Hybrid Ia Antigens: Genetic, Serologic, and Biochemical Analyses

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Ia specificities 22 and 23 were found to be determinants on hybrid Ia molecules, formed by the noncovalent binding of a 26,000–28,000 dalton beta polypeptide chain (A_e) coded by the *I-A* subregion and a 32,000–35,000 dalton alpha chain (E_α) coded by the *I-E* subregion. For expression of Ia.23 the A_e chain, coded by the *I-A* subregion, must be derived from the $H\text{-}2^d$ haplotype, while A^b, A^s, or A^k can provide the complementing beta chain for the expression of Ia.22. For expression of Ia.22 and Ia.23, most Ia.7 positive strains can provide the complementing alpha chain (E_α), with the one exception of B10.PL (E^u), which is Ia.7 positive but will not complement with A^d to express Ia.23. Antisera were also produced against hybrid Ia antigens by immunizing with F_1 cells expressing Ia.22 or Ia.23 generated by trans-complementation. These antisera detect the same specificities as conventional anti-Ia.22 and anti-Ia.23 sera produced against cis-complementing Ia antigens. It is postulated that hybrid Ia determinants are involved in recognition and generation of immune response to antigens under dual Ir gene control. It is also suggested that there are 2 types of Ia specificities: 1) allotypic Ia specificities expressed on the alpha or beta chains (for example, Ia.7 on the E_α chain) and 2) hybrid Ia specificities, which are unique interaction determinants formed by the association of alpha and beta chains (for example, Ia.22 and Ia.23). These interaction gene products may be involved in antigen recognition and presentation.

Ia antigens coded by the *I-A* and *I-E* subregions of the *H-2* gene complex consist of 2 noncovalently associated polypeptides of 32,000–35,000 (alpha) and 26,000–28,000 (beta) daltons. Two-dimensional gel electrophoresis studies of Jones et al [1, 2] and tryptic peptide studies by Cook et al [3, 4] and Silver [5] have suggested that the *I-E* subregion molecule is formed by complementation of genes in the *I-A* subregion and *I-E* subregion, with the *I-A* subregion coding for the beta chain (A_e) and the *I-E* subregion

the alpha chain (E_α). The immune response to some antigens also results from comple-
mentation of genes in the I region. Genes in the I-A and I-E subregion complement in the
in vitro murine T-lymphocyte proliferative response to GL-Phe, poly(Glu^{55} Lys^{36} Phe^9);
GLT^5, poly(Glu^{57}Lys^{38}Tyr^5); and pigeon cytochrone c [6–8]. Fatham et al [9–11]
have also shown that unique hybrid Ia determinants function as stimulating determinants
in the mixed lymphocyte reaction.

Ia.7 was the first Ia specificity mapped to the I-E (old I-C) subregion [12]. Haplo-
types k, d, j, p, r, u, v, and $w3$ are Ia.7 positive and express an I-E subregion product.
Haplotypes b, f, q, and s are Ia.7 negative, and these haplotypes, alloimmunizations
have failed to detect an I-E subregion. Private specificities were identified in the I-E sub-
region of haplotypes H-2^p (Ia.21), H-2^k (Ia.22), and H-2^d (Ia.23) [13–15]. Recently, we
showed that Ia.22 and Ia.23 are expressed on hybrid Ia molecules generated by comple-
mentation of genes in the I-A and I-E subregions [16]. Ia.22 can be generated by comple-
mentation of A^k, A^b, or A^s with E^k, E^d, or E^p and Ia.23 can be generated by comple-
mentation of A^d with E^d or E^k. In this paper, we extended these studies by examining
additional F_1 animals to determine the permissive I-E subregion alleles that can comple-
ment with A^k, A^b, and A^s to express Ia.22 and with A^d to express Ia.23. The results
show that A^k, A^b, and A^s can complement with most Ia.7 positive haplotypes to express
the Ia.22, and A^d can complement similarly Ia.23. The one exception was complementa-
tion of D2.GD with B10.PL (A^d with E^u). This exception is of interest since B10.PL is
the only Ia.7 positive haplotype that does not complement with A^d in the antigen presenta-
tion of GL-Phe (R.H. Schwartz, personal communication). Our studies suggest that hybrid
Ia molecules may be involved in antigen recognition and presentation.

MATERIALS AND METHODS

Animals

All mice used in this study were produced in our immunogenetics mouse colony.
H-2 haplotypes of strains used in this study are listed in Table I.

