TWO SEPARATE GENES REGULATE SELF-Ia AND CARRIER RECOGNITION IN *H-2*-RESTRICTED HELPER FACTORS SECRETED BY HYBRIDOMA CELLS

By PETER LONAI,* SHOSHANA BITTON, HUUB F. J. SAVELKOUL,‡ JOSEPH PURI, and GÜNTER J. HÄMMERLING

From the Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel, and the Institute of Immunology and Genetics, German Cancer Center, Heidelberg, Federal Republic of Germany

Since the discovery that T cells recognize antigen in conjunction with major histocompatibility complex (MHC)[1] products, most workers have assumed that the problem as to whether this is due to two receptors recognizing two determinants (dual recognition) or to one receptor recognizing a neoantigen (altered self) could be solved by means outside classical cellular immunological methods (1, 2). In the past few years significant advances were made in T cell culture methods. Today these cells can be grown as growth factor-dependent continuous lines (3) or as somatic cell hybrids (4). These clonable lines represent homogeneous sources of the T cell receptor. The hybridoma technology used in this context to "immortalize" T cells is a somatic genetic approach, suitable for analyzing whether a trait is controlled by one or more loci. This question appears to be at the center of the altered self/dual recognition controversy. As an initial step in solving this problem, we studied *H-2*-restricted carrier-specific helper factors secreted by *H-2* heterologous T cell hybrids.

We recently reported (5) the isolation of a number of *H-2* homologous and heterologous helper factor producing T cell hybrids. One of these, deriving from *H-2* homologous fusion partners, was described in detail (6). The factor of this clone, similarly to T cell factors and receptors, contained determinants shared with immunoglobulin heavy chain variable regions (IgV_H) (6–11) and also with Ia antigens. Significantly, its helper activity was *H-2* restricted (6). In our experiments presented here, we were guided by the idea that the factors secreted by the *H-2* heterologous clones, if they are also *H-2* restricted, could be used to study whether both *H-2* genomes of the fusion partners are involved in *H-2*-restricted helper factors. Insofar as an *H-2*-restricted T cell factor represents the receptor of the cells secreting it, such an approach could be helpful in understanding *H-2* restriction in general.

* Address correspondence to P. Lonai, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

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[1] *Abbreviations used in this paper:* CGG, chicken gamma globulin; C_H, immunoglobulin heavy chain constant region; HGG, human gamma globulin; IgV_H or V_H, immunoglobulin heavy chain variable region; MHC, major histocompatibility complex; NIP, (4-hydroxy-5-iodo-3-nitrophenyl) acetyl; NP, (4-hydroxy-3-nitrophenyl) acetyl; OVA, ovalbumin; PFC, plaque-forming cells; V_κ and V_λ, variable region of κ or λ light chains, respectively.

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We assumed that a hybridoma constructed by fusing chicken gamma globulin (CGG)-immunized C57BL/6 (*H-2*^{b}) mouse T cells with BW-5147 (AKR, *H-2*^{k}) murine lymphoma contains only one CGG-specific locus but might contain two self *H-2*-specific loci. If such a clone secretes two CGG-specific factors, one restricted for *H-2*^{b} and the other for *H-2*^{k}, this would indicate that the anti-carrier locus and the anti-self *H-2* loci are genetically independent. Such independence suggests that the dual specificity of *H-2*-restricted T cell factors is determined by two specific units that are controlled by separate genes and supports the validity of dual recognition concepts. Moreover, the experiment could also provide information on the V_H or *Ia* nature of these units.

We report results obtained from studying a number of *H-2* heterologous hybridoma clones, each of which produces two helper factors, one restricted to the normal cell partner and the other to the lymphoma partner of the fusion hybrid.

