H-40, AN ANTIGEN CONTROLLED BY AN *Igh* LINKED GENE AND RECOGNIZED BY CYTOTOXIC T LYMPHOCYTES

I. Genetic Analysis of *H-40* and Distribution of Its Product on B Cell Tumors

By JAMES FORMAN, ROY RIBLET, KATHRYN BROOKS, ELLEN S. VITETTA, and LEE A. HENDERSON

From the Department of Microbiology, University of Texas Health Science Center at Dallas, Texas 75235; and the Institute for Cancer Research, Philadelphia, Pennsylvania 19111

The *Igh* complex is on the murine 12th chromosome and contains the genes (*Igh-C*) that encode the constant (C) portion of μ, δ, γ, α, and ϵ immunoglobulin (Ig) heavy chains together with *J*, *D*, and *V* region genes that encode their corresponding variable (V) regions (1–3). In addition to these *Igh* genes, there are several other linked genes, some of which may play a role in immune reactivity. For example, a series of antigens have been described: Tsu, Tind, Tthy, and Tpre, which are controlled by genes that map telomeric to the *Igh* locus (reference 4, and R. Riblet, E. Eicher, and B. Taylor, unpublished data). These antigens are limited in their tissue expression to T cell subsets and have been detected on T cell factors that have immunoregulatory activity (5–7). Lyb-7 is an antigenic determinant controlled by a gene linked to the *Igh-V* side of the complex and is selectively expressed on a B lymphocyte subset (8). In contrast, the *Igh*-linked minor histocompatibility *H(Igh)* (9) and prealbumin (*Pre-1*) genes (10) presumably encode molecules that are unrelated to the immune system.

We have previously observed that immunization of C.B-20 (Igh^b) mice with *Igh* congenic spleen or surface (s) Ig^+ tumor cells of BALB/c (Igh^a) origin elicits both tumor rejection and the generation of cytotoxic T lymphocytes (CTL)[1] that recognize an antigen controlled by an *Igh*-linked gene (11, 12). We term this gene *H-40*. Surprisingly, the expression of the CTL target antigen is coincident with the expression of sIg. While the possibility exists that the antigen is in fact a sIg determinant, in this report we demonstrate that the gene controlling the expression of this antigen maps away from the *Igh* locus, in the region of *Pre-1*. Besides providing information on the genetics of *H-40*, we also present further evidence to indicate that the expression of this antigen requires the presence of sIg.

This work was supported by National Institutes of Health grants CA-23115, AI-13111, AI-11851, AI-13797, CA-06927, and CA-05539. R. R. is supported by an appropriation from the Commonwealth of Pennyslvania. L. A. H. is a Fellow supported by the Cancer Research Institute Inc.

[1] *Abbreviations used in this paper:* Con A, concanavalin A; CTL, cytotoxic T lymphocytes; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; LPS, lipopolysaccharide.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/84/06/1724/17 $1.00
Volume 159 June 1984 1724–1740

Materials and Methods

Mice. Mice were bred at the animal colonies of The University of Texas Health Science Center at Dallas (UTHSCD) and the Institute for Cancer Research (ICR). Some mice were kindly provided by Dr. M. Potter of the National Institutes of Health (Contract No. 1-CB-94326). Igh recombinant strains were bred at ICR for the analysis of the *Igh* locus (nomenclature as in reference 3) and nearby regions of chromosome 12 (references 4, 13, and R. Riblet, unpublished).

Media. Media used for culture was RPMI 1640 supplemented with 2-mercaptoethanol and 10% fetal calf serum (FCS).

Antisera. Antibodies against allotypes of IgD (Igh-5^a or Igh-5^b molecules) were monoclones obtained from the Salk Institute. The antibodies were affinity purified and utilized along with a fluoresceinated rabbit anti-mouse γ chain antibody (12) for the cell sorting experiments. Rabbit antibodies against the BCL_1 idiotype and μ chains have been previously described (14) and were used together with fluoresceinated goat anti-rabbit sera for the analysis of sIg on the subclones of BCL_1. Fluoresceinated $(Fab)'_2$ rabbit anti-mouse Ig was used to analyze sIg on B lymphoma cells. The latter two fluoresceinated reagents were purchased from Cappel Laboratories (West Chester, PA). Anti–H-2 sera were produced by immunizing H-2 congenic strains of mice.

Fluorescence-activated Cell Sorter (FACS). A FACS III (Becton-Dickinson, Palo Alto, CA) was used to analyze and sort sIgh-5^a positive cells from (C.B-20 × BALB/c)F_1 spleens using indirect immunofluorescence. Following the sort, >98% of the positive cells expressed sIgh-5^a, while >98% of the negatively sorted cells were unstained for this molecule.

Cytotoxic T Cell Assay. This has been described previously (11, 12). Briefly, C.B-20 mice were immunized with 3×10^7 BALB/c spleen cells or vice-versa. After 3 wk, the mice were sacrificed and their spleen cells were cocultured with irradiated BALB/c cells for 5 d before assay in a standard ^{51}Cr release cytotoxicity assay. Data are expressed as net isotope release, which equals the percent release of isotope from target cells in the presence of sensitized effector cells − the percent release of isotope from target cells cultured with control (nonsensitized) cells. When the data were analyzed using CTL clones, control release represented the isotope release from target cells cultured in media (spontaneous release).

The competition assay has been previously described (15). Briefly, unlabeled inhibitor cells were added to wells along with labeled target cells and effector cells. Percent inhibition of isotope release was calculated as described in the text.

Generation of CTL Clones. CTL clones were generated by culturing C.B-20 spleen cells from primed mice with BALB/c splenic stimulator cells for 5 d in bulk culture. The cells were harvested and recultured with fresh stimulator cells for 3 d after which the responder cells were plated in 96-well plates at several dilutions and cultured in media containing a rat concanavalin A (Con A) supernatant. Individual wells were scored for growth after 10 d and positive wells from plates, where the probability that each individual well was derived from a single cell was >98%, were transferred to 24-well plates. The clones were expanded and tested for activity and specificity.

Target Cells. Target cells were splenocytes cultured with lipopolysaccharide (LPS; 100 μg/ml) for 3 d or tumor cells.

Tumor Cells. WEHI231, ABE-8, and PK-3, were kindly provided by Dr. L. Lanier (University of New Mexico) and have been described (16). A20-056, M12, BALENLM17.1.11.15 and 16, and B17* represent tumors derived by Kim et al. (17) and subcloned and provided to us by Drs. C. Word and M. Kuehl, University of Virginia Medical School (18). Meth A tumor cells were kindly provided by Dr. E. Stockert, Memorial Sloan-Kettering Cancer Institute, New York, NY. BCL_1 and BCL_1X63 have been described previously (19, 20). Four subclones of the in vitro BCL_1 tumor line, 3G2, 3B3-10, B1.4.23, and B1.4.24 were obtained by limiting dilution culture (21). The subclones 3G2 and 3B3 (from which 3B3-10 was further subcloned) have been described in a previous report (21).