Antisera

To detect Ia.22, the antiserum (C3H.Q × B10.D2)F_1 anti-B10.AQR was used. This
antiserum also contains antibodies against I-A^k. This antiserum was made specific for
Ia.22 by absorption with B10.A(4R) cells (I-A^k). To detect Ia.23, the antiserum (B10.LG
× C3H/HeJ)F_1 anti-C3H.Ol was used. Although this antiserum combination has incom-
patibility at the I-A^d subregion, anti-I-A^d antibodies were not produced, making this anti-
serum specific for Ia.23. The following antisera were also used: for anti-Ia.22: (B10 ×
HTI) F_1 anti-B10.A(5R), (anti-Ia.7,22), and [B10.A(4R) × B6] F_1 anti-B10.A(2R) (anti-
Ia.7,22), absorbed with B10.D2 (Ia.7,23) cells. For anti-Ia.23: (D2.GD × B10)F_1 anti-
B10.BDR1 (anti-Ia.7,23) absorbed with B10.K cells (Ia.7) and (B10 × A)F_1 anti-B10.D2
(anti-Ia.11,23) absorbed with D2.GD (Ia.11). Parent anti-F_1 sera was produced by
immunizing parent strain mice with donor F_1 lipopolysaccharide (LPS)-stimulated spleen
blasts prepared as previously described [17]. After several weekly immunizations (5–8
times) mice were bled individually and the alloantisera tested against donor F_1 cells by

TABLE I. Composition of Recombinant Strains Used in This Study

Strain	Haplotype	K	A	B	J	E	C	S	D	TL
B10.AQR	y1	q	k	k	k	k	d	d	d	a
B10.A(5R)	i5	b	b	b	k	k	d	d	d	a
HTI	i1	b	b	b	b	b	b	b	d	a
B10.A(4R)	h4	k	k	b	b	b	b	b	b	b
B10.LG	arl	d	f	f	f	f	f	f	?	?
C3H.OL	Ol	d	d	d	d	d	d	k	k	b
D2.GD	g2	d	d	b	b	b	b	b	b	b
B10.BDR-1	g4	d	d	d	d	d	d	d	b	b
A	a	k	k	k	k	k	d	d	d	a
A.TFR5	ap5	f	f	?	?	k	k	k	d	a
B10.A(18R)	i18	b	b	b	b	b	b	b	d	a
B10.A(3R)	i3	b	b	b	b	k	d	d	d	a
B10.S(9R)	t4	s	s	?	k	k	d	d	d	a
B10.A(2R)	h2	k	k	k	k	k	d	d	b	b
B10.S(8R)	as1	k	k	?	?	s	s	s	s	b

the microcytotoxicity test. High titer antisera was pooled and absorbed with cells from the incompatible parent such that only anti-hybrid antigen activity remained.

Cytotoxic Tests

Serological tests were done by the microcytotoxic dye exclusion assay [18] with 72-hour cultures of LPS-stimulated spleen blasts as targets. In vitro absorptions were performed by incubating 200 μl of 1:10 dilution of antiserum in 100 million spleen cells for 1 h.

Immunoprecipitations and SDS Gel Electrophoresis

F_1 and parental spleen cells were cultured for 48 h in RPMI 1640 media supplemented with 50 μg/ml LPS, 5% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), 2 mM glutamine, and .05 mM 2-mercaptoethanol. LPS-stimulated spleen cells were radiolabeled in RPMI media without leucine supplemented with 20–30 μCi/ml of ^{3}H-leucine by culturing for 12 h at 37°C in a 5% CO_2 incubator. The cells were solubilized with 0.50% Triton X-100 in Tris-buffered saline, centrifuged, and the supernatants purified by lentil lectin affinity chromatography. The lectin purified lysates were tested with antisera using standard immunoprecipitation and SDS polyacrylamide gel electrophoresis previously described [19].

RESULTS

Complementations for Expression of Ia.23

We have previously shown that Ia.23 can be generated by complementation of A^d with E^k or E^d [16]. Only A^d can provide the permissive beta chain that complements with the alpha chain from the I-E^k subregion, since F_1 crosses between B10.K and all other independent haplotypes were negative for Ia.23 (data not shown). To determine the permissive I-E subregion alleles that can complement with A^d, D2.GD (A^d) was crossed to all the independent haplotypes. F_1 blast cells from 72-hour LPS cultures were tested for expression of Ia.23 by microcytotoxicity assay and immunoprecipitation analysis.

