:971–972.
  22. Knoll, J.H.M., and P. Lichter. 1994. In situ hybridization to metaphase chromosomes and interphase nuclei. In *Current protocols in human genetics*. Vol. 1. N.C. Dracopoli, J.L. Haines, B.R. Korf, D.T. Moir, C.C. Morton, C.E. Seidman and J.G. Seidman, editors. Green-Wiley, New York. 4.3.1–4.3.28.
  23. Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294–5299.
  - 23a. Nomura, N., M. Ota, K. Tsuji, and H. Inoko. 1991. HLA-DQB1 genotyping by a modified PCR-RFLP method combined with group-specific primers. *Tissue Antigens.* 38:53–59.
  24. Riley, J.L., S.D. Westerheide, J.A. Price, J.A. Brown, and J.M. Boss. 1995. Activation of class II MHC genes requires both the X box region and the class II transactivator (CIITA). *Immunity.* 2:533–543.
  25. Brown, J.A., X.F. He, S.D. Westerheide, and J.M. Boss. 1996. Characterization of the expressed CIITA allele in the class II MHC transcriptional mutant RJ2.2.5. *Immunogenetics.* 43:88–91.
  26. Reith, W., V. Steimle, and B. Mach. 1995. Molecular defects in the bare lymphocyte syndrome and regulation of MHC class II genes. *Immunol. Today.* 16:539–546.
  27. Liou, H.C., M.R. Boothby, P.W. Finn, R. Davidon, N. Nabavi, N.J. Zeleznik-Le, J.P. Ting, and L.H. Glimcher. 1990. A new member of the leucine zipper class of proteins that binds to the HLA DR alpha promoter. *Science (Wash. DC).* 247:1581–1584.
  28. Liou, H.C., M.R. Boothby, and L.H. Glimcher. 1988. Distinct cloned class II MHC DNA binding proteins recognize the X box transcription element. *Science (Wash. DC).* 242:69–71.
  29. Ivashkiv, L.B., H.C. Liou, C.J. Kara, W.W. Lamph, I.M. Verma, and L.H. Glimcher. 1990. mXBP/CRE-BP2 and c-Jun form a complex which binds to the cyclic AMP, but not to the 12-O-tetradecanoylphorbol-13-acetate, response element. *Mol. Cell. Biol.* 10:1609–1621.
  30. Andersson, G., and B.M. Peterlin. 1990. NF-X2 that binds to the DRA X2-box is activator protein 1. Expression cloning of c-Jun. *J. Immunol.* 145:3456–3462.
  31. Hasegawa, S.L., and J.M. Boss. 1991. Two B cell factors bind the HLA-DRA X box region and recognize different subsets of HLA class II promoters. *Nucleic Acids Res.* 19:6269–6276.
  32. Ono, S.J., H.C. Liou, R. Davidon, J.L. Strominger, and L.H. Glimcher. 1991. Human X-box-binding protein 1 is required for the transcription of a subset of human class II major histocompatibility genes and forms a heterodimer with c-fos. *Proc. Natl. Acad. Sci. USA.* 88:4309–4312.
  33. Reimold, A.M., M.J. Grusby, B. Kosaras, J.W. Fries, R. Mori, S. Maniwa, I.M. Clauss, T. Collins, R.L. Sidman, M.J. Glimcher, and L.H. Glimcher. 1996. Chondrodysplasia and neurological abnormalities in ATF-2-deficient mice. *Nature (Lond.).* 379:262–265.
  34. Moreno, C.S., P. Emery, J.E. West, B. Durand, W. Reith, B. Mach, and J.M. Boss. 1995. Purified X2 binding protein (X2BP) cooperatively binds the class II MHC X box region in the presence of purified RFX, the X box factor deficient in the bare lymphocyte syndrome. *J. Immunol.* 155:4313–4321.
  35. Chang, C.H., J.D. Fontes, M. Peterlin, and R.A. Flavell. 1994. Class II transactivator (CIITA) is sufficient for the inducible expression of major histocompatibility complex class II genes. *J. Exp. Med.* 180:1367–1374.
  36. Steimle, V., C.A. Siegrist, A. Mottet, B. Lisowska-Grospierre, and B. Mach. 1994. Regulation of MHC class II expression by interferon-gamma mediated by the transactivator gene CIITA. *Science (Wash. DC).* 265:106–109.
  37. Silacci, P., A. Mottet, V. Steimle, W. Reith, and B. Mach. 1994. Developmental extinction of major histocompatibility complex class II gene expression in plasmacytes is mediated by silencing of the transactivator gene CIITA. *J. Exp. Med.* 180:1329–1336.
  38. Chang, C.H., S.C. Hong, C.C. Hughes, C.J. Janeway, and R.A. Flavell. 1995. CIITA activates the expression of MHC class II genes in mouse T cells. *Int. Immunol.* 7:1515–1518.
  39. Chang, C.H., S. Guerder, S.C. Hong, W. van Ewijk, and R.A. Flavell. 1996. Mice lacking the MHC class II transactivator (CIITA) show tissue-specific impairment of MHC class II expression. *Immunity.* 4:167–178.
  40. Calman, A.F., and B.M. Peterlin. 1987. Mutant human B cell lines deficient in class II major histocompatibility complex

- transcription. *J. Immunol.* 139:2489-2495.
41. Heston, L., M. Rabson, N. Brown, and G. Miller. 1982. New Epstein-Barr virus variants from cellular subclones of P3J-HR-1 Burkitt lymphoma. *Nature (Lond.)*. 295:160-163.
  42. Blanck, G., M. Lok, K. Kok, E. Downie, J.H. Korn, and J.L. Strominger. 1990. Gamma-interferon induction of HLA class II mRNAs in dermal fibroblasts studied by RNase protection analysis. *Hum. Immunol.* 29:150-156.
  43. Pesando, J.M., and L. Graf. 1986. Differential expression of HLA-DR, -DQ, and -DP antigens on malignant B cells. *J. Immunol.* 136:4311-4318.
  44. Pesando, J.M., L. Graf, and P. Hoffman. 1986. HLA-DP can be expressed with or without -DR molecules on a malignant B cell line. *J. Immunol.* 137:1932-1936.
  45. Lucey, D.R., A. Nicholson-Weller, and P.F. Weller. 1989. Mature human eosinophils have the capacity to express HLA-DR. *Proc. Natl. Acad. Sci. USA.* 86:1348-1351.