USE OF SOMATIC CELL GENETICS TO STUDY CHROMOSOMES CONTRIBUTING TO ANTIGEN PLUS *I* RECOGNITION BY T CELL HYBRIDOMAS*

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The nature of the receptor(s) on T cells for antigen (Ag) plus major histocompatibility complex (MHC)[1] products remains one of the most controversial issues in modern immunology. A consensus has not been reached on a single property of these molecules. Even the question of whether there are one or two receptors has not been resolved. Although theories about Ag/MHC receptors can by and large be separated into two groups, i.e., those that favor recognition of Ag and MHC by different receptors (dual-recognition theories) and those that favor recognition of some Ag/MHC complex by a single receptor (altered-self theories), at present there is no compelling evidence in favor of one or the other. Our own experiments (1), and those of others (2, 3), have suggested that MHC products and Ag cannot be recognized independently by the T cell receptor(s), but there is contradictory evidence (4, 5).

One of the continuing controversies over the T cell receptor(s) for Ag/MHC has been whether genes associated with immunoglobulin or MHC loci contribute products which comprise at least part of the receptor. Although careful analysis has suggested that V_H or idiotype-bearing components can be found on certain types of T cells and, more often, their Ag-binding products (6–12), it has been difficult to demonstrate the presence of V_H or idiotype on normal T cells or MHC-restricted T cell lines (13, 14). Genes mapping near the *Ig-h* locus have also been implicated in Ag recognition by T cells (15–17). Other experiments have suggested that Ag-binding T cell products bear Ag coded by the MHC locus (18–20), although whether these comprise an essential part of the T cell receptor for Ag has yet to be demonstrated.

With these points in mind we thought it would be valuable to map the T cell receptor(s) for Ag/MHC using somatic cell genetic techniques. This paper contains our first set of results on this subject. T cell hybridomas have been constructed by fusing Ag/*I*-specific T cell blasts from various strains of mice, bearing easily identifi-

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[1] *Abbreviations used in this paper*: B10, C57BL/10.SgSn; Con A, concanavalin A; HAT, hypoxanthine-aminopterin-thymidine; $H\text{-}2^{Rb}$, the *H-2* haplotype associated with the (16.17) metacentric chromosome in Rb(16.17)7Bnr mice; IL-2, interleukin 2; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex.

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able metacentric chromosomes, to a T cell hybridoma, FS6-14.13.AG2.1, by standard techniques (1). In two cases we found that Ag/*I* specificity was retained in a resulting hybridoma that had lost both incoming marked chromosomes, which suggests that the chromosomes in question, 4, 6, 16, and 17, did not contribute gene products to the specific portions of the Ag/*I* receptor(s) in these hybridomas, i.e., that genes mapping on the same chromosome as κ, λ, or *H-2* did not contribute to specific Ag/*I* recognition by T cells (21–23).

Materials and Methods

Animals. C57BL/10SgSn (B10), B10.A, B10.D2 nSn, and B10.M mice were bred in our vivarium from breeding triplets purchased from The Jackson Laboratory, Bar Harbor, ME. B10.S(7R) and B10.AQR animals were bred from breeding triplets kindly supplied by Dr. D. Shreffler, St. Louis, MO and Dr. C. David, Rochester, MN, respectively.

The Robertsonian mice used in these studies were bred in our vivarium from animals obtained from Dr. Eva Eicher, The Jackson Laboratory. These animals were Rb(4.6)2Bnr, RB(8.12)5Bnr, and Rb(16.17)7Bnr. Parenthetical numbers indicate the two chromosomes that have fused to yield metacentric chromosomes in each mouse strain. For example, Rb(4.6)2Bnr has 38 chromosomes and no free chromosomes 4 or 6, but two metacentric chromosomes each made up of one chromosome 4 fused to one 6. For brevity the Bnr nomenclature will be omitted from the names of these mice in the rest of this paper.

Production of Hybridomas. T cell hybridomas were constructed as previously described (1). Briefly, mice were immunized in the base of the tail with 100 μg keyhole limpet hemocyanin (KLH) (Calbiochem-Behring Corp., American Hoeschst Corp., San Diego, CA) in complete Freund's adjuvant. 7 d later periaortic and inguinal lymph node cells were harvested (24) and cultured for 4 d in Click's medium, 1% fresh syngeneic mouse serum, and 15 μg/ml KLH. At this point dead cells were removed on Ficoll-Hypaque gradients and the T cell blasts expanded for 3 d in interleukin 2 (IL-2) (25).

At the time we received the three Robertsonian strains discussed in this paper, their *H-2* haplotypes (and minor loci types) were unknown and in fact it was not clear that the mice of each strain were uniform homozygotes at *H-2* or at any minor histocompatibility locus. Therefore, to avoid mixed lymphocyte reactions which might lead to T cell blasts with confusing specificities or to cytotoxic T cells during the in vitro phases of the generation of Ag-specific T cell blasts, T cells from individual mice were cultured separately. These cells were not pooled until the moment of fusion.

After expansion of T cell blasts in IL-2, dead cells were again removed on Ficoll-Hypaque gradients and the remaining cells were fused with the T cell hybridoma FS6-14.13.AG2.1. FS6-14.13.AG2.1 secreted IL-2 when induced with concanavalin A (Con A) (Sigma Chemical Co., St. Louis, MO) and was produced by fusing normal BDF_1 T cells to the AKR thymoma BW5147 (26). The nature of its receptors for Ag/*H-2*, if any, was unknown. The fusion protocol was as published (1). Usually $\sim 6 \times 10^7$ T cell blasts were fused to 1.5×10^7 FS6-14.13.AG2.1 cells and hybrids were plated in four to six 96-well microculture plates. Hypoxanthine-aminopterin-thymidine (HAT) was added 24 h after fusion. Medium was changed every 5 d and hybrids started to overgrow microculture wells about 11 d after fusion. As they grew up, hybrids were transferred to larger culture vessels, tested for Ag/*I* reactivity, frozen away, karyotyped, and cloned and recloned at limiting dilution where appropriate. In later fusions, hybridoma primary and cloning cultures were supplemented with the supernatant of an HAT-sensitive B cell lymphoma, A20-2J (27, 28), or the lymphoma cells themselves as conditioned medium or feeders respectively. These additions markedly increased the numbers of hybrids produced and their plating efficiencies.