I-E subregion alleles from Ia.7 negative haplotypes *b, f, g,* and *s* do not complement for expression of Ia.23, as the following F_1 animals were negative for Ia.23: (D2.GD × B10)F_1 (A^dE^b); (D2.GD × B10.M)F_1 (A^dE^f); (D2.GD × B10.Q)F_1 (A^dE^q); and (D2.GD × B10.S)F_1 (A^dE^s). The following F_1 crosses gave strong microcytotoxicity reactions: (D2.GD × B10.P)F_1 (A^dE^p); (D2.GD × B10.RIII)F_1 (A^dE^r); (D2.GD × B10.SM)F_1 (A^dE^v), and (D2.GD × C3H.W3)F_1 (A^dE^{w3}) (Table II). These reactions were confirmed by immunoprecipitation analysis (Fig. 1). In all cases, parent strains were negative by the microcytotoxicity assay and by the immunoprecipitation analysis (data not shown). One F_1 cross (D2.GD × B10.PL) (A^dE^u) was negative by the microcytotoxicity assay. This F_1 cross was also negative by immunoprecipitation analysis. A very strong microcytotoxicity reaction was seen in the F_1 cross (D2.GD × A.TFR5)F_1 (A^dE^k). This reaction was also confirmed by immunoprecipitation analysis. All the I-E molecules in this F_1 cross are generated by trans-complementation, since we previously showed that in the parent strain, A.TFR5, A^f does not complement with E^k to generate I-E molecules [20].

Complementations for Expression of Ia.22

In our preliminary study [14], we showed that Ia.22 can be generated by trans- or cis-complementation of A^b, A^s, or A^k with E^k or E^d. To determine the permissive *I-E* subregion alleles that can complement with A^b, A^s, or A^k, B10.S (A^s), B10 (A^b), and B10.A(4R) (A^k) were crossed to Ia.7-positive haplotypes. In all the F_1 crosses that we examined so far for the expression of Ia.22, all Ia.7-positive haplotypes can complement with A^b, A^s, and A^k to yield strong microcytotoxicity reactions (Table III).

To biochemically confirm the expression of Ia.22 in these F_1 animals, sequential immunoprecipitations were done in which the *I-A* subregion molecules were removed with antisera specific for the *I-A* subregion prior to testing with anti-Ia.22 serum. This

TABLE II. Cytotoxic Results of F_1's for Expression of Ia.23

F_1 combination	Derivation of A and E alleles	Anti-Ia.23 titer[a] [B10.LG × C3H] anti-C3H.OL
D2.GD × B10	A^d/E^b	0
D2.GD × B10.M	A^d/E^f	0
D2.GD × B10.Q	A^d/E^q	0
D2.GD × B10.S	A^d/E^s	0
D2.GD × A.TFR5	A^d/E^k	>160
D2.GD × B10.P	A^d/E^p	20
D2.GD × B10.RIII	A^d/E^r	40
D2.GD × B10.PL	A^d/E^u	0
D2.GD × B10.SM	A^d/E^v	20
D2.GD × C3H.W3	A^d/E^{w3}	40
D2.GD × B10.WB	A^d/E^j	80
D2.GD × B10.A(5R)	A^d/E^k	>160
D2.GD × B10.K	A^d/E^k	20
D2.GD × B10.A	A^d/E^k	40

[a]The antisera was negative against parental cells used in this study.

was necessary since unabsorbed anti-Ia.22 serum also contains activity against the *I-A* subregion. Antiserum specific for Ia.22 after absorption with B10.A(4R) was too weak for immunoprecipitation studies. The results of one such sequential immunoprecipitation study [B10.A(4R) X B10.PL] F_1 are shown in Figure 2. By sequential immunoprecipitation analysis, we have been able to confirm all the microcytotoxicity reactions. All parent strains were negative for Ia.22 by sequential immunoprecipitation analysis with 2 surprising exceptions. B10.S was found to be weakly positive for Ia.22, suggesting that Ia.7-negative E^S can complement to some degree with A^S. However, crosses between B10.S and Ia.7-positive strains yield much stronger reactions for Ia.22. B10.RIII previously listed as negative for Ia.22 [15] was found to be positive for Ia.22 by sequential precipitation analysis, suggesting the complementation of A^rE^r can also generate Ia.22. The complementations that are positive for Ia.22 are summarized in Table IV.