Materials and Methods

Mice. C57BL/6, C57BL/10, C3H.SW (*H-2*^{b}); B10.BR, C3H/DiSn (*H-2*^{k}); B10.A (4R) (*H-2*^{h4}), C3H.Q (*H-2*^{q}), B10.M (*H-2*^{f}); (C57BL/6 × BALB/c)F_1 (*H-2*$^{b/d}$), (C3Heb × BALB/c)F_1 (*H-2*$^{k/d}$), and (C3Heb × C57BL/6)F_1 (*H-2*$^{k/b}$) mice, bred at the Laboratory Animal Breeding Center of The Weizmann Institute, were used at an age of 2–3 mo.

Immunization and In Vitro Helper Assay. These were described previously (5, 6, 12). Briefly, anti-(4-hydroxy-5-iodo-3-nitrophenyl)acetyl (NIP) antibodies were detected by counting NIP-specific plaques produced by NIP-ovalbumin (OVA)-primed spleen cells in the presence of hybridoma-derived helper factors or CGG-specific syngeneic T cells. NIP-CGG was added to the cultures.

Construction of the Hybridoma Cells. Construction of the hybridoma cells was described previously (5). All lines and clones used were derived from the fusion of BW-5147 lymphoma cells and NP-CGG-primed C57BL/6 splenic T cells.

Designation of the Hybridoma Clones. The designation of the hybridoma clones expresses their origin and stage of cloning. For example, in the designation T77-146-C3/3, the first three characters stand for the fusion experiment, the second group for the well from which the line was isolated, and the third signifies the first limiting dilution cloning, whereas the last signifies the second limiting dilution cloning. Because after fusion the cells were seeded at a density that allowed growth in not more than 60% of the wells, a clone like T77-146-C3/3, for all purposes, underwent three cycles of limiting dilution cloning.

Serological Reagents. Anti-V_H-, anti-variable region of the λ light chain (V_λ), and anti-variable region of the κ light chain (V_κ) specific affinity-purified antibodies were prepared by immunizing rabbits with the appropriate myeloma protein fragments, as previously described (13–15). Previous experiments have suggested that anti-$V_{H\text{-}M315}$ have a selectively lower affinity to AKR V_H than to the V_H region of C57BL/6 or other mouse strains. This allotype-like characteristic is a unigenic trait linked to the *Igh-1* allotype (16).

Monoclonal anti-Ia antibodies were purified from B-hybridoma culture fluids. The antibodies used had the following specificities: anti-Ia.ml (anti-I-A^k, clone H 118-490 R1); and anti-Ia.m4 (anti-I-A^b, clone B 17-123 R2). These antibodies have been described previously in detail (17).

H-2K^k, H-2K^b (+I-A^b), H-2D^k, and H-2D^b antigen expression on the hybridoma cells was detected by a complement-dependent cytotoxic assay using the following respective alloantisera: (A.TL × C3H.OL)F_1 anti-C3H, (B10.A(4R) × A.SW)F_1 anti-C57BL/10, (A.TL × B10.A)F_1 anti-B10.BR, and (B10.A(5R) × A/J)F_1 anti-B10.A(2R), which were gifts from Dr. D. B. Murphy and Dr. H. O. McDevitt of Stanford University, Stanford, Calif.

Affinity Chromatography. Affinity chromatography on antibody-Sepharose and on normal spleen cells has been described previously (5, 6, 12).

Results

Antigen Specificity and Titer of the Helper Factors. Table I shows a representative experiment that demonstrates carrier (CGG)-specific helper activity in the CGG-

TABLE I

*Carrier Specificity and Titer of Helper Factors Secreted by Clone T77-146-C3**

	PFC/10^6 cells in the presence of	
	NIP-CGG	NIP-HGG
B cell control	25 ± 13	33 ± 12
T cell help‡	280 ± 8	263 ± 20
Factor-CGG-Sepharose eluate, dilution 10^{-2}	250 ± 30	56 ± 18
Factor-CGG-Sepharose eluate, dilution 10^{-3}	196 ± 52	12 ± 20
Factor-CGG-Sepharose eluate, dilution 10^{-4}	206 ± 20	20 ± 10
Factor-CGG-Sepharose eluate, dilution 10^{-5}	230 ± 15	40 ± 30
Factor-CGG-Sepharose eluate, dilution 10^{-6}	220 ± 21	15 ± 12
Factor-CGG-Sepharose eluate, dilution 10^{-7}	100 ± 30	ND§
CGG-Sepharose effluent, dilution 10^{-2}	75 ± 13	98 ± 36
CGG-Sepharose effluent, dilution 10^{-3}	20 ± 12	25 ± 15
CGG-Sepharose effluent, dilution 10^{-4}	60 ± 15	15 ± 8

* (C3H × B6)F_1 B cell source was used.
‡ Separate CGG- or HGG-specific helper cell sources.
§ Not done.

