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Class II-antigen-negative patient and mutant B-cell lines represent at least three, and probably four, distinct genetic defects defined by complementation analysis

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ABSTRACT **Expression of class II major histocompatibility complex antigens in defective B-lymphoblastoid cell lines from patients with class II antigen deficiency and from *in vitro* mutants generated with the same phenotype was studied. By heterogenetic fusion experiments, at least three, and probably four, complementation groups were defined. Furthermore, clone 13 (a DR^-, DP^-, but DQ^+ cell line) appeared to belong to the RJ2.2.5 complementation group, for which all other members are DR^-, DP^-, and also DQ^-. Thus, it is hypothesized that the cell lines of this group lack the activity of a gene that can differentially regulate the DR/DP and the DQ promoters.**

The human class II major histocompatibility antigens (DR, DQ, and DP) are a family of surface molecules expressed by cells involved in antigen presentation (1). Class II antigen deficiency (a subtype of the "bare lymphocyte syndrome") is an inherited autosomal recessive severe combined immunodeficiency associated with loss of class II antigen expression at the surface of the cells (2–5). This defect, which occurs at the transcriptional level (6), cannot be corrected by γ-interferon (7). The genes of the major histocompatibility complex are apparently intact and pedigree analyses of families have demonstrated that this syndrome is unlinked to the major histocompatibility complex (8). Moreover, expression of DR was restored by fusion of a patient cell line with a laboratory-mutant class II-negative cell line (9), directly demonstrating transactivation and the occurrence of two complementation groups. Similarly, complementation was obtained with two patient cell lines (10). Here, the fusion complementation assay, using a relatively large number of class II-defective patient cell lines and mutant B-lymphoblastoid cell lines (BLCLs), has shown that genetic defects in at least three, and probably four, transactivating factors can lead to this phenotype, a failure to transcribe and express class II genes. Similar, although not identical, results are being reported by C. Seidl, C. Saraiya, T. Mattioni, Z. Osterweil, Y. P. Fu, and J. S. Lee (personal communication).

MATERIALS AND METHODS

Laboratory-Generated Mutants. BLCL 9.22.3, generated by *in vitro* mutagenesis of BLCL T5.1, has a deletion involving all the DR and DQ genes of one chromosome and the DRα gene of the second. Thus, the surface phenotype is DR^-, DP^+, DQ^+ (11). RJ2.2.5, RM2, and RM3 are derived from the human Burkitt lymphoma cell line Raji, (12, 13) and 6.1.6 is from the normal BLCL T5.1 (14); these four BLCLs, selected after *in vitro* mutagenesis for the loss of surface DR antigens, were also negative for DP and DQ expression. Clone 13 is a mutant BLCL [a subclone of P3HR1 whose parent was the Burkitt lymphoma cell line Jijoye (15, 16)], which expresses DQ selectively, but not DP or DR (17). Cell-fusion experiments have shown that these five cell lines are deficient for transactivating factors that regulate class II gene expression (17–22).

Patient Cell Lines. The Epstein–Barr virus-transformed BLCLs derived from class II-deficient patients have been described elsewhere: TF (23), BCH (24), Ramia, Nacera, and Bequit (6), BLS1 and BLS2 (25), and SJO (26). All are deficient for the surface expression of all three isotypes of class II antigens. The cell lines were maintained in RPMI 1640 medium supplemented with 10% (vol/vol) heat-inactivated fetal calf serum, 2 mM glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml). γ-Interferon was kindly given by Biogen.

Transient heterokaryons were prepared from these cell lines essentially as described (10, 27).

RESULTS AND DISCUSSION

To determine whether the patient cell lines belong to different complementation groups, heterokaryons were prepared; 48 hr after the fusions, the cells were stained with anti-DR monoclonal antibody LB3.1 (28). The nuclei of the heterokaryons were unfused when the cells were stained. Therefore, if DR expression was rescued, transactivating factors must have been involved. Eight patient cell lines were tested: TF, BCH, Nacera, Ramia, Bequit, BLS1, BLS2, and SJO. All were defective for the transcription of all three isotypes of class II antigens DR, DQ, and DP. All possible combinations of fusion experiments between the cell lines were performed (except Ramia × Bequit), and are summarized in Table 1. Only the BCH, TF, and BLS2 cell lines initially contained no detectable class II-expressing cells. Nacera, SJO, and BLS1 contained less than 1% and Ramia and Bequit contained, respectively, 6% and 9% of cells expressing (usually weakly) class II antigens. Thus, for these last two cell lines, complementation on fusion was difficult to be certain of against the high background. The fusion experiments allowed definition of three complementation groups A, B, and C (Table 2). Reciprocal complementation (9) (i.e., expression of the class II genes of both cell lines) has not been demonstrated but is likely since fusion of each mutant cell line with 9.22.3, a DRα gene-deleted BLCL, allowed the expression of DR antigens. Fig. 1 shows three examples of fusion experiments between groups A and B (BCH–Nacera), B and C (Nacera–TF), and A and C (BCH–TF), on which the data in Table 1 are based. The control homokaryons TF–TF, BCH–BCH, and Nacera–Nacera did not express DR (photos not shown, but see ref. 9), but the heterokaryons expressed high levels of DR antigen. A Northern blot analysis, for each complementation group, confirmed that the transcription of DRα genes was restored by fusion experiments (Fig. 2). TF and SJO, the only two group C members, exhibited a very low