Detection of Ia.22 and Ia.23 With Parent Anti-F_1 Sera

Our studies show that Ia.22 and Ia.23 can be generated by both cis-complementation and trans-complementation. These studies were carried out using antisera raised against strains where Ia.22 and Ia.23 were generated by cis-complementation. To demonstrate that antisera can be produced against Ia.22 and Ia.23 generated by trans-complementation, cells from F_1 animals were used for immunization.
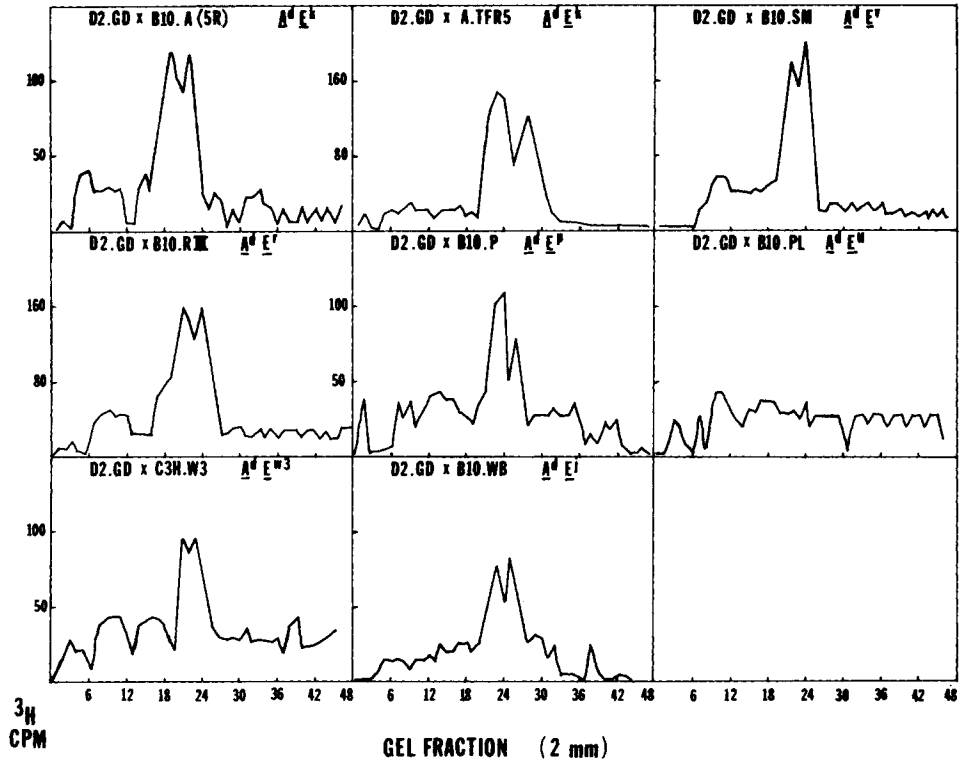

Fig. 1. SDS polyacrylamide gel patterns of molecules precipitated with anti-Ia.23 serum, (B10.LG X C3H)F_1 anti-C3H.OL. In each analysis, the antigen preparation was from radiolabeled LPS-stimulated lymphoctyes.

TABLE III. Cytotoxic Results of F_1's for Expression of Ia.22

F_1 combination	Derivation of A and E alleles	Anti-Ia.22 titer[a] [C3H.Q × B10.D2] anti-B10.AQR [abs with B10.A(4R)]
B10.A(4R) × B10.D2	A^k/E^d	80
B10 × B10.D2	A^b/E^d	80
B10.5 × B10.D2	A^s/E^d	40
B10.A(4R) × B10.P	A^k/E^p	20
B10 × B10.P	A^b/E^p	20
B10.A(4R) × B10.RIII	A^k/E^r	40
B10 × B10.RIII	A^b/E^r	40
B10.S × B10.RIII	A^s/E^r	40
B10.A(4R) × B10.WB	A^k/E^j	160
B10 × B10.WB	A^b/E^j	40
B10.S × B10.WB	A^s/E^j	40
B10.A(4R) × B10.PL	A^k/E^u	40
B10.S × B10.PL	A^s/E^u	40
B10.A(4R) × B10.SM	A^k/E^v	40
B10 × B10.SM	A^b/E^v	80
B10.S × B10.SM	A^s/E^v	40
B10.A(4R) × C3H.W3	A^k/E^{w3}	40
TBR-3 × A.TFR5	A^k/E^k	20
129 × Balb/c^{db}	A^b/E^d	40
B10.S × A.TFR5	A^s/E^k	40
B10.A(4R) × DBA/2	A^k/E^d	160
B10.A(4R) × A.TFR5	A^k/E^k	80
B6 × Balb/c	A^b/E^d	20
B6 × DBA/2	A^b/E^d	40
B10.S × CBA/N	A^s/E^k	20
A.TH × Balb/c	A^s/E^d	20
B10.S(8R) × C3H.OL	A^k/E^d	160
B6 × B10.BDR-2	A^b/E^d	40
129 × Balb/c	A^b/E^d	80
B6(TL+) × B10.BDR-2	A^b/E^d	40
A.BY × A.TFR5	A^b/E^k	80

