ALPHA AND BETA CHAINS OF SB AND DR ANTIGENS ARE STRUCTURALLY DISTINCT

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The human major histocompatibility region, *HLA-D*, controls the expression of a number of polymorphic antigens exhibiting limited tissue distribution. Two sets of antigens, SB and DR, controlled by separate loci within the *HLA-D* region (1–3), exhibit similar functions relating to antigen presentation and T cell regulation (4–7). Recent studies have demonstrated that a monoclonal antibody, I-LR1, is reactive with some of the allelic products of the *SB* locus (8). Using the I-LR1 antibody to isolate SB antigens, it has been shown that the two chains resemble the alpha and beta chains of the DR antigens in molecular weight as measured by sodium dodecyl sulfate (SDS) gel electrophoresis (9). These and other parallels between SB and DR antigens suggest a structural relationship between the two sets of antigens. This study describing a portion of the amino-terminal amino acid sequences of these molecules delineates that relationship and suggests that the alpha and beta chains of the SB antigens are structurally distinct from the DR alpha and beta chains and that SB antigens, like DR antigens, are homologues of the murine I-E antigens.

Materials and Methods

Cells. The human Epstein-Barr virus-transformed lymphoblastoid B cell line, PRIESS (DR4/4) (10), was obtained from J. Kaufman, Harvard University, Cambridge, MA. The SB phenotype of this line (SB3/4) was established by primed lymphocyte typing using the same cellular reagents and general techniques previously described (11); however, like other lymphoblastoid cell lines used as stimulator cells, PRIESS was inactivated by treatment with mitomycin C and was used at a lower stimulator cell concentration (2).

Monoclonal Antibodies. The monoclonal antibodies I-LR1 and IIIE3 have been described (8, 9, 12). Their ability to bind to human cells was examined using the method of Williams (13).

Previous studies (8) of I-LR1 (by population association and by studies of antibody inhibition) indicated that I-LR1 binds to SB3 but not to SB4 allelic products. The inference that I-LR1 would bind to SB3 but not SB4 on PRIESS was confirmed by experiments demonstrating that the response of SB3-primed cells to PRIESS was inhibited (>50%) by the addition of I-LR1 antibody, but the response of SB4-primed cells to PRIESS was not inhibited (<10%) (8). I-LR1 was unable to block cellular binding of an anti-DR antibody, IIIE3, to PRIESS (Gabriel Nunez, personal communication).

IIIE3 was not able to inhibit SB-specific proliferation using techniques previously described (8). Prior studies indicate that IIIE3 appears to recognize a subset of DR antigens (12).

Isolation of Radiolabeled Antigens. Cells were radiolabeled in culture with [³H]amino acids, lysed with Nonidet P-40, and affinity purified on a lentil lectin column to isolate a glycoprotein

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pool as previously described (12).

Antibody Affinity Columns.   Immunoglobulin was partially purified from ascites by ammonium sulfate precipitation at 50% saturation. After dialysis, the preparations were coupled to Sepharose 4B or Sepharose CL-4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) at a ratio of 2 mg protein/ml resin (14). Glycoprotein pools isolated from 1–2 × 10^8 cells were passed over 6 ml antibody affinity columns and the adherent antigens eluted using 0.05 M diethylamine (15). In some preparations, the I-LR1 eluate was dialyzed and repassed over an I-LR1 column. The I-LR1-reactive antigens were usually removed by affinity chromatography before chromatography on the IIIE3 column. The antigens were isolated from the adherent pools by precipitation with acetone.

SDS Gel Electrophoresis.   IIIE3-reactive antigens were electrophoresed under reducing conditions on 13.5% SDS gels (16). I-LR1-reactive molecules were electrophoresed under reducing conditions on 12.5% gels containing twice the normal concentration of N,N'-methylene-bis-acrylamide (0.66% bis-acrylamide). Tube gels were fractionated into a 0.05% SDS solution, and aliquots were counted to determine the positions of the alpha and beta polypeptides. Preparative isolations of alpha and beta chains from these gels have been previously described (12).

Amino Acid Sequence Determination.   Isolated alpha and beta chains labeled with a single [³H]amino acid were sequenced on a Beckman 890C sequencer (Beckman Instruments, Fullerton, CA) as previously described (12). Butyl chloride fractions were evaporated under N_2, scintillation fluid was added, and the radioactivity in each fraction was determined using a Beckman LS8100 liquid scintillation counter.