Results

Expression of a CTL Target Antigen on Mice Carrying Different Igh Haplotypes. We have previously shown that C.B-20 (Igh^b) animals immunized with BALB/c (Igh^a) splenocytes generate, when re-challenged in vitro, CTL that recognize a target antigen, H-40, controlled by a gene linked to the *Igh* locus (12). In order to determine the expression of this antigen in different mouse strains, we tested C.B-20 anti-BALB/c effector cells against LPS-activated splenic lymphoblasts obtained from a panel of strains bearing different *Igh* haplotypes. Since the CTL activity is $H\text{-}2^d$ restricted (see below), non-$H\text{-}2^d$ strains were mated to C.B-20 mice and the $H\text{-}2^d$ heterozygotes were tested for the expression of the antigen.

The data in Fig. 1 (*A–C*) demonstrate that C.B-20 anti-BALB/c CTL lyse LPS-activated lymphoblasts from BALB/c (Igh^a), C.AL-20 (Igh^d), and C57L (Igh^a) strains. In most experiments, the net release from these target cells ranged between 15–40% at E/T = 100. In contrast, target cells from strains BAB-14 ($Igh\text{-}V^a$ $Igh\text{-}C^b$), C58 (Igh^a), and NZB (Igh^e) were not killed. A summary of results for the strains tested is listed in Table I. All target cells were concomitantly tested for their sensitivity to lysis by anti-$H\text{-}2^d$ CTL and were noted to be susceptible to lysis (Fig. 1, *E–H*).

BALB/c anti-C.B-20 CTL were also analyzed for their cytotoxic potential against a limited panel of target cells. Although the lytic activity of these effector cells was usually much less than that observed for the C.B-20 anti-BALB/c CTL, they killed C.B-20, BAB-14, and B10.D2, but not C.AL-20 or NZB targets (see (Fig. 1 *D*; Fig. 3, *A*, *B*, and *D*; and Table I). Thus, these results indicate that there are at least three alleles, as defined by CTL, for the *H-40* gene. Strains with the *a* allele include BALB/c, C57L, DBA/2, C.AL-20, and AKR; strains with the *b* allele include C.B-20 and B10.D2, and strains with a third and possibly null allele include NZB.

Polymorphism of H-40 as Detected by CTL Activity. The previous data indicates that mice carrying the *Igh* haplotypes of *a*, *c*, *d* (with the exception of C58) have the same allele for *H-40*. However, each of these strains could share a common epitope while at the same time expressing a unique specificity, each of which would define a different allele. To address this issue, we tested C.B-20 anti-BALB/c effector cells against ^{51}Cr-labeled targets in the presence of unlabeled inhibitor cells taken from other antigen-positive strains.

C.B-20 anti-BALB/c CTL lyse BALB/c target cells and this activity was completely blocked by the presence of an excess of unlabeled BALB/c LPS lymphoblasts, as expected (Fig. 2, *left*). C.AL-20 and DBA/2 target cells were killed by the effector cells (Table I and Fig. 1). Further, when these cells were used as inhibitors, they blocked the cytotoxic effect to the same extent as BALB/c inhibitors. When the C.B-20 anti-BALB/c CTL were tested against either C.AL-20 (Fig. 2, *middle*) or DBA/2 (*right*) targets, equivalent blocking was observed with either BALB/c, DBA/2, or C.AL-20 inhibitors.

These data indicate that at least for the three *Igh* haplotypes tested, that there is no detectable polymorphism of this gene.

CTL Recognize an H Antigen Controlled by a Gene Linked in or Near Igh-C and Their Specificity Is Further Restricted by H-2. We (12) previously demonstrated

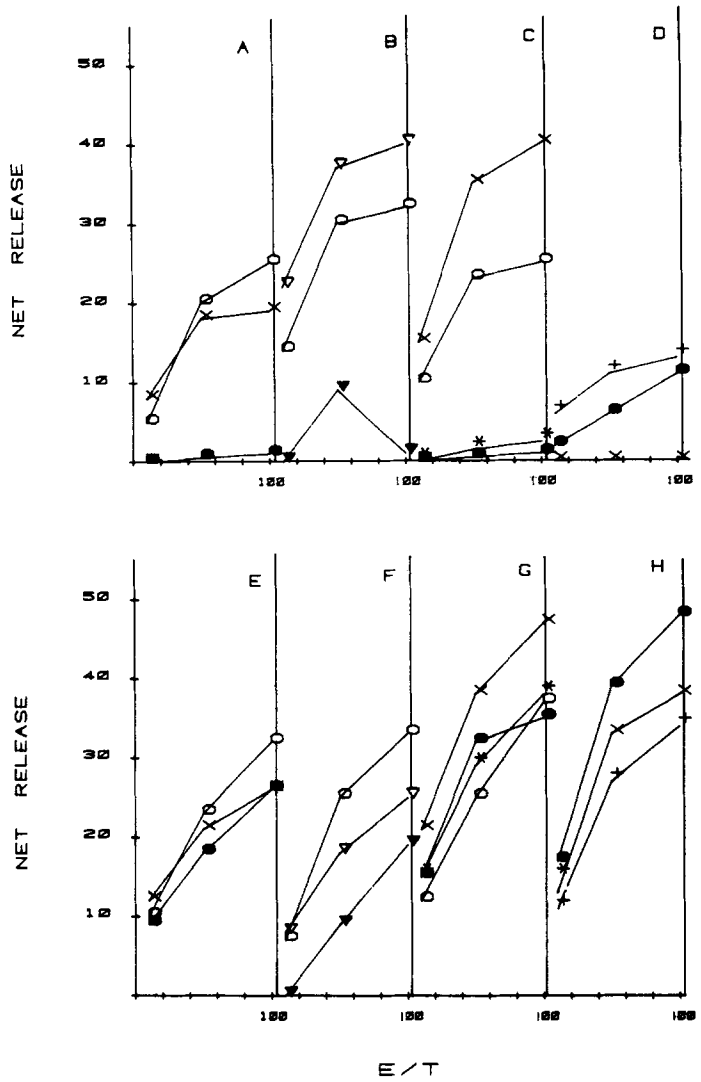

FIGURE 1. CTL recognize H-40, an antigen controlled by an *Igh*-linked gene. C.B-20 anti-BALB/c (*A–C*), BALB/c anti-C.B-20 (*D*), or B10.Q anti-BALB/c (*E–H*) CTL were tested against the following LPS splenic lymphoblast target cells. O, BALB/c; ●, BAB-14; ×, C.AL-20; ▽, (C.B-20 × C57L/J)F_1; ▼, (C.B-20 × C58/J)F_1; +, C.B-20; *, NZB. Data expressed as net isotope release.

that C.B-20 anti-BALB/c CTL recognize a target antigen controlled by a gene near the constant region (*Igh-C*) side of the *Igh* locus. Rolink et al. (22) reported that BALB/c anti-C.B-20 recognized 2 minor H antigens, one controlled by a gene linked on the *Igh-C* side, the other linked on the *Igh-V* side of the *Igh* complex. Although these antigens appear to be different than the one described here and previously (11, 12), we tested the specificity of BALB/c anti-C.B-20 CTL.