[a]The absorbed antisera was always tested against parental cells and was negative.

Two combinations were used to raise anti-Ia.22 sera: 1) B10.D2 anti-(B10 × B10.D2)F_1 and 2) A.TFR5 anti-[B10.A(4R) × A.TFR5]F_1. The B10.D2 anti-(B10 × B10.D2)F_1 antiserum was absorbed with B10 spleen cells until no activity remained against B10. However, antibody activity was still present with LPS blast cells of the F_1, but not the 2 parents (Table V). The following F_1's were negative with the absorbed antiserum: (B10 × B10.LG)F_1 (K^bK^d); (B10 × D2.GD)F_1 (A^dA^b); and [B10 × B10.A (18R)] (D^bD^d). This shows that the residual acitivity against (B10 × B10.D2)F_1 was not due to hybrid antigens generated in K, A, or D molecules. Recombinants B10.A(3R) (A^bE^k), B10.S(9R) (A^sE^k), and B10.A(2R) (A^kE^k) were positive, suggesting the

TABLE IV. Summary of Ia.22 Expression

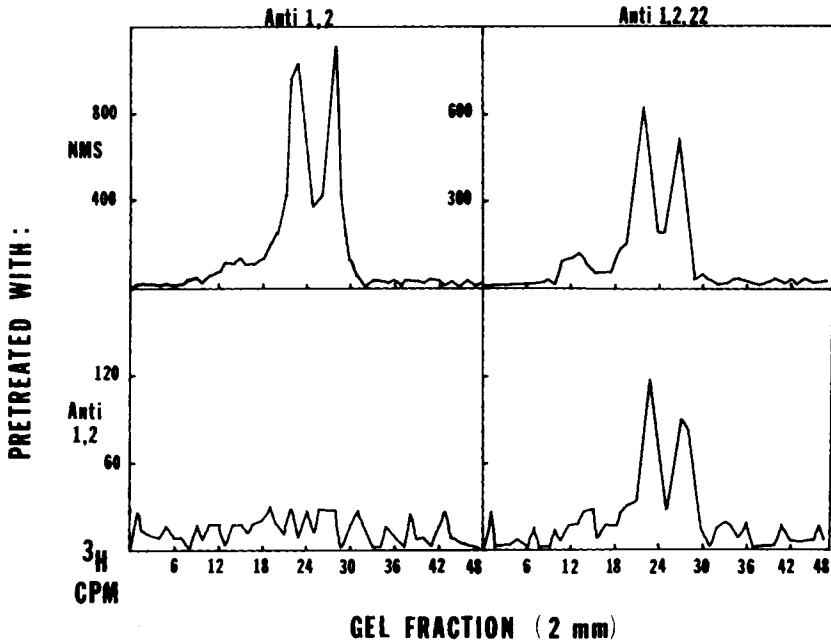| I-A allele	I-E allele											
	b	d	f	j	k	p	q	r	s	u	v	w3
b	–	+	–	+	+	+	–	+	–	NT	+	NT
d	–	–	–	–	–	–	–	–	–	–	–	–
f	–	–	–	–	–	–	–	–	–	–	–	–
j	–	–	–	–	–	–	–	–	–	–	–	–
k	–	+	–	+	+	+	–	+	–	+	+	+
p	–	–	–	–	–	–	–	–	–	–	–	–
q	–	–	–	–	–	–	–	–	–	–	–	–
r	–	+	–	+	+	+	–	+	–	+	+	+
s	–	+	–	+	+	NT	–	+	(+)	+	+	NT
u	–	–	–	–	–	–	–	–	–	–	–	–
v	–	–	–	–	–	–	–	–	–	–	–	–
w3	–	–	–	–	–	–	–	–	–	–	–	–

NT = Not tested.

