Brief Definitive Report

MODULATION OF HLA CLASS II ANTIGEN EXPRESSION BY TRANSFECTION OF SENSE AND ANTISENSE DRα cDNA

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The HLA class II molecules of the human MHC are cell surface glycoproteins that play a critical role in the immune response regulation. At least three HLA class II isotypes have been described (HLA-DR, -DQ, and -DP), each composed of an α and a β chain (1). In addition to conventional HLA class II molecules, the existence of hybrid molecules formed by transcomplementation (hybrid HLA-DQ) and interisotypic pairing (HLA-DRα-DQβ) has been previously reported (2, 3). In the context of our understanding of immune regulation and HLA class II-linked autoimmune diseases, it is of particular interest to modify the class II phenotype by creating or suppressing individual specificities. To modulate the expression of class II molecules, human EBV-transformed B cell lines were transfected with sense and antisense DRα cDNA. We show that quantitative variation of the DRα chain leads to de novo expression or extinction of the DRα-DQβ dimer.

Materials and Methods

Northern Blot. Pellets of 2×10^7 cells were treated with 0.2 M Tris, 25 mM EDTA, 1% SDS, 0.1 M LiCl, Dextran T-40, and phenol-chloroform. The RNAs were precipitated, resuspended in 5 mM Tris, 2 M LiCl for an overnight precipitation, and resuspended in Tris-EDTA. RNAs (20 μg) were run in 1% agarose gel and transferred to gene screen membrane. The membrane was successively hybridized with probes specific for DRα, DRβ, DQα, and DQβ (4). Dehybridization was performed in 5 mM Tris, 0.2 mM EDTA, 1 mM $NaPO_4$, 0.1 × Denhart's solution overnight at 68°C, and controlled by a 48-h exposure. The scanning of the bands gives a number proportional to the intensity and surface of the signal. Table I must be read horizontally to compare the same ratio in different cells.

Western Blot and Dot Blot Analyses. Transfer to nitrocellulose was performed as described (5). The binding of the mAb was revealed by a biotinylated anti-mouse antibody and a peroxidase-labeled streptavidin complex. For the dot blot, lysates corresponding to 5×10^5 cells were mixed in 100 mM KCl, 20 mM NaCl, 2 mM $NaCO_3$, 2 mM $MgCl_2$, 5 mM Hepes, 0.7% NP-40 before spotting on nitrocellulose. Detection was performed as above.

Immunoprecipitation and Two-dimensional Gel. This technique has been described in detail elsewhere (6). After immunoprecipitation with mAb and protein A-sepharose, the pellets were resuspended in a 9 M urea buffer and run in IEF (Fig. 2) or nonequilibrium pH gradient electrophoresis (NEPHGE) (Fig. 3*b*) and SDS-PAGE.

Transfection. We have modified the vector pHebo so that cDNAs are expressed from the human cytomegalovirus promoter (7). The DRα cDNA, previously shown to be functional in a retrovirus vector (8), was cloned in sense and antisense orientation. This vector (pSANIIa)

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TABLE I
mRNA Ratios in B Lymphoblastoid Cell Lines

Ratio	Cell lines			
	FJO	CA	Isa	Mic
DRα/DRβ	1.25	1.45	4.66	6.80
DQα/DQβ	2.53	0.63	0.87	0.84
DRα-DQβ interisotypic heterodimer	–	–	+	+

20 μg of total RNA from the four cell lines were run in 1% agarose gel, transferred to gene screen membrane, and the same blot was successively hybridized with ^{32}P-labeled specific probes. The autoradiograms were scanned to give a number proportional to the surface and intensity of the bands. The table must be read horizontally to compare the DRα/β or DQα/β ratios in the different cells.

has been shown to produce sense and antisense RNA and to restore the expression of the DR complex in a DRα$^-$ cell line (J. Sands and J. L. Strominger, manuscript in preparation). Electroporations were performed at 250 V/chamber containing 5 × 10^6 cells and 10 μg of DNA. Cells were maintained in 250 mg/ml of hygromycin.