BALB/c anti-C.B-20 CTL lysed C.B-20 LPS lymphoblasts, as expected (Fig.

TABLE I

Strain Distribution of the CTL Target Antigen Controlled by an Igh-Linked Gene

	Target cells								
Igh haplotype . . .	BALB/c *a*	C.B-20 *b*	NZB *n*	C58* *a*	C57L *a*	CBA/J *v*	DBA/2 *c*	AKR *d*	A/J *e*
Effector cells									
C.B-20 anti-BALB/c	+‡	–	–	–	+	–	+	+	–
BALB/c anti-C.B-20	–	+	–						

Effector cells were tested against splenic LPS lymphoblasts.

* Since the CTL are *H-2* restricted (see Fig. 3), non $H\text{-}2^d$ strains were mated to C.B-20 ($H\text{-}2^d$) and the F_1 cells were used as targets.

‡ Indicates net isotope release ranged between 15–40%; – indicates net isotope release was <5%. All target cells were concomitantly tested with anti-$H\text{-}2^d$ CTL and were equally sensitive to lysis.

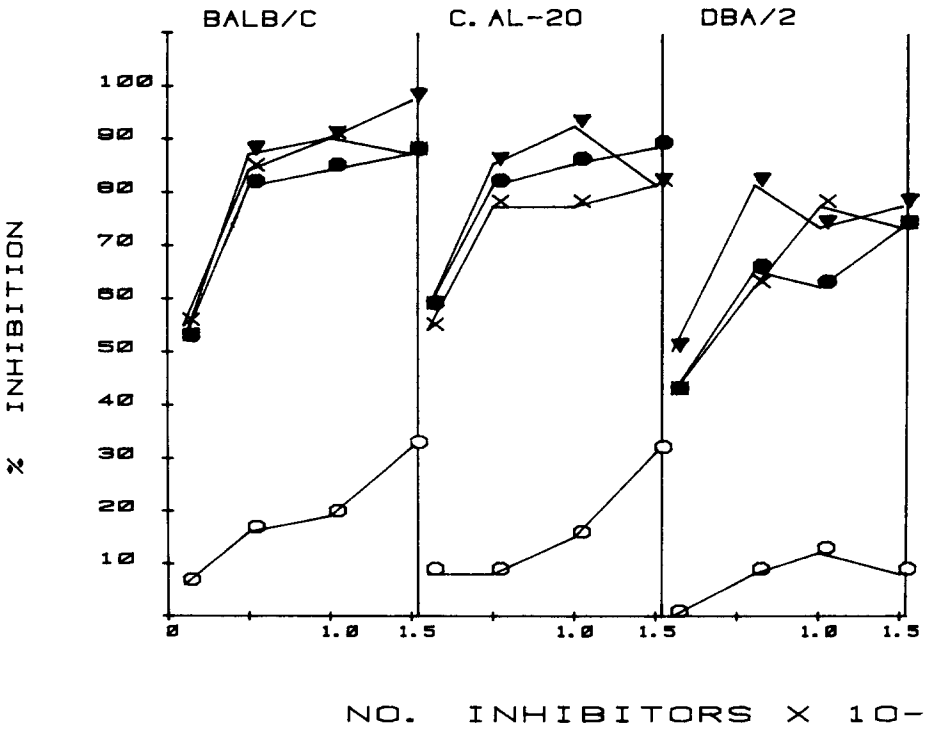

FIGURE 2. Evidence for limited polymorphism of *H-40*. C.B-20 anti-BALB/c effector cells were tested for their ability to lyse 10^4 BALB/c (*left*), C.AL-20 (*center*), or DBA/2 (*right*) LPS lymphoblasts in the presence of unlabeled inhibitor cells. Inhibitor cells were LPS lymphoblasts from O, C.B-20; ●, BALB/c; ×, C.AL-20; or ▼, DBA/2 animals. Net release was 31, 26, and 26 from BALB/c, C.AL-20, and DBA/2 target cells, respectively, in the absence of inhibitor cells at E/T = 100. Percent inhibition = 1 – Net release of isotope in the presence of inhibitor cells/Net release of isotope in the absence of inhibitor cells × 100.

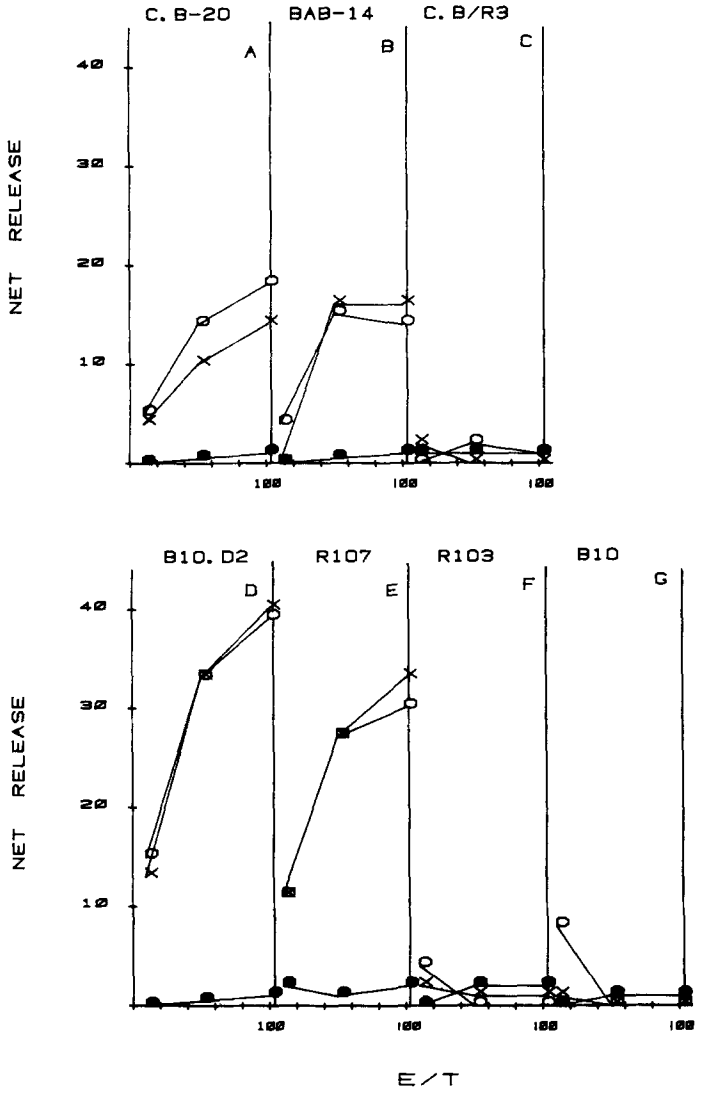

FIGURE 3. BALB/c anti-C.B-20 CTL recognize an antigen controlled by a gene linked to *Igh-C* and their specificity is restricted by a *D*-end *H-2* gene. (*A–C*) BALB/c mice were primed with C.B-20 spleen cells in vivo. Three months later their spleen cells were removed and cultured with C.B-20 (○), BAB-14 (×), or C.B/R3 (●) stimulator cells. After 5 d the effector cells generated were tested against LPS splenic lymphoblast target cells from C.B-20 (*A*), BAB-14 (*B*), or C.B/R3 (*C*) animals. (*D–G*) Effector cells were generated as described above and tested against LPS lymphoblasts from B10.D2 (*D*), B10.D2(R107) (*E*), B10.D2(R103) (*F*), and C57BL/10(B10) (*G*) animals.