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Abbreviation: BLCL, B-lymphoblastoid cell line.

Table 1. Complementation experiments

	Complementation analysis													
	9.22.3	Nacera	Ramia	Bequit	BLS1	SJO	TF	BLS2	BCH	RJ2.2.5	RM2	RM3	Cl. 13	6.1.6
9.22.3	−													
Nacera	+	−												
Ramia	(+)	(−)	(−)											
Bequit	(+)	(−)	ND	(−)										
BLS1	+	−	(−)	(−)	−									
SJO	+	+	(+)	(+)	+	−								
TF	+	+	(+)	(+)	+	−	−							
BLS2	+	+	(+)	(+)	+	+	+	−						
BCH	+	+	(+)	(+)	+	+	+	−	−					
RJ2.2.5	+	+	(+)	(+)	+	+	+	−	−	−				
RM2	+	+	(+)	(+)	+	+	+	−	−	−	−			
RM3	+	+	(+)	(+)	+	+	+	−	−	−	−	−		
Cl. 13	+	+	(+)	(+)	+	+	+	−	−	−	−	−	−	
6.1.6	(+)	ND	ND	ND	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(−)

+, HLA-DR-positive heterokaryons; −, HLA-DR-negative heterokaryons or homokaryons; ND, not done. Data in parentheses were obtained for cell lines with high background, which are, therefore, less certain.

growth rate compared to all the other cell lines used in this study. Thus, the factor missing in group C may also be involved in growth regulation in addition to its class II major histocompatibility complex gene regulatory function.

The analysis was extended to the laboratory mutant BLCLs RJ2.2.5, RM2, RM3, and 6.1.6, which are also defective for all class II antigens (Table 1). As shown (9), TF (group C) can complement RJ2.2.5 (group A). RM2 and RM3 do not complement each other (22) and are also in group A. Although TF and 6.1.6 were reported to belong to the same group (9), these cell lines complemented each other in the present experiments. Interpretation of the results was difficult because of the high percentage (7%) of 6.1.6 revertants that expressed class II antigens weakly before fusion. However, the fusion experiments were performed several times and gave reproducible results, when read blindly by three laboratory members. Moreover, SJO, which fell into the same complementation group as TF, also appeared to complement 6.1.6. Since 6.1.6 can complement members of each group, these data place this cell line in a fourth complementation group. Northern blot analyses of DRα expression in heterokaryons using 6.1.6 were not conclusive (data not shown) because of high background due to a residual class II mRNA transcription in this cell line (Fig. 2). To demonstrate further that 6.1.6 represents a fourth complementation group, more quantitative experiments such as complementation using *in vitro* transcription analysis might be possible. The complementation groups are summarized in Table 2.

Finally, clone 13 is a special case of a line that does not transcribe DR and DP but does express DQ. Interestingly, clone 13 belongs to group A, although it possesses a phenotype different from the other members. Given that all the members of this group must possess a mutation in the same gene, the product(s) of this gene cannot be specific for DQ expression only (assuming that only one gene encoding a transactivating factor is altered in each cell line). Different mutations of this gene must result in at least two phenotypes: DR^-, DQ^-, DP^- or DR^-, DQ^+, DP^-. One possible explanation is that a single factor normally activates or binds to at least two proteins (or DNA regions), each specific for DR/DP or DQ gene transcription (Fig. 3*A*). Such a factor would thus possess at least two recognizing or activating regions; the protein would be nonfunctional in the DQ-negative cell lines, but only the DR/DP-specific region would be inactive in clone 13. Another possibility is that a single gene could generate different mRNAs by way of differential splicing and thus different transcription factors (Fig. 3*B*). The product of these mRNAs would be able to activate either DQ or DR and DP genes. A common region would be involved in activation, but each factor would possess its own specificity domain. Mutation of one or the other of these domains would alter the ability of the protein to bind the DR/DP or DQ promoters and lead to differential expression of these antigens, thus result-

