THE CYTOTOXIC T CELL RESPONSE TO THE MALE-SPECIFIC HISTOCOMPATIBILITY ANTIGEN (H-Y) IS CONTROLLED BY TWO DOMINANT IMMUNE RESPONSE GENES, ONE IN THE MHC, THE OTHER IN THE Tarα-LOCUS

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The response of T-cells to antigen involves the recognition of two elements: the MHC-encoded restricting element (R)[1] and the foreign antigen (X). Therefore, the genetic control of immune responsiveness is expected to involve complementation between genes encoding R (the MHC and β_2-microglobulin [*B2m*] loci) and those encoding the T-cell receptor (the Tarα- and Tarβ-loci [reviewed in references 1 and 2]), because the responsiveness of T-cells to antigen is dependent on allele-specific recognition of MHC-encoded restricting elements ("restrictive recognition of antigen"). Thus far, investigations have dealt with only one of the components involved in immune responsiveness, the R component. Only alleles of the genes encoding restricting elements, MHC (3) or *B2m* (4), have been shown to affect T-cell responsiveness to antigen X. We show here that T-cell responsiveness to antigen is also controlled by alleles of the v_T-genes that encode the T-cell receptor. We have analyzed the cytotoxic T-cell response to the male-specific histocompatibility antigen, H-Y, in a set of nine recombinant inbred (RI) strains of mice made from SJL/J(J) and BALB/cKe(C) progenitors. This set was chosen because the two parental strains SJL/J(J) and BALB/cKe(C) are "nonresponders" to H-Y, whereas the (C × J)F_1, is a "responder." Therefore, the existence of two dominant complementing genes is implied. If one of these complementing genes specifies R, we would expect it to map in the MHC (5–7), since SJL/J and BALB/cKe express the same allele of *B2m* (8). We show that if one of the two complementing loci is the MHC, the second gene, v_α-2, maps to the Tarα-locus that encodes the α-subunit of the T-cell receptor. These results

R. Epstein, G. Sham, and M. Cohn were supported by National Institutes of Health Animal Resource grant P40 RR 01641, training grant CA 09254, and the National Instute of Allergy and Infectious Diseases grant AI 05875; J. Womack was supported by U.S. Department of Agriculture grant 83-CRSR-2-2234; J. Yague and E. Palmer were supported by National Institutes of Health grant AI 18785.

[1] *Abbreviations used in this paper:* β_2m, beta-2 microglobulin; R, MHC-encoded restricting element, Class I (RI) or Class II (RII); X, any antigen; RI, recombinant inbred strain.

permit us to explain directly the effect of the MHC haplotype on the immune response (see Discussion).

The Tarα-locus has been shown to be linked within 3 cm to *Np-2* on chromosome 14 (9). The experiments described here also provide a fine mapping of the Tarα-locus based on an analysis of three chromosome 14 markers, v_α*-1*, v_α*-2*, and *Es-10*. v_α*-1* is a variable region gene in the Tarα-locus, the alleles of which are distinguished by a restriction fragment length polymorphism; v_α*-2* encodes the cytotoxic T-cell responsiveness marker to H-Y; and *Es-10* encodes an esterase found in RBC, the variants of which are distinguished by their electrophoretic mobilities (10). The allelic differences between these three markers in BALB/cKe(C) and SJL/J(J) permit their mapping in this set of RI strains. Two recombinants between these three markers are identified. One occurs between the *Es-10* and v_α*-1*/v_α*-2* loci in the RI strain, (C × J)6, and the other in the RI strain (C × J)4, between v_α*-1* and v_α*-2*. These findings place v_α*-1* between *Es-10* and v_α*-2* (3 ± 4 cM from both markers). The order and orientation of the Tarα-locus relative to other chromosome 14 markers is: *Es-10*—v_α*-1*—v_α*-2*—[c_α—*Np-2*]—centromere.

Materials and Methods

Mice. The BALB/cKe(C) colony was derived from a breeding pair of BALB/cAn/NIH mice obtained from M. Potter in November 1962, and has been maintained at the Salk Institute animal facility by brother-sister mating. SJL/J(J) were obtained from the Jackson Laboratory, Bar Harbor, ME. SJA/20 (congeneic with SJL/J except at the Igh locus which is derived from BALB/c) were from R. Riblet at the Medical Biology Institute, La Jolla, CA, and SJL/P from M. Potter at the National Institutes of Health (maintained by Litton Bionetics, Kensington, MD). (BALB/cKexSJL/J)F_1 and (BALB/cKexSJL/J)RI (Recombinant Inbred strains) were bred at the Salk Institute, San Diego, CA.

Immunization with the Male-specific Antigen H-Y. Female mice were injected in the hind footpads with 20 μl of balanced salt solution containing 5×10^6–2×10^7 splenic lymphocytes from syngeneic males. 3 wk later, the mice received a second injection with 1–2×10^7 syngeneic male splenic lymphocytes intraperitoneally. 1 wk later, the females received a third intraperitoneal boost with 1–2×10^7 syngeneic male splenocytes. After another interval of at least 3 wk, spleens were removed aseptically and cell suspensions were made. The female responders and the irradiated male stimulators (3,000 rad from a cobalt irradiator) were cultured, each at a concentration of 2×10^6 cells/ml in RPMI media supplemented with 12% FCS, cocktail, glutamine, gentamycin, and either 10% rat spleen Con A supernatant or EL4 supernatant. Cells were cultured in a 5% CO_2, 10% O_2, 85% N_2 atmosphere on a rocking platform, then assayed on day 4.

Cytotoxic Assay. Target cells were incubated with 200 μCi of ^{51}Cr in RPMI at 37°C. Targets were washed three times with medium, and resuspended at a concentration of 3×10^5/ml. 100 μl of labeled targets was added to Linbro 96-well microwell plates (Linbro Chemical Co., Hamden, CT) containing 100 μl of an appropriate dilution of effectors. Cells from immunized females were harvested, centrifuged, washed with RPMI, and resuspended so that the initial dilution resulted in an E/T ratio of at least 50:1. Responders were titrated using twofold serial dilutions. Effectors were incubated with labeled targets for 4 h, and assayed for lytic activity. After centrifugation at 600 *g*, an aliquot (100 μl) of supernatant was collected from each well and radioactivity assayed in a Beckman 8000 gamma counter (Beckman Instruments, Fullerton, CA). The specific ^{51}Cr release was calculated using the formula: Specific ^{51}Cr release = {[experimental release (cpm)] − [background release (cpm)]}/{[total counts] − [background release (cpm × 100)]}.