3*A*). However, they also lysed BAB-14 (*B*) but not C.B/R3 target cells (*C*). Since BAB-14 shares constant region genes and C.B/R3 shares variable region genes with C.B-17 (and C.B-20, see Table II), this indicates that the gene controlling the target antigen maps to or near the *Igh-C* genes, concordant with our previous data using C.B-20 anti-BALB/c CTL (12). This is further supported by the finding that BALB/c anti-C.B-20 primed spleen cells can be restimulated in vitro

with C.B-20 or BAB-14 stimulator cells but not C.B/R3 to generate CTL that lyse C.B-20, BAB-14, and B10.D2 (Igh^b), but not C.B/R3 target cells (Fig. 3, *A–D*).

In order to map which end of the *H-2* complex controls the specificity of these CTL, we tested the BALB/c anti-C.B-20 CTL against B10.D2 (Igh^b) target cells as well as H-2 recombinant strains on the C57BL/10 background. The data in Fig. 3 demonstrate that B10.D2(R107) cells ($H\text{-}2K^bD^d$) are lysed while B10.D2(R103) ($H\text{-}2K^dD^b$) and C57BL/10 ($H\text{-}2K^bD^b$) cells are not. This maps the restricting gene to the right of the *S* region and most likely is either *H-2D* and/or *H-2L*.

The CTL Target Antigen Is Not Allelically Excluded. Allelic exclusion of *Igh* loci has been demonstrated by the finding that in heterozygotes only one of the two possible Igh allotypes is expressed or secreted by individual B cells (23). Presumably this is due to inappropriate gene rearrangements on the nonexpressed chromosome (24). Since *H-40* is linked to the *Igh* locus, we determined whether this gene is also allelically excluded. Because the target antigen is expressed on sIg^+ but not sIg^- cells (reference 12, and following sections), we used the FACS III to sort for $sIgD^+$ cells in (BALB/c × C.B-20)F_1 (Igh^a/Igh^b) animals using a monoclonal anti-Igh-5^a (anti-δ) antibody. The sorted cells, both bright and dull, as well as stained but unsorted cells were placed in culture together with LPS. After 3 d, the cells were harvested and used as target cells in the CTL assay.

(BALB/c × C.B-20)F_1 spleen cells, either untreated or treated with anti–Igh-5^a antibodies (but unsorted), were sensitive to lysis by the C.B-20 anti-BALB/c CTL (Fig. 4, *left*). Although the antibody-treated, but unsorted cells were less sensitive to CTL-mediated lysis than were the untreated cells, this finding was not observed in a second experiment. Importantly, the Igh-5^a positive and negative cells, which were sorted before culturing, were equally sensitive to lysis. We also monitored the different target cell populations for their sensitivity to lysis by anti-H-2^d effector cells (Fig. 4, *right*). No difference was noted.

These data indicate that both sets of B lymphoblasts express the BALB/c target antigen. Therefore, there is no allelic exclusion of *H-40*.

Localization of the Igh-linked Locus That Controls the CTL Target Antigen. The target antigen for the C.B-20 anti-BALB/c CTL is selectively expressed on sIg^+ lymphoblasts and tumor cells (12). Further, we previously demonstrated that while the sIg^+ tumor BCL_1 expressed the antigen, the BCL_1X63 hybridoma, which secretes BCL_1 Ig but is sIg^-, does not (12). This correlative data would suggest that the CTL recognize a determinant on sIg, itself. Therefore, it would be predicted that the locus controlling the antigen would map in the *Igh-C* region. We utilized strains that have undergone recombination in their 12th chromosome in this region to address this question.

We (12) previously showed that BALB/c but not BAB-14 target cells expressed the antigen recognized by C.B-20 anti-BALB/c CTL, indicating that the target antigen maps on the *C* rather than the *V* side of *Igh*. A similar result, in that they did not lyse lymphoblasts from the C.B/R3 strain which shares the *V* but not *C* region of *Igh* with C.B-20 (Fig. 3*C*, see Table II for strain genotypes) was observed using BALB/c anti-C.B-20 CTL. A further analysis was carried out on a series of *Igh* recombinant strains using C.B-20 anti-BALB/c effector cells.

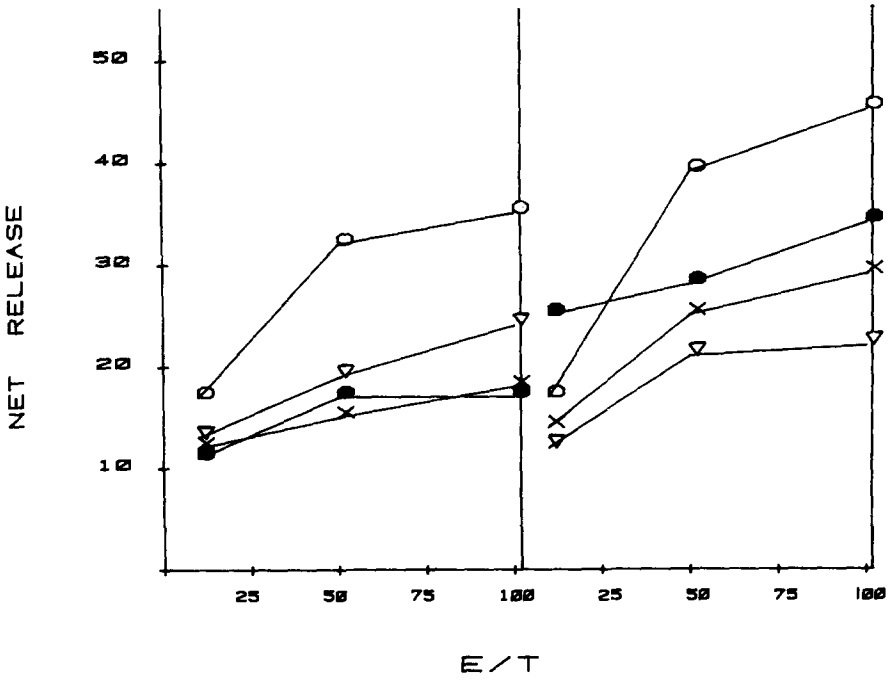

FIGURE 4. H-40 is not allelically excluded. (BALB/c × C.B-20)F_1 spleen cells were sorted for sIgh-5^a positive or negative cells using the FACS III. The cells were cultured with LPS and used 3 d later as target cells in the presence of C.B-20 anti-BALB/c (*left*) or B10.Q anti-BALB/c (*right*) effectors. LPS lymphoblast target cells were untreated (○), stained but unsorted (▽), sIgh-5^a positive (×), or sIgh-5^a negative (●).