TABLE II

Genetic Restriction of Helper Factor T77-146-C3

B cell source	*H-2*	B cell control	T cell help	Helper factor
			PFC/10^6	
C3H/DiSn	*k*	35 ± 22	215 ± 34	203 ± 27
B10.A(4R)	h^4	44 ± 18	306 ± 12	424 ± 49
C3H.SW	*b*	54 ± 31	428 ± 52	616 ± 68
C57BL/10	*b*	44 ± 20	366 ± 23	327 ± 41
C3H.Q	*q*	176 ± 45	860 ± 98	185 ± 22
B10.M	*f*	89 ± 32	327 ± 67	66 ± 12

Sepharose-purified supernatant of one of the *H-2* heterologous hybridoma clones, T77-146-C3. It can be seen that the factor did not have significant helper effect in the presence of NIP-human gamma globulin (HGG), a closely related but heterologous carrier coupled with the homologous hapten. Binding of the factor to CGG-Sepharose also demonstrates its carrier specificity. Also shown is that these cells secrete helper factors in very high titers. In all of the subsequent experiments to be reported, the factors were used at the dilutions of 10^{-3} or 10^{-4}. Experiments with other *H-2* heterologous clones gave similar results (data not shown).

Two H-2-restricted Helper Factors in the Culture Fluid of Clone T77-146-C3. H-2 restriction was investigated by using B cell sources of different genetic origins. Table II demonstrates that the supernatant of this clone, which derives from the fusion of an $H\text{-}2^b$ T cell with an $H\text{-}2^k$ lymphoma, was active only towards B cell sources expressing $H\text{-}2^k$ or $H\text{-}2^b$ gene products but not towards those that express $H\text{-}2^q$ or *f* ($H\text{-}2^q$ and *f* were chosen as foreign *H-2* types because they contain the least shared Ia specificities with haplotypes *b* and *k* [18]). The results shown in Table I demonstrate that the culture fluid has activity also towards $H\text{-}2^{k/b}$ F_1 hybrid antibody-forming cells. It follows that T77-146-C3 helps its parental *H-2* types and their F_1 hybrid but not antibody-forming cells of unrelated *H-2* types. This finding is compatible with the assumption but does not prove that the clone produces two helper factors, each

specific for the carrier, CGG, and restricted by the *H-2* type of one of the two parental cells.

To investigate this assumption, we absorbed the supernatant on normal spleen cells of C3H/DiSn ($H\text{-}2^k$) or C3H.SW ($H\text{-}2^b$) mice, and the absorbed culture fluid was then tested on B10.A(4R), $H\text{-}2^{h4}$ (a recombinant of $H\text{-}2^b$, and $H\text{-}2^a$ between subregions *I-A* and *I-B*) and C57BL/10 ($H\text{-}2^b$) antibody-forming cultures (Fig 1.) This experiment was based on previous results (6) with a genetically restricted hybridoma that was constructed from $H\text{-}2^k$ homozygous cells; the helper factor of this clone, T85-109-45, could be absorbed on spleen cells that were syngeneic with it at $I\text{-}A^k$.