The terms nonresponder and responder are relative; the term nonresponder, in these mapping experiments, means not detectable under our conditions of immunization and assay.

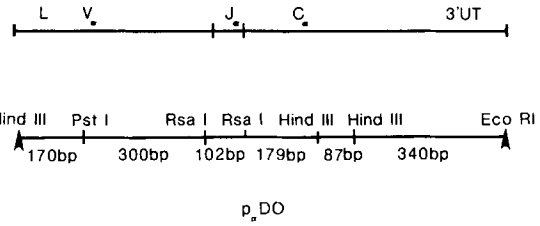

FIGURE 1. Restriction map of *Tarα*-locus and description of p_αDO fragments used as probes. A restriction map of the 1.2-kb cDNA clone p_αDO is shown. The entire clone (Hind III-Eco RI fragment) was hybridized to the blot shown in Fig. 4*a*. The 470-bp Hind III-Rsa I v_α-containing fragment was used in the blots shown in Fig. 4, *b–c*. A c_α-probe (including the 3′ untranslated [UT] region) was made by isolating the 606-bp Rsa I-Eco RI fragment.

Preparation of Plasmids. Allelic differences within the Tar*α*-locus were identified using p_αDO (11) as a probe (Fig. 1). This plasmid contains a cDNA insert derived from the hybridoma DO.11.10 (12), and includes the rearranged v_α, j_α and c_α gene segments. p_αDO was isolated from a cDNA library (kindly provided by C. Coleclough, Roche Institute of Molecular Biology, Nutley, NJ) prepared from DO.11.10 RNA. A 180-bp constant region probe was isolated from this clone by cutting with Hind III and Rsa I. A 475-bp fragment containing the v_α-region was isolated using the same enzymes. The fragments were purified by electroelution onto Whatman DE-81 paper (Whatman Inc, Clifton, NJ). After washing in a microfuge tube with 10 mM Tris (pH 8.0), 1 mM EDTA, and 100 mM LiCl, fragments were eluted from the paper using 10 mM Tris, 1 mM EDTA, 20% EtOH, and 1,000 mM LiCl, and EtOH precipitated (with 2 vol of ETOH). After centrifugation, the supernatants were discarded and the pellets lyophilized and resuspended in TE (10 mM Tris, 1 mM EDTA). 200 ng of each fragment was nick-translated as described by Maniatis et al. (13).

Identification of Tcrα DNA Restriction Site Polymorphisms. High molecular weight mouse liver DNA was prepared by a modification of the method of Blin and Stafford (14) as described by Cory and Adams (procedure B [15]). DNA was digested with the appropriate restriction enzyme for 18 h at 37°C, and electrophoresed in 1% agarose gels (30 V for ~18 h) in Tris borate buffer. Transfer to nitrocellulose membranes was according to the method of Southern (16). Filters were baked for 2 h in a vacuum oven at 80°C, and hybridized to nick-translated probes according to the procedure of Wahl et al. (17). After washing, the filters were autoradiographed on Kodak XAR film (Eastman Kodak Company, Rochester, NY).

Electrophoretic Identification of Es-10 and Hbb Alleles. Murine RBC lysates were prepared by collecting blood from the retro-orbital sinus of the eye into Alsever's solution (115 mM dextrose, 70 mM NaCl, 30 mM Na citrate. RBC were washed in 900 mM NaCl and frozen. RBC lysates were applied to cellulose acetate plates (Titan III; Helena Laboratories, Beaumont, TX) which were presoaked in pH 9.0 EBT buffer (200 mM EDTA, 350 mM borate, 900 mM Tris). Plates were blotted dry before the application of the mouse RBC lysate. Electrophoresis was for 40 min at 220 V with an electrode buffer made by diluting the EBT buffer sixfold. Staining was accomplished using an agar overlay which consists of 20 ml warm 2% agar mixed with an equal volume of a filtered 0.2 M phosphate solution (pH 7.0) containing 30 mg 4-methyl-umbelliferyl acetate (Sigma Chemical Co., St. Louis, MO). Bands were visualized under UV light after 15 min incubation at room temperature.

Results

The Response of the (BALB/cKexSJL/J)F_1 to H-Y Is the Result of Complementation Between Two Gene Loci. The data (summarized in Table I) show the following: (*a*) After immunization of females with syngeneic spleen cells bearing H-Y,

TABLE I
Distribution of Immune-related Loci in (C × J)RI Strains

Strain	Response assigned to allele of:		CHR 17	CHR 14		CHR 6		CHR 12	CHR 16
	H-Y	v_α-2	H-2	v_α-1	*Es-10*	*Tcrb*	*Igκ*	*Igh*	*Igλ*
BALB/cKe(C)	−	c	d (c)	c	c	c	c	c	c
SJL/J(J)	−	j	s (j)	j	j	j	j	j	j
(C × J)F_1	++	c/j	d/s (c/j)	c/j	c/j	c/j	c/j	c/j	c/j
(C × J)1	−	c	d (c)	c	c	c	c	c	c
(C × J)3	−	c	d (c)	c	c	j	c	j	j
(C × 1)4*	++	c	s (j)	j	j	j	j	c	c
(C × J)6‡	+++	c	s (j)	c	j	j	j	c	j
(C × J)8	+	j	d (c)	j	j	j	j	j	c
(C × J)9	−	c	d (c)	c	c	j	j	j	j
(C × J)10	−	c	d (c)	c	c	j	j	j	j
(C × J)11	−	c	d (c)	c	c	c	j	j	j
(C × J)15	−	j	s (j)	j	j	j	c	j	j
SJL/P	++	c	s (j)	c	ND	j	j§	j§	c

c, BALB/cKe allele; j, SJL/J allele; BALB/cKe is *H-2*^{d} (c), whereas SJL/J is *H-2*^{s} (j) haplotype; −, no response; +, response.

