Brief Definitive Report

LOSS OF A DNA-PROTEIN COMPLEX CORRELATES WITH EXTINGUISHED MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II EXPRESSION IN A HUMAN B CELL

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Multiple different genetic defects can lead to the loss of constitutive class II MHC (Ia) expression in B cells (1). Somatic cell hybridization between spontaneous or experimentally selected human Ia^- mutant B cell lines have divided the class II MHC expression mutants into at least three complementation groups. The defect in one such Ia mutant cell line, RJ 2.2.5, has been postulated to be in a positive transacting factor that is active across species, because it is complemented by a locus, αIr-1, that maps to mouse chromosome 16 (2). Class II Eα and DQβ upstream *cis*-active DNA elements transfected into RJ 2.2.5 cells do not act as transcriptional elements (3, 4). Nevertheless, these DNA motifs appear to bind nuclear factors in RJ 2.2.5 indistinguishable from those in the wild-type parent Raji. Thus, the absence of a positive transacting factor in RJ 2.2.5 could not be correlated with the absence of a DNA binding activity for a class II gene.

We recently determined the 5′ upstream region of the murine MHC class II Eβ gene that is necessary for constitutive Eβ expression in murine B cells (5). This 126-bp sequence contains three consensus elements, W, X, and Y (6), that are highly conserved in evolution. The W, X, Y sequence functions as a tissue-specific transcriptional element that is exclusively active in MHC class II-positive cells. In murine nuclear extracts, there are two distinct protein complexes termed complex A and complex B that bind the W, X, Y sequence. Complex B may be similar to previously identified CCAAT box-binding proteins NF-Y or YB1 (7, 8), and its presence does not correlate with constitutive or induced class II expression. Complex A, however, appeared to be novel, and is present in nuclear extracts of murine cells that express MHC class II genes. Here we examine complex A activity in extracts from normal and class II-deficient human B cells.

Materials and Methods

Cell Cultures and Transfections. Culture medium and cells have all previously been described (2, 5, 9). Txls5c17 (Txl) is a secondary transfectant of RJ 2.2.5 cells stably transfected with

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M12.4.1 DNA, and is class II positive (10). The cells were all transfected by the DEAE-dextran method and chloramphenicol acetyl transferase (CAT) activity assayed at 48 h after transfection, as described (11).

Plasmid Constructions and Oligonucleotides. The pH-H is a plasmid that contains 130 bp of Eβ upstream sequence containing W, X, and Y consensus motifs subcloned into the Sma I site of pUC 18 (11). The constructs used for CAT assays are depicted in Fig. 1 and reference 11. The oligonucleotides used for binding and competition, and their purification, are described elsewhere (9, 11).

Electromobility Shift Electrophoresis Assay (EMSA). Preparation of nuclear extracts was by a modification of the method of Dignam (12), and EMSA assays were performed as described (9). The Hae fragment probe (Hae) was generated by digesting pH-H with Bam HI, end labeled with [γ^{32}P]ATP and polynucleotide kinase, digested with Eco RI, and isolated by gel electrophoresis. For EMSA analysis, a total sample of 10 μl included 1 ng of labeled probe (10,000 cpm), 2–4 μg of protein from nuclear extract, and 4 μg of poly dI-dC (Pharmacia Fine Chemicals, Piscataway, NJ) as a nonspecific competitor. Samples for competition analysis were processed as for EMSA, except that the nuclear extracts were preincubated with water or competitor DNA for 5 min at room temperature before addition of labeled probe.

Results and Discussion

RJ 2.2.5 is a mutant of the class II-positive cell Raji, which was generated by γ irradiation and negative immunoselection (2). Although the RJ 2.2.5 cells have lost class II expression, their class II structural genes are intact, since fusion to an Ia^+ mouse cell restores human class II. Further studies of these hybrids have identified a locus on mouse chromosome 16, aIr-1, that complements the defect in these human cells (2). Since the RJ 2.2.5 mutant lacks a non-species-specific trans-acting factor, it was of interest to determine if the 126-bp-positive element containing W, X, and Y sequences (−192 to −66 relative to the transcription start site) was active in these cells. To investigate this further, it was necessary to determine whether the 126-bp element of murine origin could function in human cells. For this purpose, the human class II-positive parental cell line Raji was transfected with plasmid constructs containing 5′ upstream sequences of the murine Eβ gene fused to the CAT gene (Fig. 1). Fig. 1, Lanes *3* and *4* demonstrate that constructs containing the three conserved motifs W, X, and Y, whether contained within a 2.6-kb construct (−2679 to −66) or in a 126-bp (−192 to −66) construct, are expressed in human B cells. Interestingly, the same constructs transfected into the class II-negative mutant of Raji, RJ 2.2.5, do not express above the enhancerless control plasmid (Fig. 1, lanes *7* and *8*). Furthermore, constructs containing individual W, X, or Y motifs alone do not enhance CAT activity in Raji and RJ 2.2.5 cells (not shown). These data suggest that all three conserved motifs are necessary to function as a positive transcription element in human B cells, consistent with our earlier data in murine B cells. This positive element does not function in human class II-negative mutant cell line.