In spite of this, the plating efficiency of hybridomas varied markedly, ranging from 1 to 50% on first cloning. Hybrids were cloned at limiting dilution such that individual culture wells contained 0.36, 1.1, 3.3, or 10 cells/well. Clones for further analysis were always chosen from the plates that showed growth at the highest dilution of cells plated. Recloning was often necessary to confirm results obtained with lines that may not have been clonal (i.e., picked from 10, 3.3, or 1.1 cells/well plates).

Fusions to Robertsonian T cells were named Rb to distinguish them from other fusions in the laboratory. Fusions were numbered in the order in which they were done, e.g., Rb, 2Rb, 3Rb, etc. As hybridomas were picked from the wells in which they were plated after fusion, they were numbered according to their order of appearance, e.g., 3Rb5, 3Rb6, etc. Cloning was indicated by a period (.) and clones were numbered according to the order of their appearance, e.g., 3Rb22.1, 3Rb22.2, 3Rb22.3. Subclones were similarly indicated.

B cell hybridomas, able to present Ag in the context of their *H-2* products to T cells, were produced as previously described (28). Briefly, spleen cells were depleted of T cells (29) and fused at ratios of 5:1 to the HAT-sensitive B cell lymphoma A20-2J (kindly supplied to us by Dr. David McKean, Mayo Clinic, Rochester, MN, and produced by the efforts at different times of Drs. Asofsky, Kuehl and McKean and their collaborators). HAT was added at 24 h to cultures, medium was changed every 5 d, and hybrids began to overgrow wells by about day 12. Hybrids were tested for their ability to present KLH to T cell hybridomas from the same strain of mouse. Successful hybrids were cloned at limiting dilution in HAT with A20-2J cells as feeder cells, retested for Ag presentation, and frozen away. Three B cell hybridomas, LRb 15.1, LRb 25.24, and LRb 32.2, were prepared and used in the experiments described in this paper. All of these hybridomas are able to present KLH in the context of the (unknown) *H-2* type encoded by the Robertsonian chromosome (16.17) borne by Rb(16.17)7 mice.

Assay for Ag/H-2 Recognition by T Cell Hybridomas. T cell hybridomas were assayed for their ability to recognize Ag/*H-2* as previously described. Briefly, 10^5 T cell hybridomas were plated in 0.2 ml complete tumor medium (1) with 10^6 4,000 rad-irradiated spleen cells or 10^5 B lymphoma cells as Ag-presenting cells in the presence or absence of 1 mg/ml KLH. No response was ever observed to Ag in the absence of Ag-presenting cells. In some cases T cell hybridomas were incubated with 4 μg/ml Con A in separate wells. 24 h later, the supernatants from these cultures were serially diluted and assayed for the presence of IL-2 by their ability to support the growth of the IL-2-dependent T cell line HT-2 (kindly given to us by Dr. James Watson, Aukland, New Zealand). 24 h after addition of HT-2, cultures were scored under the microscope. In each serial dilution, the first well to contain <90% live HT-2 cells was said to contain 1 U of IL-2. In this paper, results are given as units of IL-2 per milliliter as measured by this assay (1).

Preparation of Karyotypes. Sample karyotypes were prepared from each of the three Robertsonian strains of mouse used in these studies by standard methods. Lymph node cells from these animals were incubated at 2×10^6/ml in 4 μg/ml Con A for 3 or 4 d. At this point, colcemid (Sigma Chemical Co.) was added to a final concentration of 0.2 μg/ml and the mixture was incubated for a further 4 h at 37°C. Cells were then spun down, resuspended in hypotonic solution (1 part fetal calf serum:7 parts double-distilled water or 0.075 M potassium chloride), and incubated for a further 30 min in a 37°C water bath. Cells were again spun down and resuspended in 7 ml of fixative (3 parts methanol:1 part glacial acetic acid). After incubation at room temperature for at least 20 min cells were washed three times with fixative and resuspended to a suitable density. Drops of cell suspensions were then allowed to fall ~2 ft onto ethanol-cleaned glass coated with ice-cold water. Slides were air dried, stained with Giemsa, and examined microscopically. Good spreads were photographed using a 100 × oil immersion objective.

Karyotypes of hybridoma cells, including FS6-14.13.AG2.1, were prepared by similar means. It was found, however, that the appearance of decipherable chromosome spreads depended critically on the cell line and the exact composition of hypotonic solution used. A suitable hypotonic solution had to be devised empirically for each hybridoma. Hypotonic solutions used varied from the compositions described above for normal T cell blasts, to such mixtures as 1 part fetal calf serum:11 parts double-distilled water:11.5 parts 0.4% sodium citrate.

For each cell line or clone, >10 chromosomal spreads were examined to ensure that low-frequency cells with unexpected karyotypes did not contaminate any given cell line. When hybridoma T cell clones were identified with Ag/*H-2* reactivity in the absence of any Robertsonian chromosomes, up to 50 chromosome spreads were examined to confirm this lack.