Table 2. Summary of the four complementation groups and their phenotypes

	Source			Expression		
Group	Patient cells	Laboratory mutant cells	Phenotype	Class I	Class II	Growth
A	BCH BLS2	Clone 13 RJ2.2.5 RM2 RM3	Type II	++++	−	++++
B	BLS1 Nacera (Ramia) (Bequit)		Type III	±	±	++ or +++
C	TF SJO		Type III	±	−	+
D		(6.1.6)	?	++++*	±	+++

Data in parentheses were obtained for cell lines with a high background and are, therefore, less certain. For the patient cells, class I expression refers to the surface expression before Epstein–Barr virus transformation.

*After Epstein–Barr virus transformation.

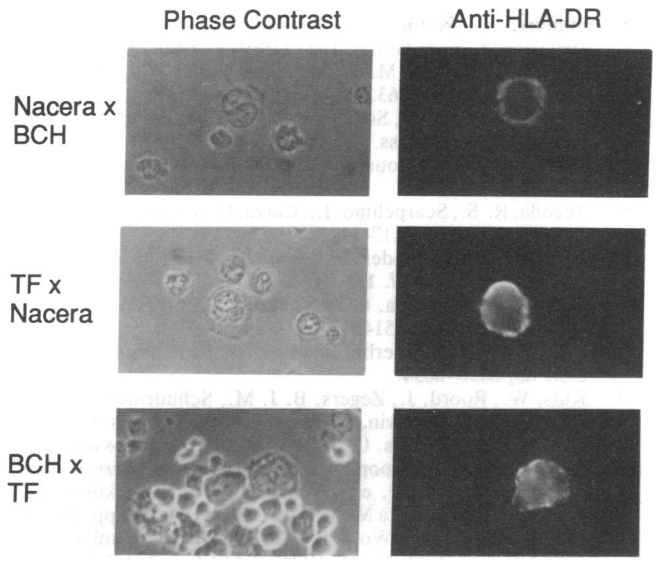

FIG. 1. DR expression at the surface of heterokaryons obtained with groups A–B (BCH–Nacera), B–C (Nacera–TF), and A–C (BCH–TF). Cells were washed with phosphate-buffered saline (PBS) 48 hr after fusion and incubated for 30 min with anti-DR monoclonal antibody LB3.1 in PBS containing 2% heat-inactivated fetal calf serum and 0.02% sodium azide. The washed cells were further stained with fluorescein isothiocyanate-conjugated goat anti-mouse antibody. Phase-contrast and fluorescence micrographs are shown. (×400.)

ing in different phenotypes within a single complementation group.

Thus, all the available class II-defective BLCLs (i.e., *in vitro*-derived mutant and patient cell lines) fall into at least three, and probably four, complementation groups. Furthermore, a patient whose B cells were defective for class II and CD21 antigens expression has also been reported (29, 30). Unfortunately, immortalization of the patient B cells with Epstein–Barr virus was impossible, since CD21 is the receptor for this virus. It might have represented a fifth complementation group. Although it is usually assumed that interferon-γ cannot correct the defect of class II expression in patients or their cell lines (7), class II genes were reported to be inducible in this patient's monocytes, but not in B cells (30). A member of each of the four complementation groups identified (BCH, BLS1, TF, and 6.1.6) was tested for the responsiveness to interferon-γ (data not shown); none expressed detectable levels of class II antigens at the surface by fluorescence-activated cell sorter analysis after a 48-hr incubation in complete medium supplemented with interferon-γ (400 units/ml).