TABLE II

C.B/R Igh Recombinant Strains Indicate That the Igh Locus Does Not Control the CTL Antigen

Strain	Allele/Antigen					Presence of target antigen
	Dex*		Igh		Pre-1	
BALB/c	+		*a*		*a*	+
C.B-17	−		*b*		*o*	−
BAB-14	+	/‡	*b*		*o*	−
C.B/R3	−	/	*a*		*a*	+
C.B/R4	−	/	*a*		*a*	+
C.B/R6	+	/	*b*		*o*	−
C.B/R8	+		*a*	/	*o*	−
C.B/R9	−		*b*	/	*a*	+
C.B/R10	−		*b*	/	*a*	+

C.B-20 anti-BALB/c CTL were tested against LPS-stimulated splenic lymphoblasts as described in Table I.

* Defined by idiotypic marker on anti-dextran antibodies (13).

‡ Indicates region of cross-over.

TABLE III

C.B.AL Igh Recombinant Strains Indicates That the Locus Controlling the Target Antigen Is Telomeric to Tsu

Strain	Allele/Antigen								CTL Antigen
	Dex		Igh-C		Tsu^d		Pre-1		
BAB/14	+		*b*		−		*c*		−
C.AL-9	−		*d*		*d*		*a*		+
C.B.AL/1	−	/	*b*		−	/	*a*		+
C.B.AL/2	−	/	*b*		−		*c*		−
C.B.AL/3	−	/	*b*		−		*c*		−
C.B.AL/4	−	/	*b*		−		*c*		−
C.B.AL/5	−	/	*b*		−		*c*		−
C.B.AL/6	−	/	*b*	/	*d*		*a*		+

See Table II for explanation.

The C.B/R series of *Igh* recombinant strains were derived from (C.B-17 × BALB/c)F_1 × C.B-17 backcross animals. The presence of the CTL antigen on strains C.B/R3 and C.B/R4 and its absence on BAB-14 and C.B/R6 further confirm that the locus maps to the right of the crossover between the gene controlling the Dex idiotype and *Igh* (Table II). Strains C.B/R8, R9, and R10 have undergone recombination between the *Igh* and *Pre-1* locus. The fact that C.B/R8 is antigen negative but is *Igh*^{a}, whereas, C.B/R9 and C.B/R10 are *Igh*^{b} but express the CTL antigen, indicates that the gene maps to the right of those crossovers between *Igh* and *Pre-1*.

The C.B.AL series of Igh recombinant strains were derived from (BAB-14 × C.AL-9)F_1 × C.AL-9 backcross animals. It is apparent from the testing of these strains (Table III) that the CTL locus again maps with *Pre-1*. C.B.AL/4 and C.B.AL/5 strains are negative for the antigen, indicating that the locus maps to the right of the Dex idiotypic marker. Further, the finding that the C.B.AL/1 strain expresses the antigen but does not carry the *Tsu*^{d} allele indicates that the gene maps to the right of the *Tsu* locus.

Taken together, these data indicate that a gene that maps distal to *Igh* and, as of yet unseparated from *Pre-1*, controls an antigen selectively expressed on sIg$^+$ cells and recognized by CTL. Further, in the accompanying report we demonstrate that this antigen elicits tumor rejection in allotype congenic recipients.[2] We have termed this gene *H-40*.

Expression of the CTL Antigen on B Lymphomas. We previously showed that the CTL antigen is expressed on sIg$^+$ LPS stimulated splenocytes, but not sIg$^-$ Con A–stimulated splenocytes (12). Further, an analysis of a variety of myelomas, T cell tumors, and BCL_1, the sIg$^+$ BALB/c tumor, indicated that only BCL_1 expressed the target antigen. The availability of a further number of sIg$^+$ lymphomas has now allowed us to extend these studies.

Six B lymphomas were tested for the presence of H-40 (Table IV). The only lines that were positive for the antigen were subclones of BALENLM17; viz.,

[2] Henderson, L., R. Ciavarra, R. Riblet, and J. Forman. 1984. H-40, an antigen controlled by an *Igh*-linked gene and recognized by cytotoxic T lymphocytes. II. Recognition of H-40 as a tumor antigen in leukemic animals. Submitted for publication.

TABLE IV

Sensitivity of sIg$^+$ B Lymphoma Cells to Lysis by Anti-Igh CTL

Experiment	Tumor*	Strain	Ig isotype	Relative sIg expression‡	Cell type	Net release§
1	ABE-8	BALB/c	μ	—	Pre-B lymphoma	0(42)
	WEHI-231	(BALB/c × NZB)F_1	μ,κ	m	B lymphoma	3(51)
	2PK3	BALB/c	γ_{2a},κ	l	B lymphoma	0(51)
	—	BALB/c			LPS splenic lymphoblasts	42(38)
2	A20-056	BALB/c	$\mu,\gamma_{2a},\kappa,\lambda$	l	B lymphoma	5(32)
	M12	BALB/c	κ		B lymphoma	3(26)
	BALENLM17.1.11.15	BALB/c	μ,κ,λ	h	B lymphoma	34(29)
	BALENLM17.1.11.16	BALB/c	μ,κ,λ	h	B lymphoma	33(32)
	B17*	BALB/c	μ,κ,λ	h	B lymphoma	21(31)
	—	BALB/c			LPS splenic lymphoblasts	38(31)
3	Meth A	BALB/c			Fibrosarcoma	1(19)
	—	BALB/c			LPS splenic lymphoblasts	32(33)

* Tumors in Experiment 1 were obtained from Dr. L. Lanier, those in Experiment 2 were derived by Asofsky and co-workers (17) and provided to us by Drs. C. Word and M. Kuehl, and those in Experiment 3 from Dr. E. Stockert (see Methods and Materials). The B17* tumor is a variant of BALENLM17 (M. Kuehl, personal communication).

‡ Data taken from references 16–18.

§ Net release at E/T = 100. Numbers in parentheses represent net release using anti-H-2^d CTL at E/T = 100.

BALENLM17-1.11.15, 16, and B17*. These subclones express a relatively high concentration of sIg (17). Although H-40$^+$ cells express sIgM, a putative precursor of the sIgM$^+$ cell represented by the pre-B lymphoma, ABE-8, does not express the antigen. Meth A tumor cells have been reported to express an antigen controlled by a gene on the 12th chromosome that interacts with a T cell–derived suppressor factor (25). However, this tumor does not express the CTL target antigen. The presence of sIgM does not ensure expression of the antigen, since WEHI231, a tumor that has a high density of sIgM by FACS analysis (reference 26, and data not shown), does not express the antigen. The fact that the tumor is derived from a (BALB/c × NZB)F_1 animal where one parent (NZB) is antigen negative cannot account for this result, since we showed that there is no allelic exclusion of H-40 (see Fig. 4).