Results

Karyotypes of Parental Cells. Cells of the T cell hybridoma FS6-14.13.AG2.1 and T cell blasts from the three Robertsonian strains were karyotyped as described in

Materials and Methods. Sample karyotypes are shown in Figs. 1 A–D, respectively. Karyotypes of Robertsonian mice were as expected, each containing 36 telocentric chromosomes and one pair of metacentric chromosomes of size varying with the strain of origin of the cells. FS6-14.13.AG2.1 proved to have ~82 chromosomes, one of which was metacentric. This metacentric chromosome was easily distinguished from any metacentrics in the Robertsonian mice, by virtue of having one pair of arms markedly shorter than the other, as well as very pronounced constrictions near the centromere on the short arms. Parenthetically, this metacentric chromosome was apparently not inherited by FS6-14.13.AG2.1 from its tumor parent, BW5147. The BW5147 from which FS6-14.13.AG2.1 was derived was of mixed karyotype, and although several metacentric chromosomes were present in the line, none resembled exactly the metacentric chromosome in FS6-14.13.AG2.1 (30).

Hybridomas Derived from Rb(4.6)2 T Cell Blasts. Four Rb(4.6)2 animals were primed with KLH; Ag-specific T cell blasts were prepared from these animals and fused to

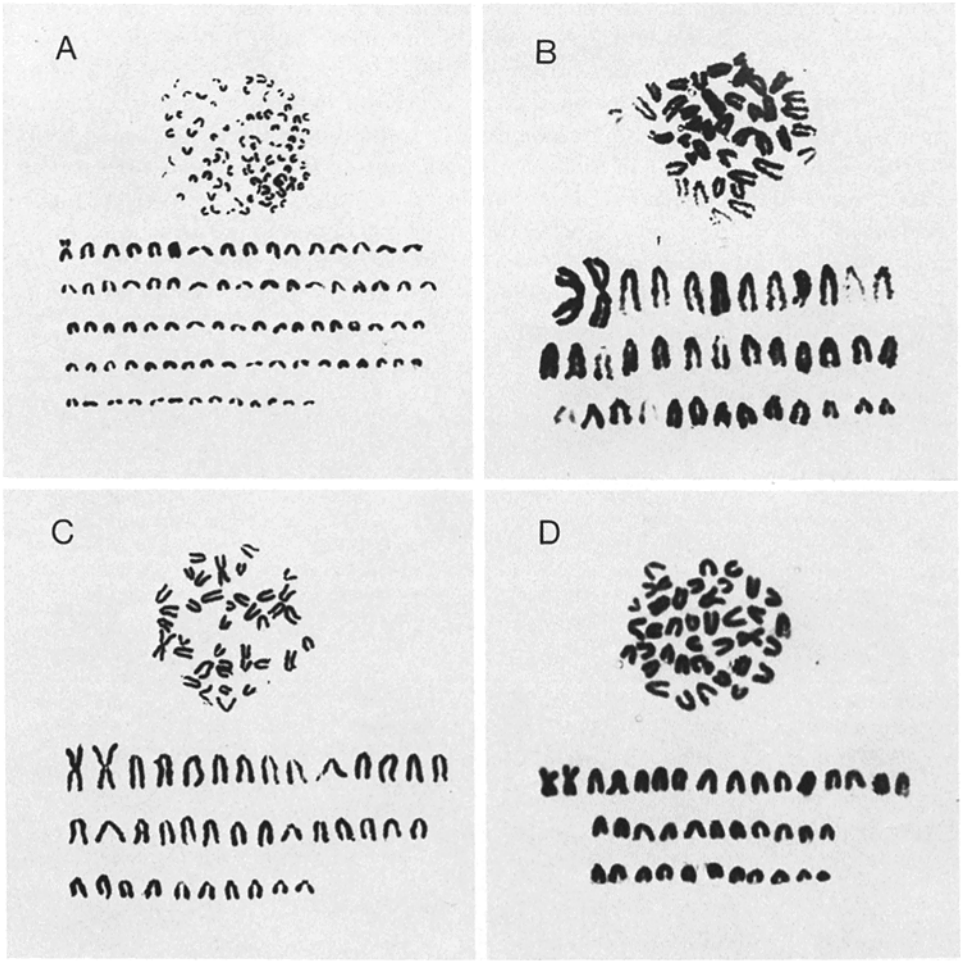

FIG. 1. Karyotypes of the parent cells used in fusions. (A) FS6-14.13.AG2.1 (B) Rb(4.6)2 T cell blast (C) Rb(8.12)5 T cell blast (D) Rb(16.17)7 T cell blast.

FS6-14.13.AG2.1 as described in Materials and Methods. Hybrids were picked and screened for their ability to respond to KLH. The response of each T cell hybridoma was tested in the presence of Ag-presenting cells from three different Rb(4.6)2 animals, since, as noted earlier, the *H-2* haplotype and indeed, *H-2* uniformity of these animals was uncertain at the time we started these experiments. Of 20 lines tested, 9 responded to KLH in the presence of Rb2-irradiated spleen cells. No difference in response was noted when these positive T cell hybridomas were screened with Ag-presenting cells from different Rb(4.6)2 animals. Five of these responsive hybridomas were chosen for cloning and further study. Cloned T cell hybridomas were screened for response with or without KLH in the presence of Rb(4.6)2-irradiated spleen cells. Sample Ag/*H-2* responsive clones were screened for activity with KLH and Ag-presenting cells from an array of B10 congenic animals and simultaneously karyotyped. Some representative examples of this analysis are shown in Table I.