* (C × J)4 is a recombinant within the Tarα-locus (Fig. 2).

‡ (C × J)6 is a recombinant between *Es-10* and v_α-*1* in the Tarα-locus.

§ There are no known differences between SJL/J and SJL/P at these loci. The *Igh* locus has not been compared with the same detail in SJL/J and SJL/P as has the *Igκ* locus (C. Huppi, National Institutes of Health, Bethesda, MD, personal communication). BALB/cKe and SJL/J express the same allele encoding β_2-microglobulin, i.e., *B2m*^{a}.

neither BALB/cKe(C) nor SJL/J(J) express a detectable anti-H-Y cytotoxic T-cell response (Fig. 2, *a–d*). (*b*) (C × J)F_1 mice do respond (Fig. 2*d*), and the induced anti-H-Y cytotoxic T-cells are preferentially restricted to the *H-2*^{s} haplotype by a factor that is on average 10-fold higher for *H-2*^{s} than *H-2*^{d}. (*c*) Of the nine (C × J)RI strains analyzed, three are responders ([C × J]4,6,8) and six ([C × J]1,3,9,10,11,15) are nonresponders. (*d*) Of the RI responders, two, (C × J)4 and 6, were of the *H-2*^{s} and one, (C × J)8, was of the *H-2*^{d} haplotype (Fig. 2, *b* and *c*). In all experiments, the *H-2*^{s} RI strains, (C × J)4 and (C × J)6, were 3–10-fold more responsive than the *H-2*^{d} RI strain, (C × J)8. However, the response of (C × J)8 was at least 20–100-fold greater than that of any of the strains designated as nonresponders.

These data suggest that complementation between two or more dominant genes differing between SJL/J and BALB/cKe regulate the response to H-Y. On statistical grounds, the probability of obtaining three responders out of nine RI strains analyzed is 0.16 (likely) if there were a two gene difference, assuming that either combination of alleles will permit a response. We treat the data assuming a two gene difference. To confirm gene complementation, several randomly chosen nonresponder (C × J)RI strains were backcrossed to each of the parents, BALB/cKe and SJL/J. Since a nonresponder (C × J)RI strain would be expected to have both complementing gene loci in either the SJL/J or BALB/cKe configuration, we would expect complementation to occur with only one of the two parents. In the two cases where backcrosses with both parents