(B10.A(4R) × B10.PL) F$_1$

Fig. 2. Sequential immunoprecipitation of Ia.22 molecules from [B10.A(4R) × B10.PL]F$_1$ aliquots were pretreated with normal mouse serum or anti-A^{u}A^k serum (Ia.1,2) (A.TH × B10.HTT]F$_1$ anti-A.TL. The immune complexes were removed with protein-A bearing Staphylococcus aureus. The supernatants were then divided and each half subjected to second immunoprecipitations with anti-Ia.1,2 serum or anti-Ia.1,2,22 serum [C3H.Q × B10.D2]F$_1$ anti-B10.AQR.

TABLE V. Detection of Ia.22 and 23 Using Parent Anti-F$_1$ Sera

Antisera combination		Absorbed with	Cytotoxic titer with		
Recipient	F$_1$ donor		Donor F$_1$ cells	B10.D2 (Ia.23)	B10.A (Ia.22)
B10.D2	B10 X B10.D2	B10	40	–	40
A.TFR5	B10.A (4R) X A.TFR5	B10.A(4R)	80	–	80
B10.A(5R)	D2.GD X B10.A(5R)	D2.GD	40	40	–
B10.SM	D2.GD X B10.SM	D2.GD	40	40	–
C3H.W3	D2.GD X C3H.W3	D2.GD	40	40	–
B10.PL	D2.GD X B10.PL	D2.GD	0	0	–

antibody activity in the absorbed F$_1$ antiserum has the same strain distribution as previously anti-Ia.22 sera. We have shown previously that in [B10.A(4R) X A.TFR5] F$_1$ all the I-E molecules are generated by trans-complementation and express Ia.7,22 [20]. The A.TFR5 anti-[B10.A(4R) X A.TFR5] F$_1$ serum was absorbed with B10.A(4R) cells. Residual activity was still seen with F$_1$ cells (Table V). LPS blast cells from [B10.A(4R) X A.TFR3] F$_1$ gave no reaction with this antiserum. This indicates that the hybrid antigen activity was not due to K, A, or D regions since A.TFR3 is identical to A.TFR5 except for E, C, and S subregions. The strain distribution of this antiserum was also identical to conventional anti-Ia.22 sera. The microcytotoxicity results of these 2 parent anti-F$_1$ sera were confirmed by immunoprecipitation analysis. The absorbed antisera precipitated strong Ia peaks and there was complete concordance with our serological results (data not shown).

Four combinations were used to raise anti-Ia.23 sera: 1) B10.A(5R) anti-[D2.GD X B10.A(5R)] F$_1$; 2) B10.SM anti-(D2.GD X B10.SM)F$_1$; 3) C3H.W3 anti-(D2.GD X C3H.W3)F$_1$; 4) B10.PL anti-(D2.GD X B10.PL)F$_1$. Each antisera was absorbed exhaustively with D2.GD spleen cells. Three absorbed antisera B10.A(5R) anti[D2.GD X B10.A(5R)] F$_1$, B10.SM anti-(D2.GD X B10.SM)F$_1$, and C3H.W3 anti-(D2.GD X C3H.W3)F$_1$ showed residual antibody activity with donor F$_1$ cells (Table V). All 3 antisera reacted with strains expressing the complete I region from H-2^d haplotype, as well as all the Ia.23 complementing F$_1$ combinations. Immunoprecipitation with these antisera yielded typical Ia peaks with F$_1$ donor but not with either of the parent cells (not shown). Antiserum B10.PL anti-(D2.GD X B10.PL)F$_1$ after absorption with D2.GD had no residual activity against donor cells. This confirms our previous studies, which showed that this F$_1$ (A^{d}E^u) does not generate Ia.23 specificity by trans-complementation.

DISCUSSION

Biochemical studies [1–5] have shown that the I-E molecule is a hybrid molecule formed by complementations of gene(s) in the I-A subregion coding for the beta chain and gene(s) in the I-E subregion coding for the alpha chain. Recently we reported [16] that Ia.22 and Ia.23 are generated by hybrid I-E molecules. In this paper, we extended these studies to include additional F$_1$ animals. Results from these studies show that for the expression of Ia.22: 1) the complementing beta chain must be derived from H-2^b, H-2^s, or H-2^k haplotypes; 2) the complementing alpha chain must come from Ia.7-posi-

tive haplotypes. For the expression of Ia.23 the results show that: 1) the beta chain coded by the *I-A* subregion must be derived from the *H-2^d* haplotype; 2) most Ia.7 strains can provide the complementing alpha chain from the *I-E* subregions, with the exception of B10.PL (*E^u*), which is Ia.7 positive but will not complement with *A^d*.