As shown, clones derived from all five hybridomas that were checked responded to KLH in the presence of $I\text{-}A^d$ or $I\text{-}E^d$, which suggests that the predominant *H-2* haplotype of Rb(4.6)2 mice in Dr. Eicher's colony was *d*. No response was seen in the presence or absence of KLH to spleen cells of any of five other independent *I* region haplotypes, *b*, *f*, *k*, *q*, and *s*. Most of the hybridoma clones had retained at least one Robertsonian chromosome. One set of clones, derived from hybridoma 3Rb22, was of particular interest, however. One clone, 3Rb22.1, showed variable response to KLH/$I\text{-}A^d$, depending on the day of assay, and averaged ~40 U of IL-2. No Robertsonian chromosomes could be seen in chromosome spreads of the clone. Because the plating efficiency of 3Rb22 was very poor (~1%), however, and since 3Rb22.1 was picked from a plate that had been innoculated with 10 cells/well, we concluded that the line was probably not clonal and immediately recloned it. Data from a KLH/$I\text{-}A^d$-responsive clone obtained on recloning, 3Rb22.1.14, is also shown in Table I. This

TABLE I

T Cell Hybridomas Involving Rb(4.6)2 T Cells Suggest that Genes Involved in Ag/I Receptors Do Not Map to Chromosomes 4 or 6

Rb(4.6)2 derived T cell hybridoma	Cells/well on cloning plate	Ag/*H-2* specificity	Units/ml IL-2 produced in response to Ag/*H-2*	Karyotype		
				Approximate total chromosome number	Number of metacentric chromosomes*	
					FS6	(4.6)
3Rb4.5	1.1	KLH/$I\text{-}A^d$	160	105	0	2
3Rb14.1	10	KLH/$I\text{-}A^d$	160	80	1	2
3Rb16.5	0.36	KLH/$I\text{-}A^d$	640	80	0	2
3Rb17.1	3.3	KLH/$I\text{-}E^d$	80	90	0	2
3Rb22.1	10	KLH/$I\text{-}A^d$	variable	76	1	0
3Rb22.2	3.3	KLH/$I\text{-}A^d$	320	92	1	1
3Rb22.3	3.3	0	0	72	1	0
3Rb22.4	3.3	KLH/$I\text{-}A^d$	320	94	0	1
3Rb22.1.14	0.36	KLH/$I\text{-}A^d$	320	74	1	0

* Metacentric chromosomes were noted to be either of the FS6-14.13.Ag2.1 type (FS6, Fig. 1 A) or of the Rb(4.6)2 type (Fig. 1 B).

clone was picked from a plate innoculated with 0.36 cells/well on which 25 clones grew up. A typical karyotype of 3Rb22.1.14 is shown in Fig. 2. 3Rb22.1.14 differed from the other 50 chromosome spreads examined in that no Robertsonian metacentrics were observed although the line did retain the metacentric chromosome characteristic of FS6-14.13.AG2.1. Ag/*I* specificity was therefore retained by this clone in the absence of any chromosomes 4 or 6 derived from the Ag/*I*-specific T cell blast parent, which suggests that the specific portions of T cell Ag/*I* receptors were not derived from genes on either chromosome 4 or 6, at least in this clone.

Hybridomas Derived from Rb(8.12)5 T Cell Blasts. A number of fusions have been performed with KLH-specific T cell blasts from Rb(8.12)5 animals. On each occasion, 4 or 5 mice were primed with KLH, and 7 d later draining lymph node cells from individual mice were cultured with Ag followed by IL-2, before pooling and fusion to FS6-14.13.AG2.1. From this strain, preliminary mixed lymphocyte reactions suggested that the predominant *H-2* haplotype was *b*. Hybrids were therefore screened for response to KLH with Ag-presenting cells from either Rb(8.12)5 or $H\text{-}2^b$ animals. Complete concordance was found in the ability of hybrids to respond to KLH in the presence of Rb(8.12)5 Ag-presenting cells or $H\text{-}2^b$ Ag-presenting cells. Variable yields of Ag/*I*-responsive hybridomas were obtained in these fusions. For example, in the first fusion, cells from 21 of 21 hybrid wells tested proved responsive. In the fusion named 5Rb, 9 of 39 hybrids tested were responsive. Data from some of the clones derived from these positive wells are shown in Table II.

Several points became apparent from these experiments. First the metacentric chromosome (8.12) appeared to be preferentially retained in these hybrids, since no hybridoma has yet been identified by us which has lost both of the metacentric chromosomes inherited from its normal T cell parent. This was in spite of continuous culture of some of the hybridomas for up to 6 mo. Even the appearance of hybridomas with only one Robertsonian chromosome was a relatively rare occurrence, having been seen only in clones derived from the 5Rb25 line, in spite of prolonged culture and multiple recloning of other lines as noted above.

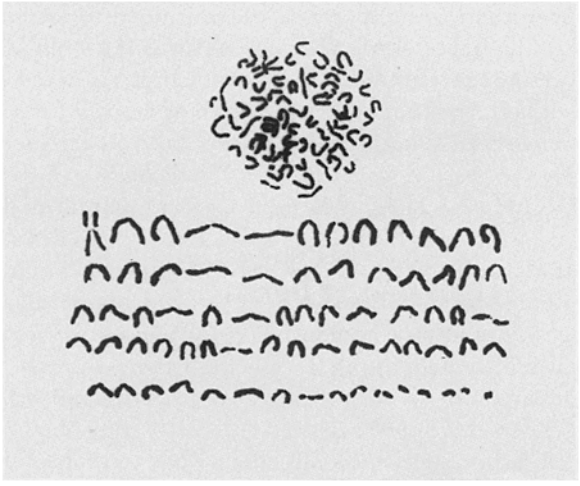

FIG. 2. Karyotype of the KLH/$I\text{-}A^d$-responsive hybridoma, 3Rb22.1.14, derived from (Rb(4.6)2 T cell blasts.