$H\text{-}2^k$ spleen cells removed the activity of factor T77-146-C3 for B10.A(4R) B cells from the culture fluid but not the activity for C57BL/10 B cell sources. The opposite result was obtained with culture fluids absorbed on spleen cells of the $H\text{-}2^b$ haplotype. Here, the absorbed culture fluid lost activity for the $H\text{-}2^b$ B cell source but remained active with B10.A(4R) cultures. These results demonstrate that the culture fluid of the *H-2* heterologous hybridoma contains two separate *H-2*-restricted helper factors. One of them expressed the anti-self specificity of the normal T cell partner ($H\text{-}2^b$) of the hybridoma, whereas the other expressed that of the BW-5147 lymphoma cell ($H\text{-}2^k$). Moreover, because the *H-2* genotype of B10.A(4R) is K^k, A^k, B^b, J^b, E^b, S^b, D^b, the results also suggest that the restriction maps to the left side of the *H-2* complex.

Association of Self Recognition with Ia. The association of self recognition with Ia was investigated by separating the two helper activities of clone T77-146-C3 on monoclonal antibody affinity columns specific for products of the *I-A* subregion of haplotype *k* or *b*. The effluents and the 0.1 N NH_4OH eluates from both columns were each probed in vitro with C3H.SW ($H\text{-}2^b$) or C3H/DiSn ($H\text{-}2^k$) spleen cells as the B cell source.

The results demonstrated that both Ia^b- and Ia^k-bearing factors are present in the culture fluid of T77-146-C3 (Fig. 2). Each column removed the helper activity towards the B cell source that carried the *H-2* haplotype corresponding to the specificity of the affinity column, and this activity could be regained in a purified form by elution from the immunosorbent.

These data suggest that clone T77-146-C3 secretes two *H-2*-restricted helper factors, one specific for CGG+H-2^b and the other specific for CGG+H-2^k. It is also evident

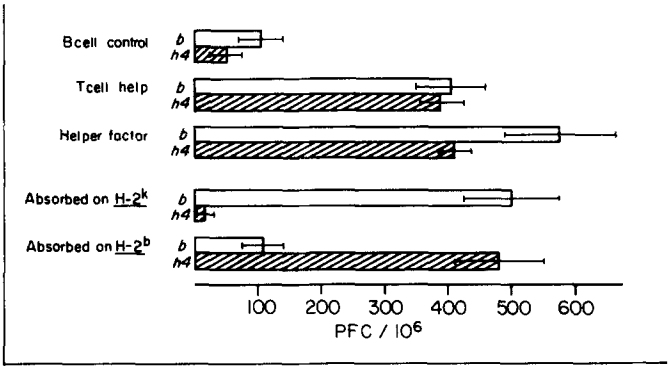

FIG. 1. Separation of an $H\text{-}2^b$- and an $H\text{-}2^k$-restricted helper factor from the culture fluid of clone T77-146-C3 by adsorbtion on C3H ($H\text{-}2^k$) or C3H.SW ($H\text{-}2^b$) spleen cells. The haplotype symbols in the second column define the origin of the B cell source.

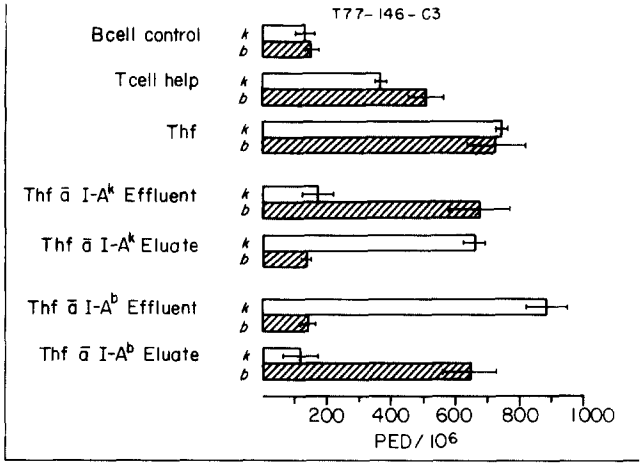

FIG. 2. Separation of *H-2*^{b} and *H-2*^{k}-restricted helper factors on anti-I-A^k and I-A^b-specific affinity columns (clone T77-146-C3). The haplotype symbols in the second column define the origin of the B cell source.