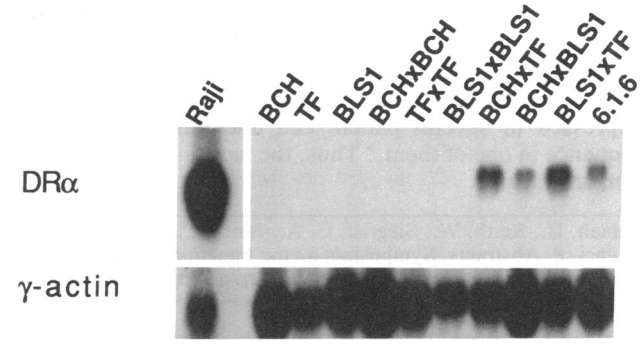

FIG. 2. Northern blot analysis showing *de novo* synthesis of DRα mRNA in hybrids between patient cell lines of all three complementation groups: A–C (BCH–TF), B–C (BLS1–TF), and A–B B (BCH–BLS1). Total mRNA (10 μg) from each sample was fractionated on a 1% agarose/formaldehyde gel and transferred to a GeneScreen*Plus* membrane in 10× standard saline/citrate. A 1-kilobase fragment of DRα gene and a 1-kilobase fragment of the γ-actin gene were used as probes. They were uniformly labeled by random priming, and hybridization and washings were performed as recommended (Promega). X-ray film was exposed to the membrane for 60 hr, except for a 15-hr exposure for the Raji DRα signal.

Some patients were reported (31) to be defective for class II antigens only (type II bare lymphocyte syndrome), whereas others were also defective for class I antigens *in vivo* (type III) (and a third group, type I, was defective for only class I). *In vitro* study of class I deficiency is difficult, since the patient class I-negative cells became class I-positive after transformation by Epstein-Barr virus. Correspondingly, the two patients whose cell lines belonged to complementation group A (BCH and BLS2) expressed normal levels of class I antigens, whereas the members of groups B and C were all defective (refs. 5, 6, 10, 26; M. Eibl, personal communication) (Table 2). Thus, at least two defects can account for the type III syndrome and appear to be distinguished by the growth rate of the cells available, but so far only one leads to type II. Although the class II antigen deficiency has been assumed to be autosomal, this point has to be reconsidered for each complementation group, since different genetic defects appear to be responsible for this syndrome.

Several specific DNA binding factors that bind to various class II gene promoters have now been cloned (32–38). It will be of interest to study their presence in the mutant cell lines and to perform transfection experiments with these and other genes for class II transcription factors that might rescue transcription of some of the class II antigens. One of these cloned DNA binding protein, RFX, binds to the X box region of the DRα promoter. By gel-retardation assays RFX did not bind to its target DNA sequence in three BLS cell lines, Ramia, Nacera, and Robert (37). It is not known whether this

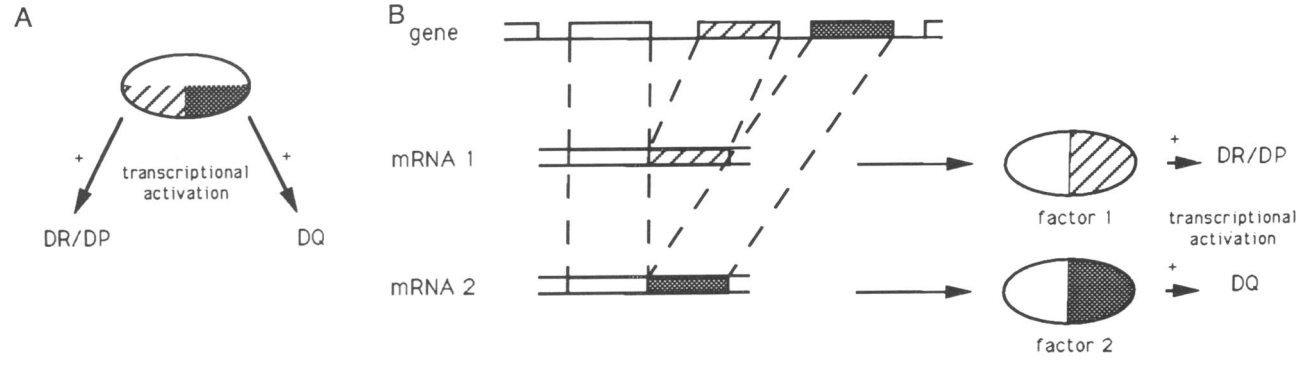

FIG. 3. Hypotheses for the defective gene of group A. (*A*) One-factor hypothesis. (*B*) Two (or more)-factor hypothesis. The cross-hatched and hatched domains are specific for DQ and DR/DP transcriptional activation, respectively.

loss of binding is a feature common to all patient cell lines, since Ramia and Nacera belong to the same complementation group. Further studies with members of the other groups should clarify this point. In addition, RFX is normally expressed in several patient cell lines and has a normal sequence in one of them.* Thus, the nature of the defect still remains elusive.

*Mach, B., Reith, W., Siegrist, C. A., Berte, C., Herrero, C., and Silaci, P. Abstracts, Symposium on the Functional Role of Antigen Presenting Cells, Baden bei Wien, Austria, July 8–11, 1990.

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