TABLE II

T Cell Hybridomas Derived from Rb(8.12) T Cell Blasts

Rb(8.12)5 derived T cell hybridoma	Cells/well on cloning plate	Ag/*H-2* specificity	Units/ml IL-2 produced in response to:		Karyotype		
			Ag/H-2	Con A	Approximate total chromosome number	Number of metacentric chromosomes*	
						FS6	(8.12)
Rb17.5	10	KLH/*I-A*^{b}	320	ND‡	90	1	2
Rb17.5.1.43	0.36	KLH/*I-A*^{b}	0	320	105	1	2
Rb17.5.1.48	0.36	KLH/*I-A*^{b}	320	320	107	1	2
5Rb14.5	1.1	KLH/*I-A*^{b}	80	40	95	1	2
5Rb17.3	3.3	KLH/*I-A*^{b}	160	ND	130	2	2
5Rb25.1	1.1	KLH/*I-A*^{b}	160	ND	71	0	1
5Rb25.14	0.36	KLH/*I-A*^{b}	320	320	81	1	1

* Metacentric chromosomes were noted to be either of the FS6-14.13.Ag2.1 type (FS6, Fig. 1, plate 1) or of the Rb(8.12)5 type (Fig. 1 C).

‡ Not done.

Second, several interesting clones of the Rb17 line were identified by prolonged culture and multiple subcloning. Rb17.5.1.43 and Rb17.5.1.48 differed by a few chromosomes but each retained both Robertsonian metacentric chromosomes. Although both subclones secreted IL-2 in response to Con A, Rb17.5.1.43 no longer responded to Ag/*I*, which suggests that it had lost at least part of the receptor(s) for KLH/*I-A*^{b}. Karyotypes of Rb17.5.1.43 and Rb17.5.1.48 are shown in Fig. 3.

Finally, a number of clones derived from 5Rb25, exemplified by 5Rb25.1 and 5Rb25.14, responded to KLH/*I-A*^{b} and bore only one Robertsonian metacentric chromosome, which suggests that no more than one chromosome 8 or 12 was required in the T cell for full Ag/*I* reactivity. In addition, some clones derived from 5Rb25 had lost the FS6 metacentric chromosome, yet continued to secrete IL-2 inducibly, which suggests that this metacentric chromosome was not obligatorily involved in Ag/*H-2* or Con A responses resulting in IL-2 production. As noted above, in spite of prolonged culture of both these and other hybridomas derived from Rb(8.12)5 mouse T cells, no hybrids have been obtained that have lost both Robertsonian chromosomes.

Hybridomas Derived from Fusions to Rb(16,17)7 T Cell Blasts. Five Rb(16.17)7 mice were primed with KLH; 7 d later, cells from draining lymph nodes were cultured with KLH followed by IL-2, and fused to FS6-14.13.AG2.1 as described in Materials and Methods. In this case, because the metacentric chromosomes included the genes coding for *H-2*, presumably inherited from their wild mouse ancestor, we felt we would be unlikely to identify a common laboratory mouse with an *H-2* haplotype such that its cells would present Ag to T cells from these animals. Rb(16.17)7 mice were scarce in our vivarium so we prepared Ag-presenting B cell hybridomas by fusion of Rb(16.17)7 splenic non-T cells to A20-2J as described in Materials and Methods. As T cell hybridomas grew up, they were screened for their ability to respond to KLH in the presence of either Rb(16.17)7 spleen cells or a panel of 30 Rb(16.17)7 B cell hybrids. By this means, three B cell hybridomas were identified

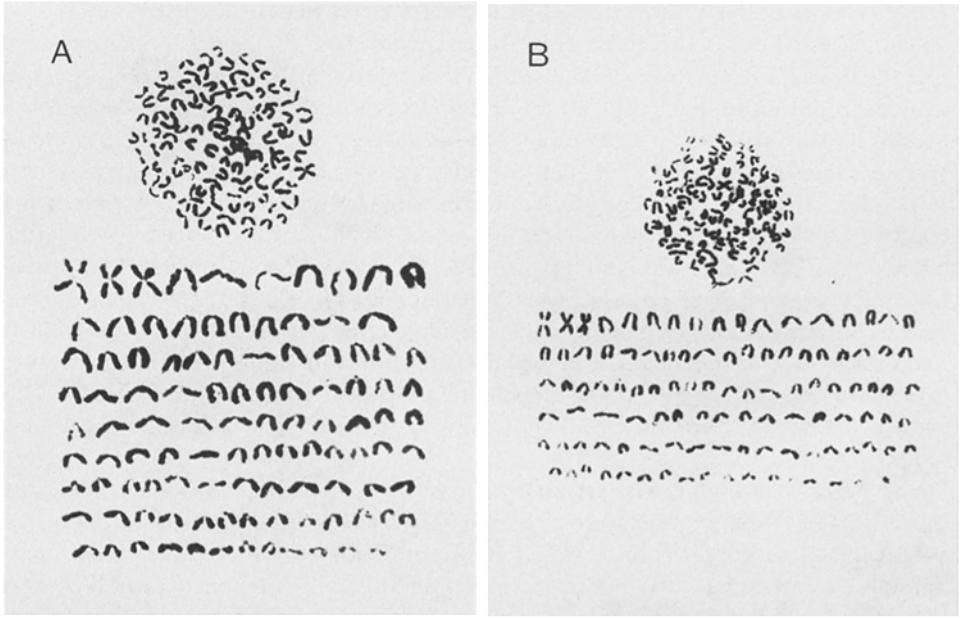

FIG. 3. Karyotypes of two hybridomas. (A) Rb17.5.1.43, a hybridoma subclone which secreted IL-2 in response to Con A but not in response to KLH/$I\text{-}A^b$. (B) Rb17.5.1.48, a hybridoma subclone which secreted IL-2 in response to either Con A or KLH/$I\text{-}A^b$.