Recently, the in vitro adapted BCL_1 line described by Gronowicz et al. (27) was subcloned (21). Two of the subclones, 3G2 and 3B3-10, were sIg$^+$ as demonstrated by FACS analysis (Fig. 5). Two other subclones, B1.4.23 and B1.4.24, are sIg$^-$ (data not shown). We tested these four subclones, the parent BCL_1 tumor, as well as the sIg$^-$ secreting hybridoma (BCL_1X63), which was produced by fusing BCL_1 to MOPC21 myeloma cells, for the presence of the CTL antigen. In these experiments both bulk-cultured CTL and a CTL clone with specificity for H-40 were used to test for the presence of the antigen. The CTL clone has the same specificity as the bulk-cultured CTL and lyses LPS cultured B lymphoblasts but not Con A–cultured T lymphoblasts (data not shown).

Both the CTL clone and the bulk-cultured effector cells lysed BCL_1 but not the sIg$^-$ (BCLX63) hybridoma, as we previously observed (12) (Fig. 6). The CTL

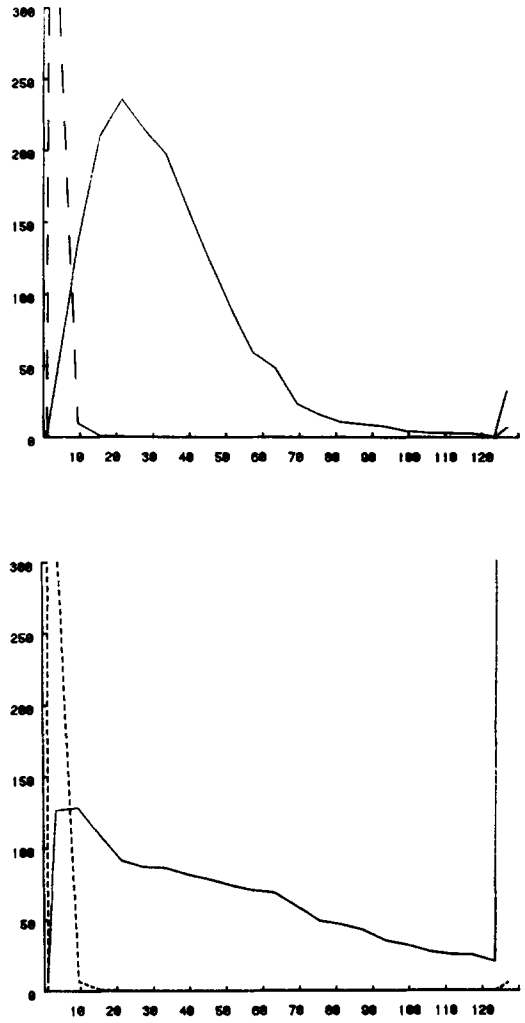

FIGURE 5. Expression of sIg on two subclones of BCL_1. 3B3-10 (*top*) or 3G2 (*bottom*) cells were stained by indirect immunofluorescence using rabbit anti-μ (solid lines) or normal rabbit serum (dashed lines) and analyzed with the FACS III. The fluorescent gain was set at 2. 10,000 cells were analyzed.

clone and the bulk-cultured cells also lysed the 3G2 and 3B3-10, but not the B1.4.23 and B1.4.24 targets. Thus, in agreement with our previous report (12) and the data presented here (Table IV), sIg^- cells do not express the target antigen. We have attempted to block the CTL clone from recognizing the target antigen on BCL_1 and 3B3 cells with rabbit antibody directed against the BCL_1 idiotype. However, these experiments have yielded negative results (data not shown).

Taken together, the above data show that only sIg^- cells (presumably sIgM) express H-40. However, the structural gene that encodes this antigen is not an immunoglobulin gene.

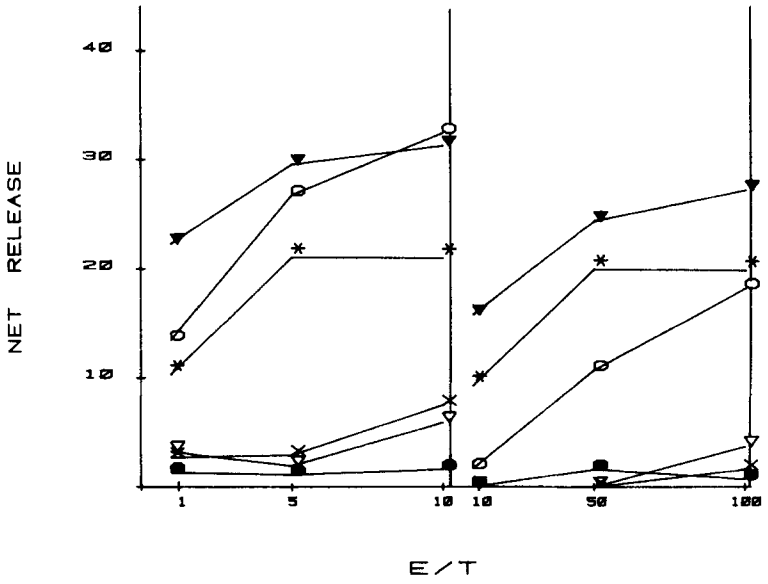

FIGURE 6. Expression of H-40 on subclones of BCL_1. C.B-20 anti-BALB/c bulk cultured (*right*) or cloned (*left*) CTL were tested for their lytic potential against the sIg^+ BCL_1 leukemia (▼), sIg^- BCL_1X63 hybridoma cells (●) and 4 BCL_1 subclones. Two of the subclones were sIg^+, 3G2 (○) and 3B3-10 (*), and two were sIg^-, B1.4.23 (▽) and B1.4.24 (×).

Discussion

The data in this report define an *Igh*-linked minor H (histocompatibility) - gene, *H-40*, which maps telomeric to the *Tsu* locus in the region of *Pre-1*. This gene controls an antigen, H-40, which is detected in vitro by CTL and in vivo by tumor rejection (11, 25). We (12) previously noted that sIg^+ LPS but not sIg^- Con A splenic lymphoblasts were sensitive to lysis by either C.B-20 anti-BALB/c or BALB/c anti-C.B-20 CTL. The same result occurs when analyzed with C.B-20 anti-BALB/c CTL clones (data not shown). Further, we (12) showed that the sIg^+ leukemia, BCL_1 (μ,δ), expressed this antigen while sIg^- tumor cells including a hybridoma (produced by fusing BCL_1 to a myeloma) that secreted the BCL_1 IgM, several myelomas, and a T cell leukemia did not express H-40. Thus, H-40 is preferentially, if not exclusively, expressed on sIg^+ cells.