To determine whether there was a difference in factors binding to any of the individual motifs, W, X, or Y, each of the individual motifs was used as a labeled target in an EMSA (Fig. 2). Comparison of extracts from the Ia^- RJ 2.2.5 (Fig. 2, lanes *2*, *4*, and *6*) cells with the Ia^+ Raji cells (Fig. 2, lanes *1*, *3*, and *5*) demonstrates that there is no difference in factors binding to W, X, or Y motifs between these two cell lines. These results are consistent with work from other laboratories

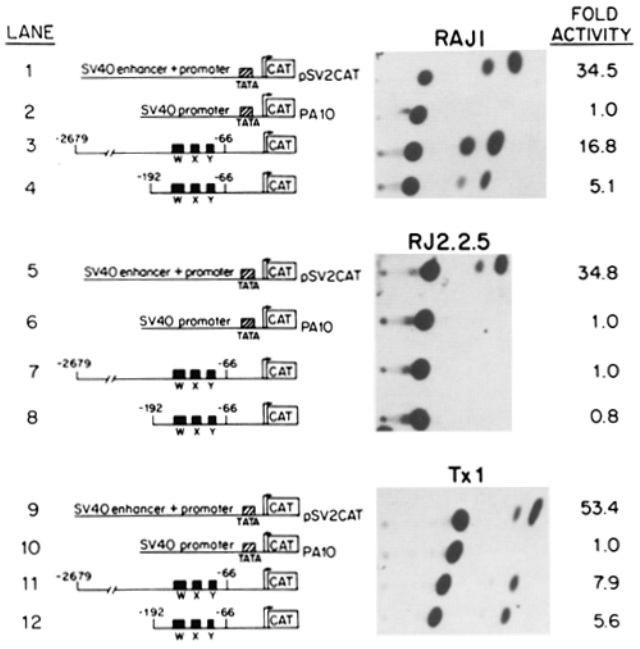

FIGURE 1. Expression of Eβ W-X-Y constructs in the Raji and Txl, but not the RJ 2.2.5, cell line. $E\beta^d$-CAT constructs as depicted were assayed for CAT activity in a transient transfection assay. The numbers for the constructs correspond to the portion of the sequence in the germ line Eβ upstream sequence. Each transfection was performed a minimum of five times and a representative experiment is shown. Fold activity represents the average CAT activity of at least three independent experiments over the CAT activity of the enhancerless control, pA10 cat_2. The cells transfected are Raji (lanes *1–4*), RJ 2.2.5 (lanes *5–8*), and Tx1 (lanes *9–12*). The constructs transfected are pSV_2 CAT (lanes *1*, *5*, and *9*), pA10 cat_2 (lanes *2*, *6*, and *10*), −2679 to −66 (lanes *3*, *7*, and *11*), −192 to −66 (lanes *4*, *8*, and *12*).

that demonstrated no difference in factors from RJ 2.2.5 or Raji cell extracts binding to the X and Y motif of the Eα and DQβ genes (3, 4).

To determine whether complex A exists in human as well as murine cells, nuclear extracts from the human class II-positive Raji were examined in an EMSA using a labeled fragment containing the W, X, Y consensus sequence (Hae) as the target probe (Fig. 3 *A*). Comparison of nuclear extracts from two murine class II-positive B cells, M12.4.1 and A20 (Fig. 3 *A*, lanes *1* and *2*), with the Raji extract (Fig. 3 *A*, lane *3*), shows that two retarded bands are noted, complex A and B, that migrate