Results

I-LR1 and IIIE3 Recognize DR-like Molecules.   A structural comparison of the DR and SB molecules was undertaken on the lymphoblastoid B cell line PRIESS. This cell line is phenotypically homozygous for DR4 (10) and, although heterozygous for SB3/4, expresses only one allelic product (SB3) to which the SB-selective monoclonal antibody, I-LR1, is known to bind. Direct binding assays confirm that the cell line binds both the I-LR1 antibody and a DR-selective monoclonal antibody, IIIE3 (12).

Antibody affinity columns containing either I-LR1 or IIIE3 were used to isolate the reactive antigens from a PRIESS glycoprotein pool. Both antibodies bound a bimolecular complex that migrated under reducing conditions in the molecular weight range of the DR antigens on an SDS polyacrylamide gel, confirming earlier studies by Nadler et al. (9). The alpha and beta chains of the I-LR1-reactive antigens were difficult to separate on a 13.5% polyacrylamide gel, and a change in the acrylamide concentration (12.5% acrylamide with twice the concentration of bis-acrylamide) was necessary to cleanly separate the alpha and beta polypeptides.

The Amino Acid Sequences of SB Alpha and Beta Chains Differ from DR Alpha and Beta Chains.   PRIESS was radiolabeled with [³H]phenylalanine and [³H]tyrosine independently. Alpha and beta chains were isolated from the I-LR1- and IIIE3-reactive preparations, and their amino-terminal amino acid sequences were determined. The results are summarized in Table I. The phenylalanine-labeled alpha chains from both I-LR1 and IIIE3 preparations are identical with phenylalanine observed at positions 12, 22, 24, and 26 in both chains. The phenylalanine-labeled SB and DR beta chains, on the other hand, differ. Phenylalanine is observed at positions 18 and 24 in the SB beta chain and at 7, 17, 18, and 26 in the DR beta chain. A variable amount (0–60%) of DR-like sequence is observed in the SB beta chain preparations depending on the method of isolation. If the I-LR1-reactive material is repassed over the I-LR1 column in the presence of a protein carrier, the DR contamination is decreased. These experiments suggest that weak cross-reactivity may exist between SB and DR antigens.

When tyrosine-labeled I-LR1 and IIIE3-reactive alpha chains are compared, a

TABLE I

Amino Acid Sequence Comparison of SB and DR Antigens

				Alpha chains			
	Position:	9	12	13	22	24	26
DR		—*	F	Y	F	F	F
SB		Y	F	—	F	F	F

						Beta chains				
	Position:	7	9	16	17	18	24	26	28	30	32
DR		F	—	—	F	F	—	(F)‡	—	Y	Y
SB		Y	Y	Y	—	F	F	—	Y	Y	—

* Indicates the absence of the assigned amino acid at that position.
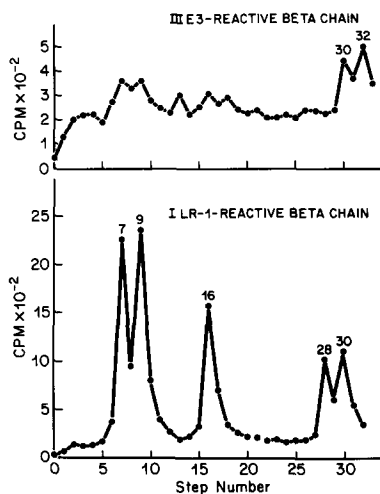‡ Tentative assignment.

FIG. 1. Linear plots of the results of two amino acids sequences of [³H]tyrosine-labeled beta chains from PRIESS. Above, the sequence of the DR beta chain isolated using the antibody IIIE3; below, the sequence of the SB beta chain isolated using the antibody I-LR1.

variation is found in the alpha chain sequences. The SB alpha chain contains a tyrosine at position 9, whereas the DR alpha chain exhibits a tyrosine at position 13. Again, depending on the method of isolation, the SB alpha chain preparation contains 0–60% DR alpha chain contamination. The tyrosine-labeled beta chain is shown in Fig. 1. Tyrosines are observed at positions 7, 9, 16, 28, and 30 in the SB beta chain, whereas the DR beta chain exhibits tyrosines at positions 30 and 32. The slight variations in the IIIE3-derived beta chain baseline are insignificant (i.e., based on the repetitive yield of the sequencer, if a tyrosine were present at position 7, for example, a radioactive peak of ~1,800 cpm would have been observed). These sequencing experiments demonstrate that both SB alpha and beta chains are structurally distinct from IIIE3-reactive DR alpha and beta chains.

Discussion

Both IIIE3 and I-LR1 monoclonal antibodies react with DR-like molecules from the human cell line PRIESS. IIIE3 appears to react with DR antigens and, by functional studies, is nonreactive with products of the *SB* locus. By functional and

cellular binding studies, I-LR1, on the other hand, appears to react only with the product of the PRIESS SB3 allele.