TABLE III

Selection of Subclones, the Factor of Which Reacts with Both Parental Haplotypes

	B cell source		Type
	H-2$^{b/d}$	*H-2*$^{k/d}$	
	PFC/10^6		
B cell control	110 ± 31	102 ± 23	—
T cell help	450 ± 65	380 ± 38	—
Factor T77-146-C3	378 ± 39	490 ± 68	b^+,k^+
C3/3	400 ± 59	371 ± 41	b^+,k^+
C3/6	168 ± 16	115 ± 43	b^-,k^-
C3/9	368 ± 43	391 ± 57	b^+,k^+
C3/10	314 ± 71	315 ± 22	b^+,k^+
C3/11	329 ± 30	172 ± 19	b^+,k^-
Factor T86-40-10	439 ± 65	397 ± 15	b^+,k^+
Factor T86-54-15	473 ± 42	356 ± 46	b^+,k^+
Factor T86-54-33	362 ± 41	94 ± 20	b^+,k^-

that the CGG+H-2^b-specific factor contains determinants controlled by *I-A*^{b}, whereas the CGG+H-2^k-specific factor determinants were controlled by *I-A*^{k}. Therefore, the antigen specificity of each factor is associated with its *Ia* type.

One could argue that this finding is due to clone T77-146-C3 containing two separate *H-2*-restricted cell populations, one restricted for *H-2*^{k} and the other for *H-2*^{b}, which have arisen from mutations in the original clone. To investigate the validity of this argument, subclones of T77-146-C3, isolated close to the establishment of the original clone, as well as helper clones deriving from an independent fusion of CGG-specific C57BL/6 T cells and BW-5147 (5), were investigated.

Table III demonstrates the results of an experiment that investigated the helper activity in nine of these *H-2* heterologous clones and subclones. Six of nine clones were active in helping both *H-2*$^{b/d}$ and *H-2*$^{k/d}$ B cell sources. These clones could be

k^+, b^+ double producers. One culture appeared to be inactive, and two produced a helper factor restricted only to the *H-2* type of the normal parental T cell (C57BL/6, *H-2*^{b}) of the hybridomas.

Four of the six b^+, k^+ clones were tested for the association of self recognition with Ia. Their culture fluids were fractionated on *I-A*^{k}-specific affinity columns. The results showed that the affinity column retained the helper factor with anti-*k* self specificity but passed the *H-2*^{b} restricted helper factor. *H-2*^{k} self specificity could be eluted from the column bound fraction, and the eluate was devoid of any *H-2*^{b}-restricted helper activity (Fig. 3). Similar results were obtained with all four clones.

It follows from the data in Fig. 3 that the four additional hybridoma clones also produce two *H-2*-restricted factors, as does clone T77-146-C3. Hence, secretion of two helper factors by *H-2* heterologous hybridoma cells, each restricted to one of the two parental *H-2* haplotypes, appears to occur frequently.

Both Helper Factors Contain Similar V_H Determinants. Anti-$V_{H\text{-}315}$ recognizes allotype-like *Igh-1*-linked determinants in V_H and reacts weakly or fails to react with AKR heavy chains or AKR T cell receptors (10, 13, 16, 19; see also Materials and Methods). This characteristic of anti-V_H was used to investigate the presence of BW-5147 (AKR)-type V_H products in the helper factors secreted by clone T77-146-C3.

CGG-Sepharose-purified culture supernatant was passed on an anti-V_H-Sepharose affinity column and tested on (C3H × C57BL/6)F_1 cultures at the dilution of 10^{-3}. Previously, it was shown that the same purified factor preparation had detectable activity up to dilutions of 10^{-5} to 10^{-6} in the same assay system (Table I). The results