TABLE III

T Cell Hybridomas Derived from Rb(16.17)7 T Cells Suggested that Genes Involved in Ag/I Receptors Do Not Map onto Chromosomes 16 or 17

Rb(16.17)7 derived T cell hybridoma	Cells/well on cloning plate	Ag/*H-2* specificity	Units/ml IL-2 produced in response to Ag/H-2	Karyotype		
				Approximate total chromosome number	Number of metacentric chromosomes*	
					FS6	(16.17)
8Rb11.2	0.34	KLH/$H\text{-}2^{Rb}$	80	9	1	1
8Rb46.1	1.1	KLH/$H\text{-}2^{Rb}$	80	118	1	2
8Rb64.3	0.34	KLH/$H\text{-}2^{Rb}$	640	90	1	1
8Rb80.2	3.3	KLH/$H\text{-}2^{Rb}$	640	72	1	0
8Rb80.6	10	KLH/$H\text{-}2^{Rb}$	160	74	1	0
8Rb80.6.2	1.1	KLH/$H\text{-}2^{Rb}$	80	73	1	0

* Metacentric chromosomes were noted to be either of the FS6 type (Fig. 1A) or of the Rb(16.17)7 type (Fig. 1C).

that could present Ag in the context of the Robertsonian *H-2* type ($H\text{-}2^{Rb}$) to our Rb(16.17)7 T cell hybrids. These were used in all further experiments.

Of 56 T cell hybrids tested for Ag/*H-2*-reactivity after this fusion, 53 reacted. Some of these were karyotyped, and on the basis of both reactivity and the presence of potentially interesting karyotypes, four lines were cloned. Data from sample clones are shown in Table III.

Clones from all four lines responded to KLH in the presence of cells bearing the

$H\text{-}2^{Rb}$. Clones derived from the 8Rb80 line were of particular interest because 15 of 16 clones tested responded to KLH in the context of $H\text{-}2^{Rb}$ type and no chromosome spreads from these 16 clones contained Robertsonian metacentrics. 8Rb80.2 is given as an example of this and a representative karyotype is shown in Fig. 4. Unfortunately, 8Rb80 had a very poor plating efficiency on initial cloning, ~1%. Clones grew up only on plates innoculated with 10 or 3.3 cells/well. To be sure that some very potent responder, containing a Robertsonian metacentric chromosome, was not present as a contaminant in our clones of this line, one clone, 8Rb80.6 was recloned. The cloning efficiency of this line was still poor, ~3%. Of six subclones picked from plates innoculated with 0.36 or 1.1 cells/well, three responded to KLH/$H\text{-}2^{Rb}$. None of these clones contained a metacentric chromosome derived from the Rb(16.17)7 T cell blasts. Data about one of these clones, 8Rb80.6.2, are shown in Table III. Taken together, these data suggest that cells of the 8Rb80 line were able to respond to KLH/$H\text{-}2^{Rb}$ in the absence of any chromosome 16 or 17 derived from its Ag/*H-2*-specific T cell blast parent.

Since $H\text{-}2^{Rb}$ had not been extensively studied, and certainly no *H-2* recombinants involving this *H-2* haplotype were available, it was not possible to prove formally that the 8Rb80 lines recognized KLH in the context of *I*-region products using recombinant animals. We therefore set out to demonstrate this by studying the inhibition of hybridoma recognition of KLH/$H\text{-}2^{Rb}$ by a collection of anti-*I* monoclonal antibodies. The results of the more interesting of these experiments are shown in Table IV.

8Rb64.2 and 8Rb80.6 showed similar patterns of inhibition. In both cases, responses were blocked with an anti-$I\text{-}A^{s,f}$ monoclonal, MKS4 (20), and with hybridoma antibodies known to be directed at a number of different *I-A* haplotypes, M5/114 and 10-3.6 (28, 29), and 8KP, which reacted strongly with $I\text{-}A^{b,f,q,r,s}$ and weakly with an I^k molecule (Dr. C. A. Janeway, Yale University Medical School, personal communication). No inhibition was seen with an anti-*I-E* reagent, Y17, which recognized *I-E* molecules of many different haplotypes (31). This suggested that both 8Rb64.2 and 8Rb80.6 recognized KLH in the context of $I\text{-}A^{Rb}$ and certainly supported

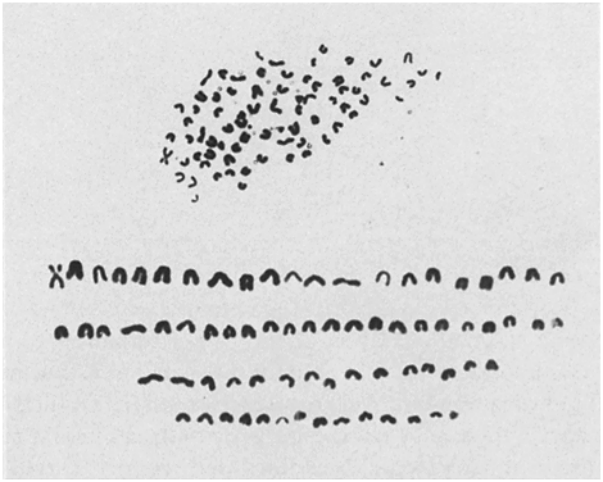

FIG. 4. Karyotype of the KLH/$H\text{-}2^{Rb}$ responsive hybridoma, 8Rb80.2, derived from Rb(16.17)7 T cell blasts.