Results and Discussion

Detection of the Mixed Isotypic Heterodimer DRα-DQβ in B Lymphoblastoid Cell Lines. Four representative human B cell lines are shown in Fig. 1. First, the HLA-DR complex was removed from unlabeled protein extract by successive immunoprecipitations with the mAb D1-12 (anti-DR complex) (9) and then immunoprecipitation with the mAb G2a5 (anti-DQβ) (3) was carried out. The first and sixth D1-12 precipitate and the G2a5 precipitate were dissociated and reprecipitated with the mAb HC2.1 (anti-DRα) (10). These immunoprecipitates were run in SDS-PAGE, transferred to nitrocellulose, and probed with HC2.1. After total removal of DR complex a DRα chain was detected in the G2a5 precipitate from Isa and Mic (Fig. 1, lanes *9* and *12*) but not from FJO and CA (lanes *3* and *6*), indicating that Isa and Mic are expressing the heterodimer DRα-DQβ. One phenomenon that may favor locus mis-

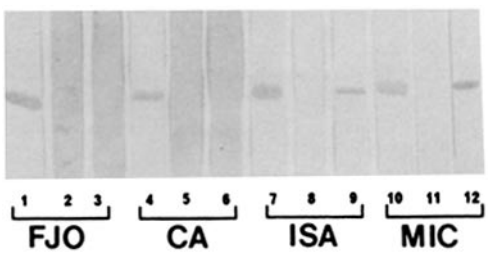

FIGURE 1. Detection of interisotypic heterodimer. Unlabeled protein extracts were precipitated six times with D1-12 (anti-DR complex) (9) before precipitation with G2a5 (anti-DQβ) (3). The first and sixth D1-12 precipitates and G2a5 precipitate were disassociated in 10% SDS, reprecipitated with HC2.1 (anti-DRα) (10), run in SDS-PAGE, transferred to nitrocellulose, and probed again with HC2.1 (Lanes *1*, *4*, *7*, and *10*) First D1-12 immunoprecipitate; (lanes *2*, *5*, *8*, and *11*) sixth D1-12 immunoprecipitate; (lanes *3*, *6*, *9*, and *12*) G2a5 immunoprecipitate after DR complex depletion. No DRα can be detected in FJO and CA (lanes *3* and *6*). In contrast DRα can be detected in Isa and Mic (lanes *9* and *12*), indicating that these cells are expressing DRα/DQβ.

matched pairing is an unbalanced expression of one chain (α or β) within at least one isotype. To determine the α/β ratios of DR and DQ transcripts, a Northern blot of total RNA from these four cell lines was hybridized with probes specific for DRα, DQα, DRβ, and DQβ and the autoradiograms were scanned. Since the system is not calibrated with a reference curve for each probe (each of which may have a somewhat different specific activity), the interpretation of the results must be restricted to the comparison of the same ratio (e.g., DRα/β or DQα/β) in the different cell lines (i.e., horizontally in Table I). The DRα/β ratio was much higher in Isa and Mic than in FJO and CA. This can reflect a higher amount of DRα transcripts and/or a lower amount of DRβ in these two cells as compared with FJO and CA. In contrast the DQα/β mRNA ratio is higher in FJO than in CA, Isa, and Mic, where it is nearly identical. Therefore, in these three cells more DQβ and/or less DQα mRNA is present relative to FJO. Taken together the results show a correlation between a high DRα/β mRNA ratio and the expression of the mixed isotypic heterodimer DRα-DQβ. Variation in the DQα/β mRNA balance could be necessary but is not sufficient by itself to induce the expression of DRα-DQβ.

Induction of the Mixed Isotypic Heterodimer DRα-DQβ by Transfection of Sense DRα cDNA. To modify the DRα/β balance in CA cells, transfections were performed with the vector pSANIIa containing the DRα cDNA in sense orientation. The mAb G2a5 (anti-DQβ) only precipitates the DQα chain from parental CA cells (Fig. 2 *A*). In contrast, after transfection of DRα cDNA the same antibody precipitated the DQα chain and an additional α chain that migrates to the position of DRα (Fig. 2 *B*). The identification of this α chain as DRα was confirmed by Western blot of G2a5 immunoprecipitations probed with HC2.1 (anti-DRα) and LC2.1 (anti-DRβ) (11). DRα was detected in G2a5 precipitate of transfected cells but not parental cells (Fig. 2 *C*). Therefore, by increasing the amount of DRα available in the cell, the expression of DRα/DQβ was induced. Thus, an imbalanced expression of one chain of an isotype can lead to the expression of new molecules.