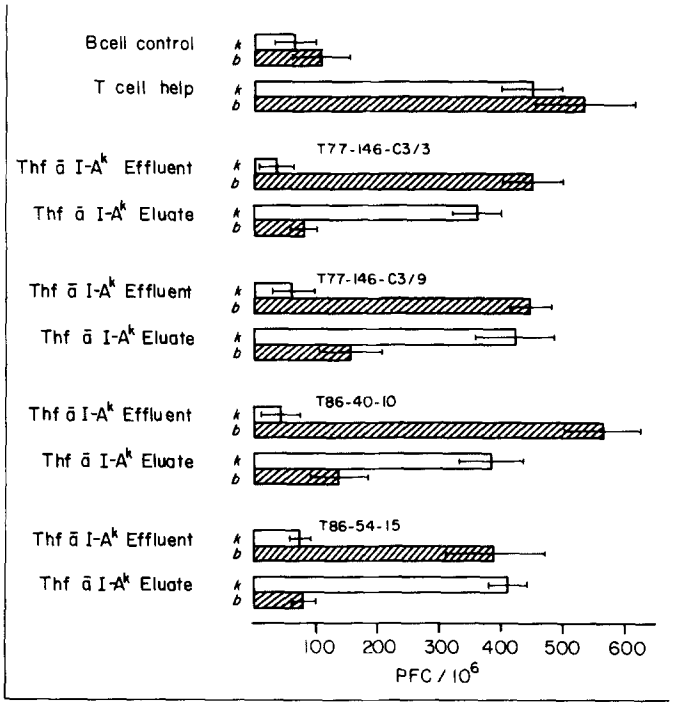

FIG. 3. *H-2* heterologous hybridomas that produce two *H-2*-restricted helper factors; a test of four additional clones and subclones. The haplotype symbols define the origin of the B cell source.

shown in Table IV demonstrate that all of the activity was bound by anti-$V_{H\text{-}315}$, suggesting that it is unlikely that one of the helper factors contains only AKR V_H. As controls, anti-V_λ and V_κ columns were used, and the results show that the factors of this clone, similarly to other *H-2*-restricted helper factors and helper cell receptors (5, 11), do not contain Ig light chain V-region determinants.

Double Producer Hybridoma Clones Express H-2K and D Specificities of Both Fusion Partners. To evaluate the phenomenon reported here, it is important to know whether double producer somatic hybrid clones carry both parental *Igh-1* and *H-2* loci. In the present study, we could test only the expression of the *H-2* gene products. Table V demonstrates that all of the five hybridoma clones, which were studied above in detail, express antigens controlled by both the *k* and the *b* allele of *H-2K* and *H-2D*. It can be observed that T86 clones appear to express less *H-2* antigens than T77-146 subclones. Additional experiments have shown that the T86 clones express low amounts of Ia^b and Ia^k, whereas the T77 series was *Ia* negative in this test (results not shown).

Discussion

The hybridoma-derived helper factors described here and previously (6) are *H-2* restricted, insofar as they affect only those B cell sources that share the left part of the *H-2* complex with the genotype of the hybridoma cells. This phenomenon is similar to *H-2* restriction as observed in various other systems, wherein T cells appear to interact directly with other cells. Therefore, it can be described operatively as a

TABLE IV

*Expression of the $V_{H\text{-}M315}$ Marker in Factors Secreted by Clone T77-146-C3**

	PFC/10^6
B cell control	75 ± 50
T cell help	854 ± 39
CGG-Sepharose eluate (diluted 10^{-3})‡	497 ± 68
Anti-V_H effluent	62 ± 28
Anti-V_λ effluent	537 ± 55
Anti-V_κ effluent	654 ± 36

* The B cell source is (C3H × C57BL/6)F_1.

‡ The same preparation was also used in the experiment shown in Table I. In other experiments, complete elution of the helper factor from anti-V_H-Sepharose was demonstrated (data not shown).

TABLE V

Expression of Both Parental H-2K and D Loci in Double Producer Hybridomas

Clone	Cytotoxic index			
	H-2K^b	H-2K^k	H-2D^b	H-2D^k
T77-146-C3	77	99	96	81
T77-146-C3/3	100	97	93	94
T77-146-C3/9	100	99	99	96
T86-40-10	19	22	60	73
T86-54-15	40	27	56	34

combined effect of carrier-specific and self Ia-specific units in the specificity defining part of the helper factor. Our experiments were planned to analyze whether these operative units are controlled by one or by more than one locus.