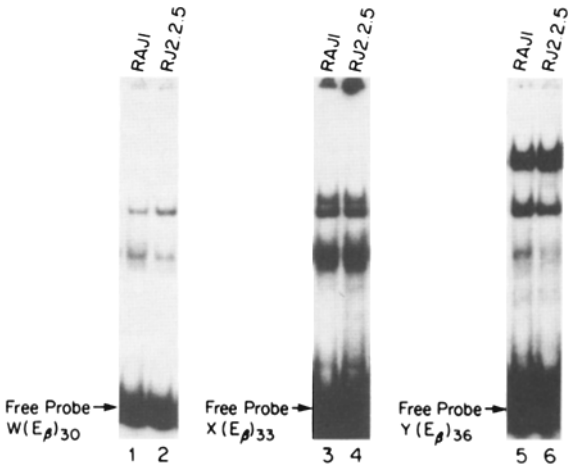

FIGURE 2. No difference in factors binding to individual W, X, and Y motifs between Raji and RJ 2.2.5 cells. EMSA with end-labeled oligonucleotide probes corresponding to the Eβ W (lanes *1* and *2*), X (lanes *3* and *4*), or Y (lanes *5* and *6*) -conserved motifs. Raji extract (lanes *1*, *3*, and *5*); RJ 2.2.5 extract (lanes *2*, *4*, and *6*).

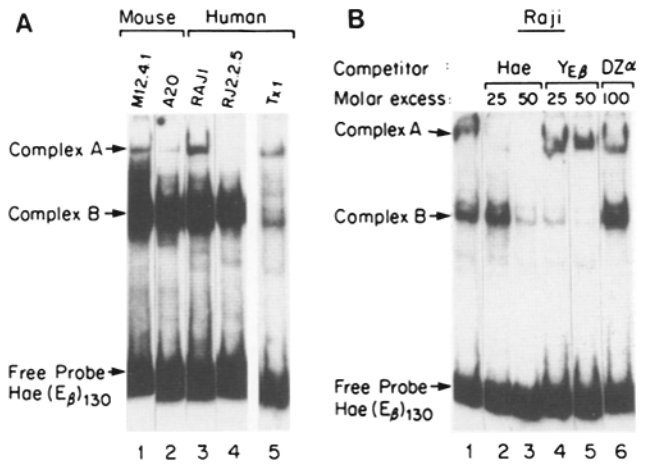

FIGURE 3. (*A*) Complex A is present in an Ia^+ human B cell, absent in an Ia^- mutant, and reappears in the Ia^+ Txl cell. EMSA of nuclear extracts from M12.4.1 (lane *1*), A20 (lane *2*), Raji (lane *3*), RJ 2.2.5 (lane *4*), and Txl cells (lane *5*). The probe is the end-labeled Hae fragment. (*B*) Competition of complex A formation in extracts from human Raji cells. The competitors are unlabeled homologous sequence (Hae) (lanes *2* and *3*), YEβ motif (YEβ) (lanes *3* and *4*), and irrelevant competitor corresponding to the X box of the human DZα gene (DZα) (lane *6*).

at the same position in all three cell lines. The complexes in human extracts bind specific DNA sequences because their formation is prevented by unlabeled homologous sequence (Fig. 3 *B*, lanes *2* and *3*), but not by 100-fold molar excess of irrelevant sequence (Fig. 3 *B*, lane *6*). Complex A is most efficiently competed by an unlabeled homologous sequence that contains all three motifs (Fig. 3 *B*, lanes *2* and *3*), while W, X, or Y sequences tested individually have little effect (not shown). Footprint analysis shows that complex A from nuclear extracts from Raji cells protects the conserved Y motif (not shown), similar to what we had observed with extracts from murine macrophages and B cells (5, 11).

We next asked whether the correlation between the presence of complex A and class II expression extended to the human class II-negative cell RJ 2.2.5. An EMSA (Fig. 3 *A*, lane *4*) demonstrates that complex A is absent in RJ 2.2.5 extracts, in contrast to the pattern seen with the parental Raji (Fig. 3 *A*, lane *3*). To ask whether the defect in class II expression in the RJ 2.2.5 cells was related to the inability to form complex A, the Txl cell line was used. Txl cells are derivatives of RJ 2.2.5 cells that have been stably transfected with mouse genomic DNA resulting in restoration of the human class II-positive status (10). If loss of class II expression in the RJ 2.2.5 cell is related to the absence of complex A, then restoration of the class II-positive status should be accompanied by complex A formation as well. EMSA with nuclear extracts from Txl cells (Fig. 3 *A*, lane *5*) indeed demonstrates the reappearance of complex A. In addition, complex A from extracts from Txl cells has the identical footprint pattern as is observed with extracts from Raji cells (not shown). Moreover, *cis*-active transcription activity of the W, X, and Y element is restored in the Txl cell line (Fig. *1*, lanes *11* and *12*), concomitant with restoration of class II expression and complex A formation.