In F$_1$ animals there are expressed 2 I-E molecules, one molecule being generated by trans-complementation and the other being generated by cis-complementation. In this study, we showed that antisera can be produced against hybrid Ia antigens by immunizing with F$_1$ cells expressing the determinant generated by trans-complementation. These antisera appear to be detecting the same specificities as conventional anti-Ia.22 and anti-Ia.23 sera produced against cis-complementing Ia antigens.

Our studies show that most Ia.7 (*I-E*)-positive haplotypes can complement to express Ia.22 and Ia.23. One exception was haplotype *H-2^u* (B10.PL), which is Ia.7 positive, but fails to complement with *A^d* to express Ia.23. Thus, only one I-E molecule (*A^{u}E^u*) generated by cis-complementation is expressed in (D2.GD × B10.PL)F$_1$. The failure to produce I-E molecules by trans-complementation was confirmed by B10.PL anti-(D2.GD × B10.PL)F$_1$ sera, which failed to produce anti-Ia.23 antibodies. A defect in the alpha chain of B10.PL can be ruled out since it can successfully complement with B10.A(4R) *(A^{k}E^u)* and B10.5 *(A^{s}E^u)* to generate Ia.22. The reason for the failure of successful complementation of *A^d* with *E^u* could be a structural incompatibility between A^d_e and E$^d_\alpha$ chains.

The immune response to several antigens have also been shown to result from complementation of genes in the *I-A* and *I-E* subregions [6–8, 21]. Of particular interest is the immune response to GL-Phe [6,7]. Strain B10.D2, which carries *A^d* and *E^d* alleles, is a high responder to GL-Phe, while D2.GD is a nonresponder even though it carries one of the responder genes. Similarly, B10 (*H-2^b*), also a nonresponder, carries a permissive allele in the *I-A* subregion. F$_1$ crosses between B10 and most Ia.7-positive strains can complement to respond to GL-Phe. Recently R.H. Schwartz (personal communication) has shown that B10.PL cannot complement with B10 in the antigen presentation of GL-Phe. These studies suggest that the immune response to GL-Phe could be initiated by the hybrid Ia determinant generated by a complementation of genes in the *I-A* and *I-E* subregion. Ia.23 could be one such determinant. Although Ia.22-positive strains B10.A (5R) (*A^{b}E^k*) and B10.S(9R) (*A^{s}E^k*) respond to GL-Phe, Ia.22-positive strain B10.A (*A^{k}E^k*) does not. If Ia.22 is involved in the immune response to GL-Phe, we have to conclude that the determinant generated by the complementation of *A^{k}E^k* is slightly different from the *A^{b}E^k* and *A^{s}E^k* products, even though all 3 are Ia.22-positive by serological criteria. Recently, on the basis of determinants recognized by alloreactive T-cell clones (Fathman et al, in preparation) and primed lymphocyte typing (Krco et al, in preparation), it is clear that the structures generated by these 3 complementations are distinct.

Our studies suggest that Ia.7 is probably a determinant on the alpha chain controlled by the *I-E* subregion, while Ia.22 and Ia.23 are either expressed on the beta chain or generated by the association of the alpha chain and beta chain to form the hybrid Ia molecule. We favor the latter possibility. We propose that there are 2 types of Ia specificities: 1) *allotypic specificities,* expressed on alpha and beta chains (for example Ia.7 on I-E alpha chain). These determinants might aid in binding of alpha and beta chains; 2) *hybrid Ia specificities,* unique interaction products formed by the association of the alpha and beta chains. These determinants might function in antigen recognition and antigen presentation (for example, Ia.22 and Ia.23 and the immune response to GL-Phe). Hybrid Ia determinants may also be associated with the I-A molecule. Recent studies by

Silver has shown that 4 hybrid I-A subregion molecules are generated in $I\text{-}A^b/I\text{-}A^k$ F_1 animals ($\beta^a \alpha^a$, $\beta^b \alpha^a$, $\beta^a \alpha^b$, $\beta^b \alpha^b$) [22]. We are currently producing parent anti-F_1 sera to detect such specificities.

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