We found that each of five *H-2* heterologous hybridoma clones produced two CGG-specific helper factors, one restricted to the *H-2* type of the normal cell partner, whereas the other restricted to the *H-2* of the lymphoma cell partner. This suggests that both the normal and the lymphoma cell partner of the fusion hybrid had the potential to express its anti-self *H-2* property in the helper factor.

The identical carrier specificity of both factors suggested that each allelic anti-self product was associated with an anti-CGG product. The question is, which of the two parental cells donated this anti-CGG receptor? Two arguments support the notion that only the one derived from the normal cell partner could be active in the hybridoma clones. First, it is unlikely that the BW-5147 lymphoma, which has no known antigenic specificity and neither produces CGG-specific helper factors nor binds CGG (5), could contribute an anti-CGG locus. Second, even if the BW-5147 genome had expressed CGG and anti-self ($H\text{-}2^k$)-specific V_H products, such a factor most likely would not have been retained by our anti-V_H-Sepharose columns. The reason for this is that anti-V_H has a selectively weak reaction with AKR heavy chains (13) and helper T cell receptors (10), and more recently it has been reported (19) that anti-V_H does not react with receptors isolated from AKR T cells.

It follows that the $H\text{-}2^b$-restricted factor carried C57BL/6-derived anti-carrier and anti-self products, whereas the $H\text{-}2^k$-restricted factor was the product of a C57BL/6-derived anti-carrier locus and an AKR-lymphoma-derived anti-self locus. This shows that the anti-self and anti-nominal antigen units are products of two independent genes. This conclusion demonstrates the validity of dual recognition concepts for the case of *H-2*-restricted helper factors.

To identify the products of these anti-carrier and anti-self loci in the helper factors, we have to rely on two known markers of T cell factors, V_H and Ia. There is evidence that cross-reactive heavy chain idiotypes and V_H framework regions are involved in the receptors and factors of T cells (7–11, 16). It is, however, not known whether both the anti-nominal and the anti-self receptor might be V_H-like. Our anti-Ia affinity separation data demonstrate that anti-self specificity in the factors is associated with the corresponding *Ia* antigens. Hence, they formally suggest that self recognition may be mediated by *Ia*. This conclusion, however, is not unequivocal because the Ia antigens of these factors could have been bound by V_H type anti-self receptors specific for the *Ia* type of the appropriate parental cell.

Therefore, there are at least two alternative hypotheses for the anti-self receptor in these helper factors. It could be either an *H-2* gene product or a V_H-like product. The first hypothesis suggests a genetically simpler situation than the separate rearrangement of two V_H regions of different specificity in one genome, and it explains the involvement of *Ia* antigens in antigen-specific T cell factors. This hypothesis also assumes a more general role for the MHC.

According to the second hypothesis, two V_H-like receptors are sufficient for *H-2*-restricted antigen recognition. Therefore, the presence of Ia antigens in *H-2*-restricted T cell factors could be functionally irrelevant, a question that was raised by Binz et al. (20). At present the two hypotheses have equal validity in explaining *H-2* restriction in T cell factors. The choice between the two interpretations could be decided by

biochemical and cytogenetic studies with T cell hybrids, in which the segregation of the relevant loci could be studied.

The mechanism of action of these helper factors can be interpreted according to the two hypotheses. The second hypothesis suggests that the factor recognizes antigen by two V_H products on the surface of B cells or possibly macrophages. As the most likely mechanism derived from the first hypothesis, it can be suggested that the factor might include a carrier-specific V_H-like product and Ia. This Ia part could recognize or could be recognized by determinants on the cooperating cells. Ia antigens could also have a stabilizing effect on the carrier-specific portion, which could explain how a supposedly low affinity receptor (21) binds antigen in the absence of MHC and coupled to Sepharose. Similar explanations were offered for antigen-specific T cell factors by Tada and Okumura (22) and by Droege (23).