TABLE IV

T Cell Hybridomas Derived from Rb(16.17)7 T Cell Blasts Recognized KLH in Association with I^{Rb}

Anti-*I* reagent added to IL-2 generation cultures	Known *I* specificity of reagent	Units/ml IL-2 produced in response to KLH/*H-2*^{Rb} by:		
		8Rb11.13	8Rb64.2	8Rb80.6
None	—	640	640	80
MKS4*	$I\text{-}A^{s,f}$	640	0	0
8KP	$I\text{-}A^{b,f,k,q,r,s}$	640	40	0
M5/114	$I\text{-}A^{b,d}$ $I\text{-}E^{d}$	40	640	40
10-3.6	$I\text{-}A^{k}$	320	80	0
MKD6	$I\text{-}A^{d,p,q}$	640	640	40
Y17	$I\text{-}E^{b/k,k,r,s/k,s/d}$	640	640	160

* Monoclonal antibodies were the gifts of Dr. C. A. Janeway (Yale University, New Haven, CT), Dr. T. A. Springer (Harvard Medical School, Boston, MA), and Dr. L. A. Herzenberg (Stanford University, Palo Alto, CA). Their properties are described in refs. 1 and 32–34.

the idea that Ag recognition by these hybridomas was *I* region-restricted. Ag/*H-2* recognition by 8Rb11.13 was blocked significantly by only one antibody tested, M5/114, and thus showed a different pattern of reactivity than either 8Rb64.2 or 8Rb80.6. The fact that M5/114 has been shown to bind to *I-A* and *I-E* molecules depending upon the *H-2* haplotype in question suggested that 8Rb11.13 was specific for KLH/$I\text{-}E^{Rb}$, but might simply indicate a different restriction site on *I-A* Rb for 8Rb11.13 as compared with the other two T cell hybridomas mapped. In any case, 8Rb11.13 seemed to be conventionally *H-2* restricted.

These conclusions were supported by another experiment, not shown here, in which the 8Rb T cell hybridomas were screened for their ability to respond to spleen cells from a panel of B10 congenic mice in the presence or absence of KLH. No reactivity was seen in the absence of KLH. In the presence of KLH, however, one hybridoma, 8Rb64.3, responded well to spleen cells from B10.S(7R) animals but not to B10, B10.D2, B10.M, B10.BR, or B10.AQR spleen cells, which again suggests that these hybridomas are restricted for Ag recognition by molecules mapping to the *I* region of $H\text{-}2^{Rb}$.

Discussion

Considerable interest centers on mapping genes that code T cell receptor(s) for Ag/*I*. In fact finding the chromosomes that do not bear the receptor(s) is probably as significant as finding the ones that do, depending on which these turn out to be. Various theoretical and experimental considerations have suggested that in the mouse genes controlling Ag/*I* receptors should be found on chromosomes bearing immunoglobulin heavy chain genes and/or MHC genes, i.e., chromosomes 12 and 17, respectively (6–12, 14–23). Since on occasion Ag-binding T cell products have been shown to bear immunoglobulin idiotypes thought to be controlled by a combination of immunoglobulin light and heavy chains, chromosomes bearing light chain genes, i.e., chromosomes 6 and 16 (21, 22), may also be implicated, although this is apparently not necessarily true (35). Since it is not clear how the free Ag- or *H-2*-binding T cells and secreted products that have often been used to establish the above

properties are related to T cell Ag receptors that are *I* restricted, it was of particular interest to examine the role of chromosomes 6, 12, 16, and 17 in T cell Ag/*I* recognition.

In somatic hybrids, T cell receptors could of course be mapped by conventional somatic cell genetics. For example, a cloned T cell hybridoma with Ag/*I* specificity could be grown for various lengths of time and recloned. The karyotypes of positive and negative subclones could then be compared, under the assumption that loss of Ag/*I* specificity would be the consequence of chromosome loss. Experiments of this type are underway in our laboratory in collaboration with Dr. Uta Francke at Yale. Unfortunately, because all normal mouse chromosomes are similarly telocentric, analysis of this type requires chromosome banding and careful examination, a lengthy and tedious procedure. No data are yet available from these experiments.

To find out more quickly whether particular chromosomes are involved in the T cell receptor(s) for Ag/*I*, we decided to make T cell hybridomas, using Ag/*I*-specific T cell blasts from Robertsonian mice that bear easily distinguishable metacentric chromosomes. KLH was chosen for these experiments because of its antigenic strength and complexity. Initially, the *H-2* haplotypes of the Robertsonian mice used in these experiments was unknown, but we felt that the use of an Ag such as KLH would probably circumvent any unexpected nonresponsiveness, such as that caused by immune response genes. T cell blasts from Robertsonian mice were thus primed with KLH, enriched for KLH/*I* reactivity, and fused to a T cell hybridoma. The T cell hybridomas resulting from these fusions were screened for KLH/*I* reactivity by their ability to secrete IL-2 in response to this combination and simultaneously karyotyped. After cloning and recloning, the presence of KLH/*I* reactivity and metacentric chromosomes derived from the normal T cell blast parent were correlated.

Using these procedures, we isolated cloned T cell hybridomas that could still respond to Ag/*I* but had lost all chromosomes 4 and 6 or 16 and 17, derived from their Ag/*I*-specific T cell blast parents. We therefore concluded that genes on these chromosomes probably did not contribute to the specific portion of the T cell receptor(s) for Ag/*I*. These hybrids presumably did retain, however, chromosomes 4, 6, 16, and 17, derived from their FS6.14.13.AG2.1 parent. It was unlikely that these chromosomes contributed any part of the specific portion of the Ag/*I* receptor(s), although some nonspecific but essential portion of the receptor(s) could be encoded on these chromosomes. This could include a membrane-anchoring peptide, for example, or a β_2-microglobulin-like structure.