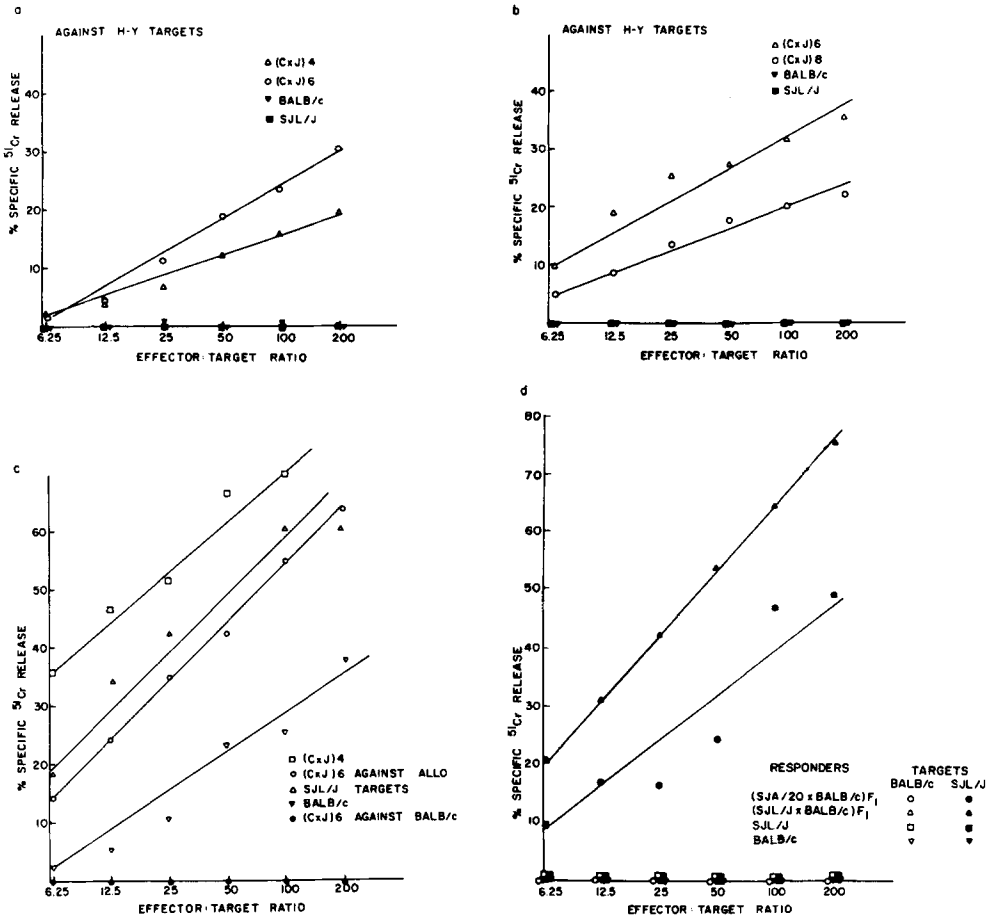

FIGURE 2. Cytotoxicity assays of responsiveness to H-Y. Cells from immunized females were always assayed on both male and female targets of BALB/c and SJL/J. To assure that the target blasts, as prepared, were susceptible to lysis by cytotoxic T-cells, an allogeneic killer (anti-*H-2*^{s} or anti-*H-2*^{d}) was included as a positive control for the targets. In addition, an aliquot of cells from every female responder under assay was immunized in vitro with an X-irradiated allogeneic stimulator, and assayed on allogeneic targets to provide a positive control for the responders. Killing by cytotoxic T-cells (percent ^{51}Cr release) is plotted as a function of the ratio of recovered splenic T-cells/labeled LPS-blasts. The data in *a* and *b* show a comparison of cytotoxic effector function by responder (C × J)RI strains with their nonresponder progenitors: BALB/c and SJL/J. Killing is shown on H-2–matched male targets. Not shown is the killing on syngeneic female targets, since response to these targets was undetectable. Only one example demonstrating H-2 restriction of the H-Y CTL response is shown in *c*. Here lysis by H-Y immunized CTLs from H-2^s (C × J)6 is measured on a mismatched H-2^d male target. Fig. 2*c* demonstrates that the splenic lymphocytes used in Fig. 2*a* could mount a response to an allogeneic H-2^k target (CBA/J). Fig. 2*d* shows the killing by (BALB/c × SJL/J or SJA/20)F_1 effectors on both BALB/cKe and SJL/J targets. BALB/cKe and SJL/J responses on male syngeneic targets were below background. Since each experiment was repeated at least five times and quantitated by statistical analysis, we use the designation +, ++, +++ (Table I) to refer to the relative magnitude of E/T ratios calculated to give 10% killing in 4 h. A negative (−) response is background lysis at >300 E/T (or defined another way, a response in which >10^8 effectors are calculated to produce 10% lysis).

TABLE II

Only One of the Two Progenitors Provides an Allele Which Complements With the Allele Present in a Given Nonresponder (C × J)RI Strain

NR (C × J)RI strain	Response to H-Y in:		H-2	v_α-allele	
	(NR × BALB/cKe)F_1	(NR × SJL/J)F_1*	Haplotype	Predicted‡	Found
(C × J)3	−	ND	d (c)	c	c
(C × J)9	−	+	d (c)	c	c
(C × J)10	−	ND	d (c)	c	c
(C × J)11	−	ND	d (c)	c	c
(C × J)15	+	−	s (j)	j	j

NR, nonresponder; −, no response; +, response; BALB/cKe (c), *H-2*^{d}; SJL/J (j), *H-2*^{s}.

* Cytotoxic killing by progeny from this backcross is shown in Fig. 3.

‡ Predicted on the basis of complementation with H-2 (see text). In all cases the predicted and observed v_α-allele are in concordance.

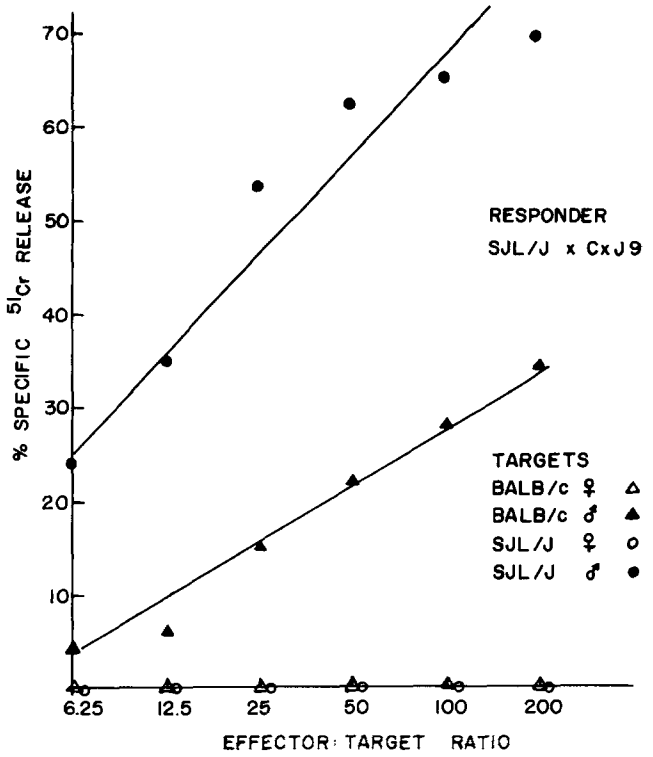

FIGURE 3. Comparison of cytotoxicity of CTL from (nonresponder × SJL/J) backcross on *H-2*^{s} and *H-2*^{d} targets. Lytic activity of immunized splenocytes taken from female progeny of the backcross (SJL × [C × J]9) immunized with male splenocytes from the same backcross by the protocol described in Materials and Methods. Targets are ^{51}Cr-labeled LPS-stimulated spleen cells from either BALB/c or SJL/J males or females. Not shown is killing in the comparable backcross (BALB/cKex[C × J]9) because lysis of all targets was equivalent to that of the background (as defined in Fig. 2).

have been completed, only one of the two crosses produced progeny expressing the responder phenotype (Table II and Fig. 3), i.e., when (C × J)9 was backcrossed to SJL/J or when (C × J)15 was backcrossed to BALB/cKe, the progeny were responders, whereas progeny from the other parental type were nonresponders.

Unresponsiveness in BALB/cKe and SJL/J Is Not Due to "Tolerance"

The data suggest that tolerance is unlikely to account for the inability of BALB/cKe or SJL/J to respond to H-Y. The concept is that the nonresponder expresses a self component so similar to the reference antigen that the animal is rendered unresponsive. Since T-cell responsiveness is in question, the way that the T-cell recognizes antigen must be considered. The T-cell recognizes either (*a*) H-Y itself, in which case unresponsiveness cannot be due to tolerance, as the (C × J)F_1 is responsive to ($H\text{-}2^s$ + H-Y) (Fig. 2), or (*b*) an interaction product between H-Y and H-2. If the antigen is assumed to be an interaction product, then the fact that the response in the (C × J)F_1 is predominantly to ($H\text{-}2^s$ + H-Y) would be interpreted as implying that there exists a self-component that mimics ($H\text{-}2^d$ + H-Y). This self-component would be present in the BALB/c female, thus accounting for its nonresponder status (such an assumption could not explain the nonresponder status of SJL/J females). Since an $H\text{-}2^d$ responder, (C × J)8, exists (Fig. 2*b*), it would be necessary to assume that it lacks the allele encoding this putative self-component. This implies that cytotoxic activity to ($H\text{-}2^d$ + H-Y) raised in females of (C × J)8 would lyse BALB/cKe female targets, providing that this self-component is present on these lymphoid blasts. Such CTL are specific for BALB/cKe male targets; no lysis of BALB/cKe female targets is detectable. Furthermore, progeny from the backcross of the $H\text{-}2^d$ nonresponder (C × J)9 with the SJL/J parent (described in the preceding section, Table II, and Fig. 3) respond to both BALB/cKe and SJL/J male targets, although "preference" is once again observed on the $H\text{-}2^s$ target. Therefore, this case of nonresponsiveness could not be due to a self-component present in the female (C × J)9 that mimics H-Y itself or an ($H\text{-}2^d$ + H-Y) interaction antigen.