The amino acid sequence data indicate that both alpha and beta chains of SB antigens differ from DR antigens. The alpha chains share four of the six positions examined and, in this limited analysis, appear homologous to each other. Both chains have phenylalanine at positions characteristic of murine I-E alpha chains (but lacking in murine I-A alpha chains) (17); thus, both subsets appear to be I-E homologues. The DR and SB beta chains are very different from one another as might be expected if the beta chains exhibit the majority of the observed polymorphism of these molecules (18, 19). Because of this polymorphism, it is difficult to categorize clearly beta chains as being I-A- or I-E-like molecules. Both subsets have structural similarity to human HLA-DR chains described previously by a number of laboratories.

Whereas the available data are consistent with the interpretation that the molecules isolated using I-LR1 and IIIE3 are SB3 and DR4, respectively, there are alternative possibilities. It is theoretically possible that the two subsets of molecules are allelic products of the same locus; however, there are two lines of evidence that suggest that they are products of distinct loci. First, the cell line used is phenotypically homozygous for DR4/4, although it is possible that the two alleles of DR4 are not identical. Alternatively, because the cell line is heterozygous for SB, both monoclonal antibodies could recognize allelic products of the SB locus. It would be surprising, however, to find that the structural differences between the alleles would be so dramatic. Second, characterization of the monoclonal antibodies indicates that the subsets are products of distinct loci. I-LR1 is known to bind to products of the SB locus but has not been observed to bind to molecules bearing DR4 determinants. Most relevant in this discussion is the finding that I-LR1 binds to the SB3 allelic product of the cell line PRIESS, as indicated by its ability to specifically block proliferation of SB3- (but not SB4-) specific responses to PRIESS. In contrast, the monoclonal IIIE3 preferentially binds gene products associated with DR antigens which are lost in a DR null mutant (S. Shaw and R. DeMars, personal communication). In addition, IIIE3 and I-LR1 also appear to recognize structurally distinct molecules from an HLA hemizygous cell line (C. Hurley and R. DeMars, personal communication).

Although these lines of evidence suggest that the SB and DR subsets are products of distinct loci, only the second, the characterization of the monoclonal antibodies, indicates that the I-LR1- and IIIE3-reactive pools correspond to SB and DR. At present this is the most likely hypothesis. In particular, it is certain both subsets are distinct from the DC/MT/DS product on the basis of sequence (20). However, it is possible that, in particular, the I-LR1-reactive subset represents a fourth family of molecules (distinct from DR, SB, and MT/DC/DS). Because I-LR1 appears to recognize epitopes on at least two DR-like molecules (SB2, SB3, and DR5) (8), it is possible that it can occasionally recognize products of yet another locus. The present data provide no evidence for this hypothesis, but it cannot be formally excluded.

Although the SB antigen appears to be I-E-like based on its alpha chain sequence, the large extent of sequence divergence between the SB and DR beta chains is surprising. Using amino-terminal amino acid sequence analysis, the presence of structurally distinct I-E-like DR beta chains has been noted (12, 21, 22); however, individual I-E-like beta chains isolated using monoclonal antibodies have appeared to be structurally very similar (12). This suggests that either the loci encoding the SB and DR beta chains may have diverged earlier in evolution than the loci encoding the multiple DR beta chains, or the SB beta chain represents an entirely new

molecular species. Further comparisons between the SB and DR beta chains will answer this question.

These data provide the first evidence for two structurally distinct nonallelic I-E-like alpha chains in the human. Multiple D region alpha chains have been previously proposed (23–26); however, the two alpha chains identified in those studies may represent the I-A and I-E alpha chain homologues (20) rather than multiple I-E-like molecules. The presence of two I-E-like alpha chains is interesting, as published studies using DR alpha chain DNA probes suggest that there is a single I-E-like alpha chain locus (27, 28). The presence of structurally distinct but related SB and DR alpha chains suggests that either the SB and DR alpha chain genes are different enough not to cross-hybridize or they share identical restriction sites and appear as a single restriction fragment, as has been suggested for some of the members of the murine H2 gene family (29). The SB and DR alpha chains could, therefore, be very different as are the SB and DR beta chains or, alternatively, the chains are so similar in structure that even the nontranslated regions of DNA surrounding the two loci share extensive homology. A closer examination of the SB and DR gene products is currently underway to determine the extent of variability present in these two sets of *HLA-D* region antigens.

Summary

Limited amino-terminal amino acid sequences of the HLA-controlled SB and DR antigens from the cell line PREISS (DR4/4, SB3/4) show differences in both the alpha and beta chain sequences of the two molecules. SB antigens, like DR antigens, appear to be homologues of the murine I-E antigens.

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