In the present study we have extended this phenotyping using a panel of B lymphomas. Our results revealed that in addition to BCL_1, other sIg^+ B lymphomas also express H-40. Detection of the CTL antigen was correlated with the expression of a relatively high density of sIg on these tumor cells. The finding that 2PK3 (γ_{2a},) and A20-056 (μ,γ_{2a}) were not sensitive to lysis while the BALENLM17 subclones BALENLM17.1.11.15, 16 (μ), B17* (μ), and BCL_1 ($\mu\delta$) were, suggests that the antigen is expressed on cells that are $sIgM^+$. Conversely, tumors with little or no detectable sIg were not killed. This included ABE-8 (a pre-B lymphoma), A20-056 and M12 (B lymphomas), and Meth A (a fibrosarcoma). This latter tumor has been reported to express an antigen that is controlled by a gene on the 12th chromosome and that interacts with a T cell–derived suppressor factor (25).

An exception to the correlation between the coordinate expression of sIgM and H-40 was the findings with WEHI231. This lymphoma (μ) has a relatively high sIg density, but is not sensitive to CTL-mediated lysis. Whether this result indicates that WEHI231 has an aberrant form of sIg or that there is a requirement for a factor in addition to sIg for H-40 expression cannot be determined at this time.

We (21) recloned the in vitro BCL tumor line of Gronowicz et al. (27) and tested four subclones for the presence of H-40. Two of the (sIg^+) subclones expressed the antigen, as detected by both bulk culture and cloned CTL, while two other (sIg^-) subclones were H-40 negative. Thus, this data suggests that the expression of the antigen is strictly dependent on a sIg positive phenotype, even though the gene controlling this antigen is separable from the *Igh* gene cluster.

It should be noted that our ability to detect H-40 is limited by the sensitivity of the CTL assay. Thus, it is possible that some of the cell populations that we describe as $H\text{-}40^-$ may, in fact, express this molecule. However, even if this is the case, it is still evident that the relative expression of H-40 on sIg^+ cells is much greater than that on sIg^- cells.

The construction of strains that are genetically recombinant in the region of the *Igh* locus enabled us to map the gene controlling this antigen. Using different sets of *Igh* recombinant strains we showed that *H-40* maps away from the genes (*Igh*) that encode Ig heavy chains. This indicates that although the antigen is expressed on sIg^+ positive cells, it is not controlled by an immunoglobulin heavy chain (*Igh*) structural gene. We further demonstrated that *H-40* maps telomeric to *Tsu* and as of yet is unseparated from *Pre-1*. This makes the gene order *Igh-V, Igh-C, Tsu, H-40/Pre-1. H*(*Igh*) maps to the *C*-region side of the *Igh* complex (9). We are currently in the process of determining the relationship between *H*(*Igh*) and *H-40*.

There are several possibilities to explain the coordinate expression of H-40 with sIg. Firstly, this finding could be coincidental. Thus, Tyler et al. (28) have described a minor H-gene that controls a CTL target antigen that is also preferentially expressed on sIg^+ cells. Since B10.K and AKR animals share the same allele for this alloantigen (D. Steinmuller, personal communication) it is unlikely that this antigen is controlled by *H-40*, since we have shown that these strains have different *H-40* alleles. Secondly, the fact that the detection system (CTL) is relatively insensitive in that only certain cell types can be used in a standard CTL assay biases the results in favor of lymphoblasts over other cell types. Accordingly, if additional cell types could be tested they might be found to be $H\text{-}40^+$. Thirdly, the CTL antigen could be sIg even though the gene controlling it maps to a locus that is distinct from the *Igh* cluster. One possibility is that H-40 is an enzyme that posttranslationally modifies sIg. Eidels (29) noted that sIgD could be expressed as δ_2L_2 or δL. Pollock et al. (30) noted an allotype-linked genetic variation in the relative expression of these two different forms of sIgD. Taken together, these findings suggest that an *Igh*-linked gene controls the subunit structure of sIgD. Thus, it is possible that in an analogous fashion there is a variant of sIgM that accounts for the H-40 epitope. In a different system, Huber (31) has defined an epitope, Iaw.39, which is expressed on the $I\text{-}A^b$ molecule. This epitope is not found in (CBA/N ♂ × C57BL/6 ♀)F_1 animals,

even though they possess a normal *I-A*^{b} gene. Apparently a gene on the Y chromosome and missing in the CBA/N mouse controls the expression of this particular specificity on I-A^b. Another possibility to explain these findings is that the antigen recognized by CTL represents a determinant formed by the interaction of two gene products; i.e., sIgM and the molecule encoded by *H-40*. According to this model, these two molecules would interact with an H-2 class I molecule to form a tri-molecular species containing the alloantigen.

Irrespective of the *Igh* haplotype, we noted that the presence of the CTL antigen was determined solely by a gene (*H-40*) that maps to the region of *Pre-1*. Thus, if *H-40* modifies sIg to create the CTL epitope, then sIg from either the *Igh*^{a} or *Igh*^{b} haplotype can be the substrate. This interpretation would also be consistent with our data showing that the expression of the antigen is not allelically excluded in heterozygous B cells.

If the expression of this antigen is coincidental with the expression of sIg rather than representing an epitope on sIg, itself, then it is possible that it represents an allelic variant of a receptor for a differentiation signal of B lymphocytes. For example, the finding that it is found on sIgM but not sIgG cells could make it a candidate for a B cell differentiation factor that potentiates the expression of a particular class of Ig (32).

We were unable to block lysis of target cells using anti-Ig antibodies. While this would suggest that the target antigen is not sIg, there are many examples in CTL systems where the target antigen is not masked by antibody directed against the antigen (33, 34). Therefore, this issue is still open at this time.

In contrast to the previous report of Rolink et al. (22), who described two CTL target antigens controlled by genes linked to the *Igh* locus, we did not detect an antigen controlled by a gene in the *V*-region of the *Igh* complex, nor did we note the antigen expressed on sIg$^-$ cells. We have generated our CTL using C.B-20 anti-BALB/c (and vice-versa) effector cells. The above investigators generated CTL with C.B-17 and BALB/c cells. The BALB/c mice they used were from a different source than the Potter stock used in this study. Therefore, it is possible that additional *Igh*-linked genes with allelic variation control antigens that are immunodominant with respect to H-40 and were defined by Rolink et al. (22). Further, since there is a difference in *Qa-2* alleles between BALB/c sublines which can give rise to anti-Qa-2 CTL (35), it cannot be ruled out that the C.B-17 anti-BALB/c reactivity that they described was directed against the Qa-2^a antigen.

The tissue distribution of most minor H-antigens is not known. Steinmuller et al. (36) have described a lymphocyte and a skin-specific minor H antigen. Carter and Wegman (37) noted an antigen detected by CTL in the spleen but not in the thymus. In this and a previous communication (12) we have described an antigen expressed on sIg$^+$ cells. Since in most cases minor H antigens are only detected by graft rejection or cell-mediated cytolysis rather than with serological reagents, the tissue distribution and further characterization of these molecules awaits clarification.

In an upcoming paper[2] we describe how H-40 acts as a tumor rejection antigen on BCL_1 tumor cells when injected into *Igh* congenic recipients.