In the next sections, it will be shown that the control of responsiveness to both (H-Y + $H\text{-}2^s$) and (H-Y + $H\text{-}2^d$) map to the complementing loci, Tarα and H-2. Under a tolerance model of unresponsiveness, this finding is unexpected, as the probability is low that the genes encoding two different self-components mimicking two different interaction antigens, (H-Y + $H\text{-}2^s$) and (H-Y + $H\text{-}2^d$), should map linked to the same loci, H-2 and Tarα. This mapping will now be discussed.

The MHC and Tarα Loci Regulate H-Y Responsiveness

As noted in the Introduction, there are two candidates for genes regulating this CTL response, the MHC and the T-cell receptor (Tar) genes. The determination of the distribution of alleles of the MHC- and Tarβ-locus in these (C × J)RI strains has been described previously (18). Now, the distribution of the alleles of Tarα in these (C × J)RI strains will be detailed.

Identification of Allelic Restriction Endonuclease Sites (v_α-1) in the Tarα-locus. When Eco RI digested liver DNAs from BALB/cKe, SJL/J, and the (C × J)RI strains were hybridized to either the complete p_αDO insert (*v*, *j*, and *c*, Fig. 4*a*) or to a probe specific for v_α (Fig. 4, *b* and *c*), three fragments were found to differ between the two parental strains. Fragments of 3.3 and 1.0 kb are present

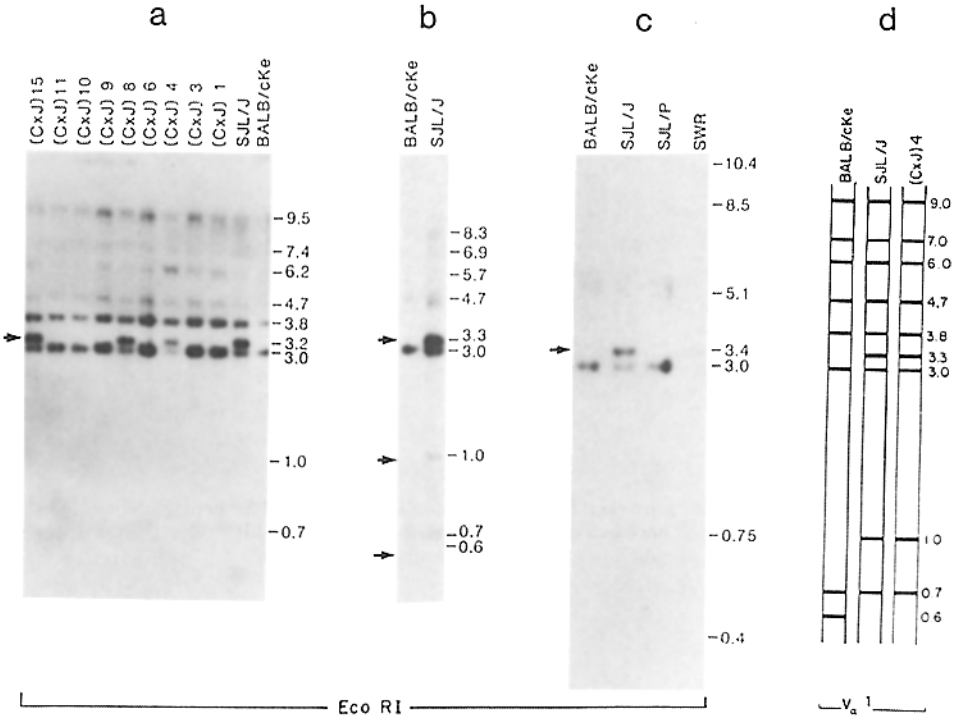

FIGURE 4. Autoradiographs of southern blots of murine liver DNAs. (*a*) The distribution of the Eco RI restriction site alleles using the p_αDO probe (see Materials and Methods) is compared in BALB/cKe, SJL/J, and (C × J)RI strains. Numbers indicate size of fragments in kilobases. Arrows are used to indicate the unique fragments. The 3.8-kb fragment is detected specifically with the c_α probe (data not shown). (*b*) The v_α-*1* restriction enzyme (Eco RI) site alleles using a v_α-probe (see Materials and Methods). (*c*) The alleles of v_α-*1* present in BALB/cKe, SJL/J, SJL/P, and SWR/J (an H-2^s mouse of independent origin expressing the v_α-1^j allele). (*d*) The distribution of restriction site fragments using the p_αDO probe are summarized in this schematic. The patterns of the haplotypes characteristic of BALB/cKe and SJL/J are compared with that of (C × J)4. (C × J)4 is SJL/J-like for v_α-*1*.

in SJL/J and absent in BALB/cKe, while BALB/cKe possesses a 0.7-kb fragment absent in SJL/J. These patterns that define the alleles of v_α-*1* are schematized in Fig. 4*d*. The typing of the (C × J)RI strains with respect to the v_α-*1* alleles (Fig. 4*a*) is summarized in Table I. The distribution of the v_α-*2* alleles that determine the response to the male-specific antigen H-Y is identical to that of v_α-*1* in eight of the nine strains. The exception, (C × J)4, must be a recombinant between v_α-*1* and v_α-*2*. Other restriction fragment length polymorphisms using BstE II, Eco RV, Kpn I, and Bgl I can be found between BALB/cKe and SJL/J with the p_αDO probe. The allelic differences generated after digestion with either Kpn I or Bgl I have been shown to be detectable with the v_α-probe. This defines these allelic sites as v_α-*1*. Since the (C × J)4 DNA displays the SJL/J v_α-*1* pattern after digestion with all of these enzymes, v_α-*1* must be 5′ of v_α-*2* (see next section).