A considerable number of reports (reviewed in 22) have demonstrated that T cell factors are composed from products of two separate *V_H* and *H-2I* genes. As a role for Ia antigens, it was suggested that they might have effector functions, such as defining the mode of action (suppression or help) and genetic restriction. This role of Ia antigens was contrasted by some with the antigen specificity of the V_H portion; they suggested that it might be functionally similar to the Fc portion of immunoglobulins (24). Our studies demonstrate that the anti-self and anti-carrier specificity of *H-2*-restricted T cell factors are controlled by two independent specificity-defining loci. Hence, if the dual specificity of these factors is not mediated by two V_H components, the Ia moiety should have a more specific role than that of defining effector functions similarly to immunoglobulin Fc, and allosteric portions might define the mode of action.

Our results are directly relevant only to *H-2*-restricted T cell factors. Because it is not known whether T cell factors represent secreted T cell receptors, it is not clear how pertinent they are for the cellular receptor. The possibility that the alternatives raised for helper factors should also be applicable to cellular antigen receptors is supported by antigen-binding experiments with the same hybridoma clones. Clone T77-146-C3 ($H\text{-}2^{b/k}$) binds CGG processed on $H\text{-}2^{b}$, $H\text{-}2^{k}$, or $H\text{-}2^{b/k}$ macrophages but not CGG processed on $H\text{-}2^{q}$ or $H\text{-}2^{f}$ macrophages (25). This finding suggests that the receptors could be products of a similar intragenomic segregation, as are the factors, and supports the validity of dual recognition concepts for the receptor of these cells.

Arguing against the possibility that Ia antigens are involved in the receptor are reports on the isolation of T cell receptors that did not contain Ia antigens (20, 26). Although it cannot be dismissed that these negative findings were due to problems of receptor isolation, one has to consider that T cell factors contain only the anti-carrier receptor of T cells coupled with Ia antigens, whereas the cellular receptor might be the product of the V_H loci of different specificity (23).

A recent report on T cell hybrids containing two antigen-specific and a lymphoma genome characterized by *H-2*-restricted lymphokine production is of interest because no segregation was observed between the original self Ia and antigen specificities in these clones (27). It is possible that homologous allelic products have higher mutual affinity than heterologous ones. Also, if this phenomenon was due to two specific gene products, it is possible that they could not separate and recombine in the membrane of the cells described.

To clarify the relationship between factors and membrane-bound T cell receptors,

it would be important to know whether the anti-self specificity of the factors is a trait that can be acquired during differentiation in a semiallogeneic environment, similar to the "learning" of restriction specificity by chimera-derived T cells (28). A simpler solution to the problems discussed above could be found by determining what effect the loss of chromosome 12 (*Igh-1*) or 17 (*H-2*) has on cellular antigen binding and factor-mediated help of our T cell hybrids.

Summary

H-2 heterologous T cell hybridomas were used to study the genetic control of dual, anti-nominal antigen and anti-self *H-2* specificity of *H-2*-restricted T cell factors. Each of four hybridoma clones produced two helper factors. One was restricted for the *Ia* type of the normal T cell partner ($H\text{-}2^b$), whereas the other was restricted for the *Ia* type of the lymphoma partner ($H\text{-}2^k$) of the somatic hybrid. This was shown by affinity separation on parental type spleen cells and on monoclonal anti-I-A-Sepharose. Both factors had carrier (chicken gamma globulin; CGG)-specific helper effect, and both bound to anti-$V_{H\text{-}315}$-Sepharose. Because the lymphoma (BW-5147) partner could not contribute a CGG-specific locus, the $H\text{-}2^k$-restricted, CGG-specific factor had to be the product of segregating anti-nominal and anti-self loci. This suggests that dual specificity is due to two independent loci and supports the validity of dual recognition concepts. Anti-self specificity was associated with homologous *Ia* alloantigens in the individual factors. Therefore, *Ia* and anti-self might be linked. Implications of the major histocompatibility complex or V_H nature of anti-self receptors and the relationship of T cell factors and receptors was discussed.

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