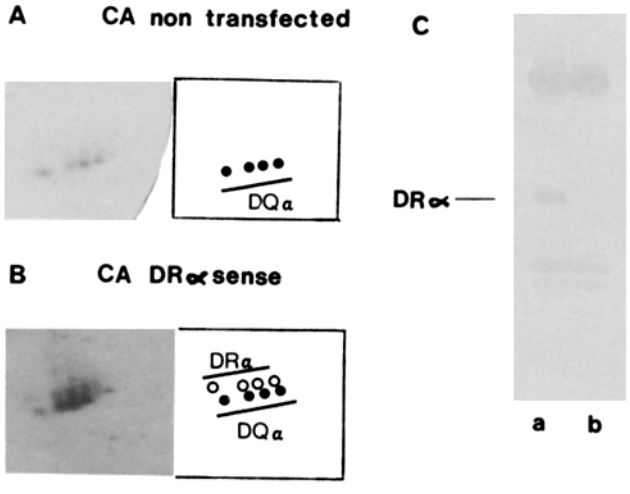

FIGURE 2. Induction of DRα-DQβ expression. (*A*) DQw1α chain precipitated with G2a5 (anti-DQβ) from parental CA cells; (*B*) DRα transfected cells immunoprecipitated with G2a5. In addition to DQα an extra α chain is detected that migrates to the position of DRα; (*C*) Western blot of G2a5 immunoprecipitates of unlabeled parental CA cells and DRα transfectants probed with HC2.1 (anti-DRα) and LC2.1 (anti-DRβ). (Lane *a*) DRα detected by HC2.1 in the transfectants. LC2.1 does not detect any DRβ, indicating that there is no contaminating DR complex. (Lane *b*) In contrast no DRα is precipitated by G2a5 from parental cells.