The Tarα-locus Is Located Between Es-10 and Np-2. BALB/cKe and SJL/J differ at the *Es-10* locus. BALB/cKe expresses allele *a* and SJL/J, allele *b*. The electrophoretic mobility of *Es-10*, obtained from the progenitors and (C × J)RI strains, is shown in Fig. 5. The distribution of the *Es-10* alleles in the (C × J)RI

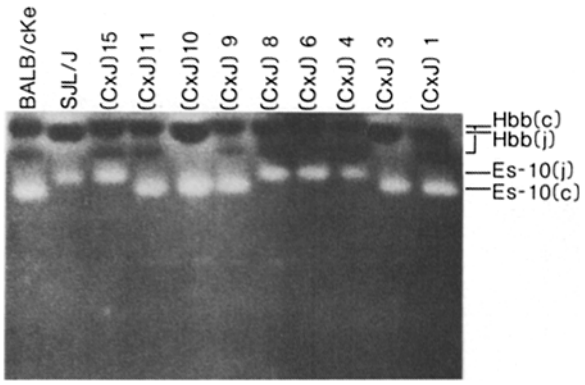

FIGURE 5. Determination of the *Es-10* and *Hbb* alleles. Shown here is a cellulose acetate gel illustrating the differences in electrophoretic mobilities of the enzyme *Es-10* (the white bands) from BALB/cKe, SJL/J, and (C × J)RI strains. The distribution of alleles of *Es-10* (found on chromosome 14) in each strain is summarized in Table I. This figure also illustrates for each strain the alleles of *Hbb*, a marker found on chromosome 7 (31). The hemaglobin β-chain (Hbb) alleles are recorded here as a datum, although not relevant to this study. A single band characterizes the SJL/J allele (j), while a diffuse band defines the BALB/cKe allele (c). (C × J)RI strains 1, 4, 6, 8, 9, 11, and 15 express the *c* allele of *Hbb*, while (C × J)RI strains 3 and 10 express the *j* allele.

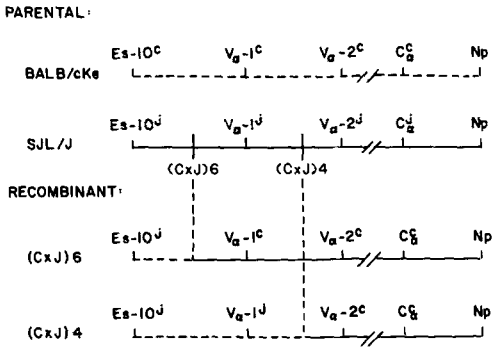

FIGURE 6. Location of v_α-*2* as defined by analysis of recombination within *Es-10* → *Np* interval. Shown here are the locations of known chromosome 14 markers relative to the *Tara*-genes which are described in the text. Arrows indicate the sites of recombination in (C × J)4 and (C × J)6. Symbols designating markers are as described below and in text. The position of the H-Y responsiveness marker, v_α-*2*, relative to the postulated site of recombination in (C × J)4 is indicated. Calculation of linkage (32): During the successive generations of inbreeding in the preparation of an RI line, there are multiple opportunities for recombination between linked loci. The probability of fixing a recombinant genotype (R) is $4r/(1 + 6r)$ where r is the probability of recombination in a single meiosis. The standard error of r is given by the square root of the sampling variance, $V(r)$ where $V(r) = r(1 + 2r)\,(1 + 6r)^2/4N$. N is the number of RI strains. *Es-10*, electrophoretic marker of erythrocyte esterase-10; v_α-$1^{c/j}$, v_α-gene marked by EcoRI restriction site alleles c or j; v_α-$2^{c/j}$, v_α-gene determining responsiveness to H-Y, c or j; *Np*, *Np-1*/*Np-2* = nucleoside phosphorylase. SJL/J and BALB/cKe possess the same alleles of *Np-1*/*Np-2*^{a} symbolized *Np*. Numbers are centimorgans.

strains is shown in Table I. *Es-10* cosegregates with the v_α-*1* restriction enzyme site allele in eight of the nine RI strains. One recombinant between *Es-10* and v_α-*1* was found, (C × J)6. (C × J)4 (described above) is recombinant between (*Es-10*—v_α-*1*) and v_α-*2* (Fig. 6).

Identification of the Genes That Regulate Responsiveness to H-Y. In order to

TABLE III
The Principle of the Genetic Analysis

Allele present at locus		Phenotype of the H-Y response	Prototype strain*
Y	*Z*		
c	c	Nonresponder	BALB/cKe, (C × J)1, 3, 9, 10, 11
j	j	Nonresponder	SJL/J, (C × J)15
c/j	c/j	Responder	(C × J)F_1
c	j	Responder	(C × J)8
j	c	Responder	(C × J)6

* See Table I.

discuss the identification of the two complementing loci, we have summarized in Table I the distribution among the (C × J)RI strains of the of *Tarα* and *Tarβ*-alleles. For comparison, the mapping of other immune-related loci (H-2 [19, 20], *Igλ* [19], *Igκ* [20], *Igh* [19]) in the (C × J)RI strains is included.

The principle of the analysis if that, given two gene loci, *Y* and *Z*, the phenotype of the animal will depend on the combination of alleles, c or j, at these loci (Table III).

The nonresponder (C × J)RI strains, like their progenitors BALB/cKe and SJL/J, must possess either the c-c or j-j alleles at loci *Y* and *Z*. The responder (C × J)RI must be c-j or j-c. As noted in the Introduction, one of the two complementing loci is expected to encode the restricting element, i.e., the H-2 locus. Since it is known that an H-2 linked gene controls H-Y responsiveness (5–7), we analyzed each RI using H-2 as the *Y* locus, and assigned the corresponding *Z* allele on the basis of the responsiveness phenotype (responder or nonresponder). By this procedure we determined that there is a direct concordance in eight out of nine (C × J)RI strains when the Tarα-locus is equated with the *Z* locus (Table I). The discordant strain (C × J)4 is predicted to be a recombinant at either H-2 or the Tarα-locus.