Unfortunately, experiments testing the presence of T cell receptor genes on mouse chromosome 12 were hindered by our inability to obtain T cell hybridomas that had lost both (8.12) metacentric chromosomes derived from the normal T cell blast parent. Mouse chromosome 12 was known to be preferentially retained in mouse-hamster hybrids (Dr. Uta Francke, personal communication). The parent tumor cell line for the fusions described in this paper, BW5147, contained 4 chromosomes similar or identical to 12 out of a total of 44–49 chromosomes (30). Both these observations suggested that growth in tissue culture selected for multiple copies of chromosome 12 in cells, and indicated that the loss of this chromosome in hybridoma cells would not occur without a selection mechanism. Our future efforts will concentrate on establishing such a selection. Meanwhile, two conclusions about the involvement of chromosome 12 gene products in T cell Ag/*I* receptors were drawn from the results in this

paper. First, a T cell hybridoma was obtained which had retained both metacentric chromosomes (8.12) from its normal T cell parent but failed to secrete IL-2 in response to Ag/*I*. This T cell hybridoma had not lost the ability to secrete IL-2, since it produced this lymphokine in response to Con A. Additionally, it differed by only a few chromosomes from a subclone derived from the same parental hybridoma, which retained the ability to respond to Ag/*I*. This observation suggested that products of genes coded on chromosomes other than 8 or 12 might contribute to Ag/*I* recognition, although the involvement of products of genes on chromosomes 8 and 12 could not be excluded.

Second, one hybridoma was found that responded to Ag/*I* and contained only one chromosome (8.12) derived from the normal T cell blast parent, which suggested that both chromosomes 8 or 12 were not required by the T cell for full expression of Ag/*I* reactivity.

Preliminary experiments, not reported in this paper, have also suggested that genes on the X chromosome did not code for specific portions of the Ag/*I* receptor(s), since Ag/*I*-specific T cell hybridomas could easily be back-selected for azaguanine resistance and HAT sensitivity, properties that are presumably dependent on the loss of all normal X chromosomes and retention of the $HGPRT^-$ X chromosome derived from BW5147. Azaguanine-resistant hybridomas of this type very often retained Ag/*H-2* specificity (1).

One important proviso should be mentioned about our experiments. All interpretations depended on the fact that translocations of DNA did not occur from metacentric chromosomes to other chromosomal material. Translocations of this type, unless they resulted in very unusual-looking chromosomes, would be unnoticed in our analysis, and indeed, may or may not be visible in more careful banding analysis of the chromosomes. Studies to examine this potential problem will be carried out in the future. Additionally, there is the possibility that the Robertsonian metacentrics might disintegrate to telocentric chromosomes in hybridomas. This is a phenomenon that has never been seen before with these types of chromosomes in tissue culture (Dr. Eva Eicher, personal communication) and we think it is unlikely in this case. Once cloned, our cells, whether or not they contained metacentric chromosomes, retained remarkably consistent karyotypes. Subcloning revealed that a few chromosomes might be lost, but on the whole, startling chromosomal abnormalities or disintegrations of metacentric chromosomes were not apparent even after prolonged culture. As we continue our experiments, these possibilities will be eliminated by the identification of multiple, independent occurrences of the same phenomena.

There is one other possibility that might invalidate our conclusions and in fact make mapping of the T cell Ag/*I* receptor(s), given our current knowledge of these structures, almost impossible. We do not know whether receptors of this type are made up of several equivalent chains, only one of which is used for each clonally distributed receptor. In the case of immunoglobulins, for example, each receptor is composed of a heavy chain, the genes for which are on chromosome 12, with one of two types of light chains encoded on either chromosome 6 or 16. If this is also true for T cell Ag/*I* receptor(s), analysis could be made impossibly difficult by our not knowing the type of complications to expect. By chance, 3Rb22.1.14 may have had a light chain-like component to its receptor(s) not encoded on chromosome 6, and 8Rb80.2 may have had a light chain-like component not encoded on chromosome 16. Until loss of

specificity is correlated with loss of a particular chromosome, we cannot be certain that this is not the case.

Overall, however, we would like to point out that our results suggested that genes encoded on chromosomes 4, 6, 16, and 17 of the mouse did not contribute to the specific portion of Ag/*I* receptor(s) on T cells. This suggested that κ, λ, and *H-2* encoded genes were not involved in these structures. Moreover, the data indicated that gene products other than those on chromosomes 8 and 12 contributed to T cell Ag/*I* specificity, although the additional contribution of gene products from chromosomes 8 and 12 has not been eliminated. Our future experiments will be devoted to confirming and extending these results.

Summary

Keyhole limpet hemocyanin (KLH)/*I* region-specific T cell hybridomas have been prepared by fusing KLH/*I*-specific T cell blasts from mice with single pairs of metacentric chromosomes to the inducible, interleukin 2 (IL-2)-secreting T cell hybridoma FS6-14.13.AG2.1. T cell hybridomas with KLH/*I* receptors were identified by their ability to secrete IL-2 in response to KLH and the appropriate antigen-presenting cells. After cloning and subcloning, KLH/*I* reactivity was correlated with the presence or absence of metacentric chromosomes derived from the KLH/*I*-specific T cell blast parent. Hybridomas were identified that had lost all chromosomes 4 and 6 or 16 and 17 derived from their normal T cell parent, but retained the ability to respond to KLH/*I*. This suggested that products of genes on these chromosomes did not contribute to the specific portions of T cell Ag/*I* receptors. These gene products would include, of course, κ and λ chains and *H-2*.

We did not obtain any T cell hybridomas that had lost both metacentric (8.12) chromosomes derived from T cells of the Robertsonian mouse strain Rb(8.12)5, so we could not draw any conclusions about the contributions of products of genes on these chromosomes. T cell hybridomas with KLH/*I* reactivity were found that contained only one metacentric (8.12) chromosome derived from this strain. Moreover, a T cell hybridoma was found that retained both metacentric (8.12) chromosomes from its normal T cell parent, but had lost KLH/*I* reactivity. These results suggested that neither two chromosomes 8 nor two chromosomes 12 were required for antigen/*I* reactivity in normal T cells and that antigen/*I* reactivity was controlled, at least in part, by genes mapping on chromosomes other than 8 or 12.

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