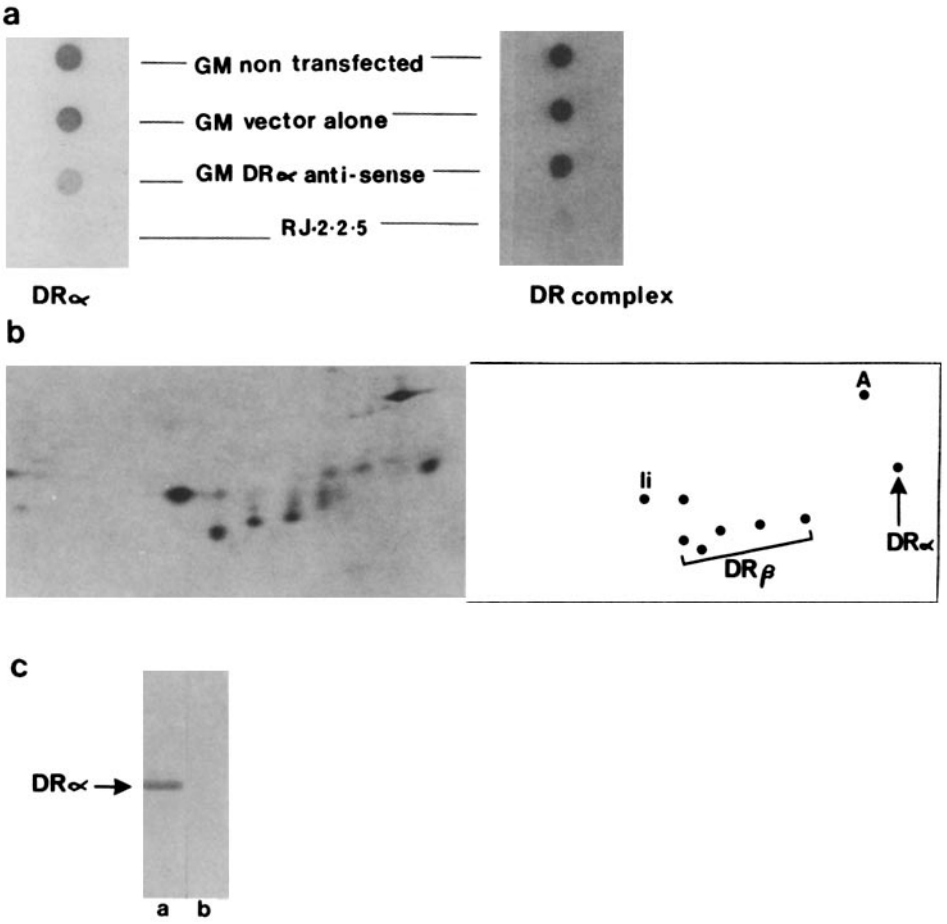

FIGURE 3. Analysis of cells transfected with DRα antisense cDNA. (*a*) Protein dot blot. RJ2.2.5 is a mutant class II$^-$ B cell line (18) used as control. The amount of DRα chain is decreased in the transfectants while the DR complex is not changed. (*b*) 2D-PAGE profile of DA6-147 immunoprecipitate (anti-DRα) of GM3163 transfected with antisense DRα cDNA. The profile reveals the presence of DRα, DRβ, and invariant (Ii) chains. In contrast to the parental cells (3) the DQβ chain, which would have appeared to the left of DRβ and below the Ii chain, is not detected. (*c*) Western blot of CA-206 immunoprecipitations (anti-DRβ, DQβ) (13) of transfected and parental GM3163 cells after complete removal of DR complex and probed with DA6-147. DRα can be detected in parental cells (*a*) but not in transfectants (*b*).

Suppression of the Mixed Isotypic Heterodimer DRα-DQβ by Transfection of the DRα Antisense cDNA. The first evidence for the cell surface expression of DRα-DQβ was obtained using the B cell line GM3163 (3). This cell line was transfected with pSANIIa containing the DRα cDNA in antisense orientation. Protein dot blot analysis was performed to determine the level of DRα expression. Total cell lysates were spotted on nitrocellulose and probed with the mAb DA6.147 (anti-DRα) and D1-12 (anti-DR complex) (12). The amount of DRα was decreased after transfection of the antisense DRα cDNA while the amount of DR complex was unchanged (Fig. 3 *a*). Analysis by immunofluorescence confirmed that no variation of the level of the surface DR complex occurred after transfection (data not shown). Thus, the amount of DRα can be decreased without any influence on the level of expression of DR

complex, indicating that all the DRα was not paired with DRβ. This DRα could be either not paired or associated with another β chain. Immunoprecipitation of transfectants with DA6-147 and two dimensional gel analyses shows that DQβ is no longer associated with DRα (Fig. 3 *b*; compare with Fig. 4 in reference 3). To confirm this result the DR complex was removed from unlabeled lysates before precipitation with the mAb CA-206 (anti-DRβ,DQβ). This last precipitate was run in SDS-PAGE, blotted on nitrocellulose, and probed with DA6-147 (anti-DRα). A DRα chain was detected in the anti-DQβ precipitate of the parental but not of the transfected cells, confirming that DRα is not associated with DQβ in the transfectants (Fig. 3 *c*). Thus the transfection of antisense DRα cDNA decreased the amount of DRα chain available for pairing and led to the extinction of the pre-existing DRα-DQβ heterodimer.

Compared with the parental CA cells, the DRα sense transfectants express an excess of DRα chain that results in the appearance of the mismatched heterodimer DRα-DQβ. In contrast the transfection of antisense DRα cDNA in cells where DRα-DQβ is pre-existing leads to the nonexpression of this heterodimer. These data indicate that the quantitative variation of one chain can affect the α-β pairing, promoting the expression or the extinction of nonorthodox HLA class II molecules. This quantitative variation only influenced the locus mismatched pairing, suggesting that the isotypically matched α-β assembly is highly efficient and stable compared with the interisotypic α-β pair. These results are in agreement with previously reported transfections of I-A and I-E genes into L cells, which showed that both the amount of mRNA and the allelic polymorphism affect the extent of cell surface expression of hybrid Aα-Aβ and Eα-Aβ heterodimers (14–16). In the mouse the expression of the isotype mismatched dimers is five to six times less efficient than for the isotype matched dimers (17, A. Sant and R. N. Germain, personal communication). In our experiments the DRα/DRβ mRNA ratio was 3–5 times higher in cells expressing DRα-DQβ than in cells not expressing this heterodimer. Taken together these results indicate that isotype-mismatched dimer can be expressed when an unbalanced chain synthesis occurs within an isotype leading to an excess of one chain or the other and could be relevant to the consideration of HLA-linked autoimmune diseases.

Summary

In the human there are three isotypic forms of MHC class II gene products (HLA-DR, -DQ, and -DP). The isotype-matched α-β dimers are predominant but isotype-mismatched dimers can also be expressed (DRα-DQβ). Here it is shown that the expression of the DRα-DQβ dimer can be correlated to a high ratio of DRα/DRβ mRNA. The DRα chain expression was modulated by transfection of a sense and antisense DRα cDNA. Overexpression of DRα promoted the appearance of the DRα-DQβ dimer. On the other hand, pre-existing DRα-DQβ dimer expression was suppressed after antisense DRα cDNA transfection. Therefore, imbalanced expression of the α and β chain from a given isotype could lead to the modification of HLA class II phenotype.

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