We have determined that the (C × J)4 mouse is not recombinant in the H-2 region. This locus was originally typed as $H\text{-}2^s$ using serological as well as alloreactivity assays (19, 20). Recently, two markers defined by restriction fragment length polymorphisms have been found to be allelic in BALB/cKe and SJL/J. The first is the A-crystallin locus which is 1.06 ± 0.83 cM from H-2 between glyoxylase (Glo-1) and H-2K (21). The second is detected using a murine genomic clone, which is believed to be situated between H-2I and H-2D (22). The region of chromosome 17 encompassing the H-2 region of (C × J)4 is SJL/J-derived by both of these criteria. Furthermore, CTL of (C × J)4 exhibit specificity for (H-Y + $H\text{-}2^s$) targets uniquely (not [H-Y + $H\text{-}2^d$] targets), indicating that the Class I antigens, in question, encode $H\text{-}2D^s$ and/or $H\text{-}2K^s$.

The most likely explanation of the phenotype of the (C × J)4 is that this strain is recombinant between the v_α-*1*-restriction site alleles and the v_α-*2*-alleles which determine responsiveness to H-Y. This explanation assumes that only two genes determine the responsiveness difference in these RI strains. In support of the "two-gene" hypothesis, the response of the offspring can be predicted when

nonresponder (C × J)RI strains are crossed to the parental strains (Table II), using the assumption that H-2 and Tarα are the only two complementing loci.

SJL/P Differs From SJL/J at v_α-1. Before discussing our interpretation of these results, it should be noted that our finding that SJL/J is a nonresponder appears to be in contradiction with a report that SJL/O (obtained from OLAC 1976 Ltd., Blackthorn, Bicester Oxon, United Kingdom) is a responder to H-Y (6). We therefore examined another available "subline" of SJL, SJL/P.

SJA/20 (congeneic with SJL/J except at the Igh locus), like SJL/J, is unable to respond to H-Y, whereas SJL/P is a responder. SJL/P is identical to SJL/J at H-2, but differs at several immune-related loci (Table I). Eco RI digested liver DNAs from BALB/cKe, SJL/P, and SJL/J were compared using the v_α-fragment as a probe. The data in Fig. 4*c* demonstrate that SJL/P resembles BALB/cKe not SJL/J. Our finding that SJL/J and SJL/P differ at Tarα is consistent with the other data presented here which suggest that the Tarα is one of the loci involved in determining the response to H-Y. Since our RI strains were constructed using the SJL/J subline, the two complementing loci, H-2 and Tarα, controlling responsiveness to H-Y, are revealed.

Discussion

The responsiveness difference between BALB/cKe and SJL/J to the male-specific antigen H-Y has been analyzed, and three points are established: (*a*) the F_1 cross between the two nonresponders, BALB/cKe and SJL/J, is a responder; (*b*) two unlinked complementing dominant genes are indicated, one in the H-2 locus on chromosome 17, and the other in the Tarα-locus on chromosome 14; (*c*) there is a hierarchy in responsiveness to H-Y which is a function of the interaction between the allele of R and the gene product encoded by the Tarα-locus; that is, the response is severalfold higher when the restricting element is *H-2*^{s}-encoded rather than *H-2*^{d}-encoded.

In the interest of brevity, we confine our discussion to an interpretation of these data in terms of a dual-recognitive, single receptor model. These experimental findings are compatible with this formulation (23, 24), and incompatible with several other formulations currently in vogue (25–28). Two loci, one H-2, and the other, Tarα, control responsiveness to H-Y, because the interaction between an R and a Tarα-encoded anti-R determines whether an individual is a responder or a nonresponder to X which is recognized with an appropriately low affinity. This implies that the affinity of the (R + anti-R) interaction determines a threshold affinity for the (X + anti-X) interaction below which an individual is a nonresponder and above which, a responder. Since the MHC is known to encode R, the Tarα-locus must encode anti-R if it is to complement with R. Thus our data are only compatible, under the dual recognitive-single receptor model, with the interpretation that it is the anti-R site which is determined by the v_α-alleles, not the anti-X site. This does not imply a universal. In other combinations of H-2 and Tarα/Tarβ, the Tarβ-locus could encode anti-R and the Tarα-locus, anti-X (see reference 24 for discussion). In fact, both cases are expected, depending on the combinations of haplotypes, H-2 and Tarα/Tarβ, that the animal expresses.

BALB/cKe(C) and SJL/J(J) are nonresponders, whereas the (C × J)F_1 is a

responder. This is interpreted to mean that the gene products specified by the BALB/cKe v_α-gene(s) encoding anti-R^d and the SJL/J v_α-gene(s) encoding anti-R^s are each of too low an affinity to permit a response to H-Y, because the affinity of the (H-Y + anti–H-Y) interaction is itself low. However, the affinity of the anti-R^s site (specified by the BALB/cKe v_α-allele) and of the anti-R^d site (specified by the SJL/J v_α-allele) is sufficient to permit a response to H-Y, since the combining sites, anti-R and anti-X, act in concert as the T-cell receptor. The implication here is that the MHC controlled responsiveness differences cannot be, strictly speaking, antigen-specific. Within the context of a given (R + anti-R) interaction, any antigen-X which interacts with anti-X at an affinity below threshold will result in unresponsiveness and above threshold, responsiveness.

The affinity of the interaction between R and anti-R is sufficient in the (C × J)F_1, the *H-2*^{s} RI strains (C × J)4 and (C × J)6, and the *H-2*^{d} RI strain (C × J)8 to permit a detectable response. Further, the fact that (*a*) the (C × J)F_1 shows a preference in responsiveness for male targets bearing *H-2*^{s} over those bearing *H-2*^{d}, and (*b*) the response by the *H-2*^{d} RI, (C × J)8, is always less than that of the *H-2*^{s} responders, (C × J)4 and (C × J)6, leads us to conclude that there is a hierarchy of affinities in the (R + anti-R) interactions, i.e., BALB/cKe anti-R^s > SJL/J anti-R^d ≫ BALB/cKe anti-R^d or SJL/J anti-R^s.

The *H-2*^{s} responders, (C × J)4 and 6, and the *H-2*^{d} responder, (C × J)8, use v_α-genes to encode anti-R^s and anti-R^d, respectively. This implies that these responders derive their cytotoxic anti–X(H-Y) specificity from the Tarβ-locus. It has been suggested that the v_β-gene pool of BALB/cKe consists of 18–30 genes, while that of SJL/J numbers 5–9 genes (29). In this instance, the difference in the size of the germline v_β-gene pool is of no apparent consequence. In fact, all of the responders, whether *H-2*^{s}- or *H-2*^{d}-restricted, possess the truncated SJL/J Tarβ-locus. This means that BALB/cKe and SJL/J do not possess distinguishable germline v_β-genes from which the recognition of H-Y is derived.