This is the first demonstration that the Ia^- RJ 2.2.5 cell lacks a DNA-binding protein complex and fails to utilize the W, X, and Y transcriptional element, both of which correlate with the loss of class II MHC expression (compare Fig. 1 *B*, lanes *3* and *4* with *7* and *8*). Restoration of both class II expression and complex A, as well as transcriptional activity of the target WXY sequence in the Txl cell line (Fig.

3 *A*, lane *5*; and Fig. 1, lanes *11* and *12*), points to a pivotal role for complex A or factors that control complex A in driving class II gene transcription. Thus, there are several lines of evidence that demonstrate a strong correlation between the presence of complex A and MHC class II expression. First, complex A is absent in extracts from a human class II-negative cell, RJ 2.2.5; second, complex A reappears in the human class II-positive Txl cell line; third, complex A is present in extracts from murine class II-positive cells; fourth, we have recently shown that complex A is related to the level of Ia expression during the development of the B cell lineage, and finally, complex A formation occurs both in nuclear extracts from macrophages exposed to IFN-γ, as well as in pre-B cells exposed to IL-4 (5, 11). Therefore, we propose that the mutation in RJ 2.2.5 is a defect in the complex A factor(s) or in another factor that regulates complex A formation.

Summary

An Eβ DNA protein complex termed complex A, whose binding activity has recently been shown to correlate with both constitutive and regulated class II expression in murine cell lines, is also present in a human B cell, Raji. The DNA involved in complex A, which includes three previously defined transcriptional motifs, W, X, and Y, is a *cis*-acting transcription element in Raji cells. Both complex A binding activity and transcriptional activity of its target sequence are absent in an Ia^- mutant subclone of Raji, RJ 2.2.5. This cell line, whose defect is complemented by a locus on mouse chromosome 16, reexpresses both class II and complex A upon transfection with mouse genomic DNA. We suggest that factors that form complex A or that regulate complex A formation account for the molecular lesion in this cell line.

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References

1. Lee, J. S. 1988. Regulation of HLA class II gene expression. *In* Immunobiology of HLA. B. Dupont, editor. Springer-Verlag New York Inc., New York. 49-62.
2. Accolla, R. S., M. Jotterand-Bellomo, L. Scarpellino, A. Maffei, G. Carra, and J. Guardiola. 1986. aIr-1: a newly found locus on mouse chromosome 16 encoding a *trans*-acting activator factor for MHC class II gene expression. *J. Exp. Med.* 164:369.
3. Koch, W., S. Candeias, J. Guardiola, R. Accolla, C. Benoist, and D. Mathis. 1988. An enhancer defect in a mutant Burkitt Lymphoma cell line. *J. Exp. Med.* 167:1781.
4. Sloan, J. H., and J. M. Boss. 1988. Conserved upstream sequences of human class II MHC genes enhance expression of class II gene in wild-type, but not mutant B-cell line. *Proc. Natl. Acad. Sci. USA.* 85:8186.
5. Finn, P. W., C. J. Kara, T. T. Van, J. D. Douhan, M. R. Boothby, and L. H. Glimcher. 1990. The presence of a DNA-binding complex correlates with Eβ class II MCH gene expression. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:1543.
6. Miwa, K., C. Doyle, and J. L. Strominger. 1987. Sequence-specific interactions of nuclear factors with conserved sequences of human class II MHC genes. *Proc. Natl. Acad. Sci. USA.* 84:4939.

7. Dorn, A., J. Bollekens, A. Staub, C. Benoist, and D. Mathis. 1987. A multiplicity of CCAAT box-binding proteins. *Cell.* 50:863.
8. Didier, D. K., J. Schiffenbauer, S. L. Winlfe, M. Zacheis, and B. D. Schwartz. 1988. Characterization of the cDNA encoding a protein binding to the MHC class II Y box. *Proc. Natl. Acad. Sci. USA.* 85:7322.
9. Boothby, M. R., H.-C. Liou, and L. H. Glimcher. 1989. Differences in DNA sequence specificity among MHC class II X box binding proteins. *J. Immunol.* 142:1005.
10. Guardiola, J., L. Scarpellino, G. Carra, and R. S. Accolla. 1986. Stable integration of mouse DNA into Ia-negative human B-lymphoma cells causes reexpression of the human Ia-positive phenotype. *Proc. Natl. Acad. Sci. USA.* 83:7415.
11. Finn, P. W., C. K. Kara, J. D. Douhan, T. T. Van, V. Folsom, and L. H. Glimcher. 1990. Interferon-γ regulates binding of two nuclear protein complexes in a macrophage cell line. *Natl. Acad. Sci. USA.* 87:914.
12. Dignam, J. D., R. H. Lebowitz, 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.