The response in these RI strains appears to depend solely upon the relationship between the v_α-allele and the H-2 haplotype, and not between the v_α- and v_β-alleles or between the v_β-allele and the H-2 haplotype. No effect of alleles at the Tarβ-locus is discernible. RI strains which carry the pair of alleles, Tarα^cTarβ^J be either responders ([C × J]4 and 6) or nonresponders ([C × J]3,9, and 10) to H-Y. Similarly, RI's expressing the combination, Tarα^JTarβ^J, differ in their responsiveness phenotype ([C × J]8 is a responder; [C × J]15, a nonresponder). RI strains that possess the identical Tarα-allele (c) but differ in their Tarβ-loci, ([C × J]1 and [C × J]3) are both nonresponders. Finally, complementation between the Tarβ- and H-2-loci does not account for the phenotype. RI strains that are *H-2*^{d} and possess the identical Tarβ-allele (j) can be either responders ([C × J]8) or nonresponders ([C ×J]3, 9, and 10). We are missing in this RI set a responder possessing the *c* allele of Tarβ. However, given the above discussion, it is not expected to affect responsiveness. Even in RI strains possessing the Tarβ^c allele, only appropriate combinations of the complementing H-2- and Tarα-loci (either Tarα^J, H-2^d] or Tarα^c, H-2^s]) should be responders.

If only two genes are involved (as is likely), alleles of the Tarγ-locus (30, 31) cannot influence this response. In any case, allelic differences between BALB/c and SJL/J at the Tarγ-locus could only be proposed as an alternative to recom-

bination as an explanation of the H-Y responsiveness phenotype of (C × J)4. The genetic control of responsiveness discussed here deals with the determination of recognitive specificity. All of the published evidence favors the assumption that only the α- and β-chains of the T-cell receptor determine the recognitive properties (antigen and MHC) of the T-cell receptor (2, 11). There is no reason to believe that the γ-chain contributes to specificity and, in any case, it is insufficiently characterized to be usefully discussed, e.g., there is no evidence that it is translated.

Our experiments do not permit us to decide whether the genetically determined responder/nonresponder phenotype being mapped is expressed in helper or cytotoxic T-cells, since, in our view, helper T-cells are required for the initial step of activation of the antigen-responsive cytotoxic T-cells, in addition to their role in lymphokine production. However, there is a hint that it is expressed in cytotoxic T-cells. The response difference to H-Y in the (C57Bl/6 × DBA/2)RI strains of the $H\text{-}2^d$ haplotype has been mapped to a gene on chromosome 2 (6), probably *B2m*. The involvement of β_2-microglobulin (a subunit of RI, the Class I restricting element) in the determination of responsiveness to H-Y in these strains suggests that it is the interaction with a Class I element that is being mapped. Since, in our experiments, BALB/cKe and SJL/J share the same $B2m^a$ allele, the effects of differences at the H-2- and Tarα-loci are revealed. Therefore, it is likely that the responsiveness difference of these RI strains is expressed in CTLs.

The Tarα-locus has been mapped to chromosome 14 of the mouse. Allelic restriction endonuclease patterns are detected with a c_α-probe when C57Bl/6J and DBA/2 DNAs are digested with Bgl I (9). These allelic differences map c_α to within 3 cM of *Np-2*, which is adjacent to *Np-1*. The *Np-1* locus has in turn been shown to be linked to *Es-10*, separated by a distance of 10 ± 2 cM (10). We have identified a (C × J)RI strain, (C × J)6, which is recombinant between the *Es-10* marker and v_α-*1*, and another RI, (C × J)4, which is recombinant between v_α-*1* and v_α-*2*. We have determined that the gene(s) encoding responsiveness to H-Y, v_α-*2*, maps within 3 cM of v_α-*1*, toward the *Np* locus. On the basis of these data, the orientation of the Tarα-locus, in the interval between *Es-10* and *Np-2* is: *Es*-10—v_α-1—v_α-2—[c_α—*Np*(1 and 2)]—centromere. Since it is not known on which side of *Np* (1 and 2) c_α is located, these markers are bracketed. The observations that (*a*) v_α-*1* is separated from *Es-10* by 3 ± 4 cM, (*b*) v_α-*2* is separated from v_α-*1* by 3 cM ± 4, (*c*) *Es-10* is separated from v_α-*2* by 8.3 ± 2.2, and (*d*) c_α is within 3 cM of *Np-2*, suggest that the gene encoding responsiveness to H-Y, v_α-*2*, is between v_α-*1* and c_α. A map is proposed (Fig. 6), showing the sites of recombination in the RI strains.

Summary

The genetic control of the cytotoxic T-cell response to the male histocompatibility antigen, H-Y, was analyzed in BALB/cKe(C) and SJL/J(J) which are both nonresponders. However, the (C × J)F_1 hybrid is a responder. Therefore, two dominant complementing genes are involved. Analysis of a set of (C × J) recombinant inbred (RI) lines reveals that these two complementing gene products are a restricting element (R) encoded by the H-2 (MHC) locus on chromo-

some 17 and a subunit of the T-cell receptor (anti-R) encoded by the Tarα-locus on chromosome 14. The order and orientation of gene segments within the Tarα-locus has also been established relative to the chromosome 14 marker, *Es-10*. The existence of two RI strains which are recombinant at chromosome 14 has made it possible to determine that this order is *Es-10*—v_α-*1*—v_α-*2*—[c_α—*Np-2*]—centromere. The implications of these data for the antigen-specific regulation of immune responsiveness are discussed in terms of the dual recognitive-single receptor model.

The authors are indebted to Dr. Rodney Langman for his many contributions to their thinking; to Dr. Paul Patek for his constructive suggestions and discussion; to Richard Dargusch, who provided the appropriate crosses and maintains the RI strains; and Sally Jo Divis for technical assistance.

Received for publication 1 October 1985 and in revised form 23 December 1985.

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