Summary

C.B-20 (*Igh*^{b}) mice challenged with BALB/c (*Igh*^{a}) spleen cells (or vice-versa) generate cytotoxic T lymphocytes (CTL) that recognize an antigen, H-40, controlled by an *Igh*-linked gene. The gene maps to the *Igh-C* region end of the *Igh* complex, telomeric to *Tsu* in the region of *Pre-1*. At least three alleles, *a*, *b*, and *c*, can be defined. Using a cold target competition assay, no polymorphism of the *a* allele was detected. Both surface Igh-5^a positive and negative spleen cells from (C.B-20 × BALB/c)F_1 animals express the *a* allele of the antigen, indicating that this gene is not allelically excluded. Recognition of the target antigen by CTL is restricted by the *D*-end of *H-2*^{d}.

The tissue distribution of H-40 was explored using both bulk-cultured and cloned CTL. The antigen is expressed on surface immunoglobulin positive (sIg$^+$) cells and correlates with the expression of sIgM. This was determined by analysis of several B lymphomas as well as of other tumors that varied in their extent of expression of sIg. Four subclones of BCL_1 were analyzed. Two of the subclones are sIg$^+$ and express H-40, while two other subclones are sIg$^-$ and H-40$^-$. Thus, these data define an *Igh*-linked gene, separate from immunoglobulin structural loci, that controls an antigen expressed on sIg$^+$ cells. Possible mechanisms to account for this finding are discussed.

We would like to acknowledge the expert technical assistance of J. Tsan and S. Byers, and B. J. Washington for her secretarial assistance.

Received for publication 30 January 1984.

References

1. Meo, T., J. Johnson, C. V. Beechey, S. J. Andrews, J. Peters, and A. G. Searle. 1980. Linkage analyses of murine immunoglobulin heavy chain and serum prealbumin genes establish their location on chromosome 12 proximal to the T (5;12) 31H breakpoint in band 12F1. *Proc. Natl. Acad. Sci. USA.* 77:550.
2. Moller, G., editor. 1981. Organization of immunoglobulin genes. *Immunol. Rev.* 59:1.
3. Green, M. C. 1979. Genetic nomenclature for the immunoglobulin loci of the mouse. *Immunogenetics.* 8:89.
4. Owen, F. L., and R. Riblet. 1984. Genes for the mouse T cell alloantigens Tpre, Tthy, Tind, and Tsu are closely linked near *Igh* on chromosome 12. *J. Exp. Med.* 159:313.
5. Owen, F. L., R. Riblet, and B. A. Taylor. 1981. The T suppressor cell alloantigen Tsu^d maps near immunoglobulin allotype genes and may be a heavy chain constant-region marker on a T cell receptor. *J. Exp. Med.* 153:801.
6. Owen, F. L. 1983. Tpre, a new alloantigen encoded in the IgT-C region of chromosome 12, is expressed on bone marrow of nude mice, fetal T cell hybrids, and fetal thymus. *J. Exp. Med.* 157:419.
7. Taniguchi, M., T. Tokuhisa, M. Kanno, Y. Yaoita, A. Shimizu, and T. Honjo. 1982. Reconstitution of antigen-specific suppressor activity with translation products of mRNA. *Nature (Lond.).* 298:172.
8. Subbarao, B., A. Ahmed, W. E. Paul, I. Scher, R. Lieberman, and D. E. Mosier. 1979. Lyb-7: a new B cell alloantigen controlled by genes linked to the IgC_H locus. *J. Immunol.* 122:2279.

9. Riblet, R., and C. Congleton. 1977. A possible allotype linked histocompatibility gene. *Immunogenetics.* 5:511.
10. Taylor, B. A., D. W. Bailey, M. Cherry, R. Riblet, and M. Weigert. 1975. Genes for immunoglobulin heavy chain and serum prealbumin protein are linked in the mouse. *Nature (Lond.).* 256:644.
11. Ciavarra, R., and J. Forman. 1981. Influence of IgH V-region genes on the growth kinetics of a murine B cell leukemia (BCL_1). *J. Immunol.* 126:54.
12. Forman, J., R. Ciavarra, and E. S. Vitetta. 1981. Cytotoxic T cells specific for antigens expressed on surface immunoglobulin positive cells. *J. Exp. Med.* 154:1357.
13. Weigert, M., and R. Riblet. 1978. The genetic control of antibody variable regions in the mouse. *Springer Semin. Immunopathol.* 1:133.
14. Krolick, K. A., P. C. Isakson, and E. S. Vitetta. 1979. Murine B cell leukemia (BCL_1): organ distribution and kinetics of growth as determined by fluorescence analysis with an anti-idiotypic antibody. *J. Immunol.* 123:1928.
15. Forman, J., and E. S. Vitetta. 1975. Absence of H-2 antigens capable of reacting with cytotoxic T cells on a teratoma line expressing a *T/t* locus antigen. *Proc. Natl. Acad. Sci. USA.* 72:3661.
16. Lanier, T., N. L. Warner, J. A. Ledbetter, and L. A. Herzenberg. 1981. Quantitative immunofluorescent analysis of surface phenotypes of murine B cell lymphomas and plasmacytomas with monoclonal antibodies. *J. Immunol.* 127:1691.
17. Kim, J. K., C. Kanellopoulos-Langevin, R. M. Mervin, D. H. Sacks, and R. Asofsky. 1979. Establishment and characterization of BALB/c lymphoma lines with B cell properties. *J. Immunol.* 122:549.
18. Word, C. 1981. Murine B Lymphomas: models for Ig expression in B cell development. Ph.D. thesis. University of Virginia, Charlottesville, VA.
19. Slavin, S., and S. Strober. 1978. Spontaneous B cell murine leukemia. *Nature (Lond.).* 272:624.
20. Krolick, K. A., C. Villemez, P. Isakson, J. W. Uhr, and E. S. Vitetta. 1980. Selective killing of normal or neoplastic B cells by antibodies coupled to the A chain of ricin. *Proc. Natl. Acad. Sci. USA.* 77:5419.
21. Brooks, K., D. Yuan, J. W. Uhr, P. H. Krammer, and E. S. Vitetta. 1983. Lymphokine-induced IgM secretion by clones of neoplastic B cells. *Nature (Lond.).* 302:825.
22. Rolink, T., K. Eichmann, and M. M. Simon. 1978. Detection of two allotype (Ig-1)-linked minor histocompatibility loci by the use of H-2 restricted cytotoxic lymphocytes in congenic mice. *Immunogenetics.* 7:321.
23. Pernis, B., G. Chiappino, A. S. Kelus, and P. G. H. Gell. 1965. Cellular localization of immunoglobulins with different allotypic specificities in rabbit lymphoid tissues. *J. Exp. Med.* 122:853.
24. Coleclough, C. 1983. Chance, necessity and antibody gene dynamics. *Nature (Lond.).* 303:23.
25. Flood, P. M., A. B. DeLeo, L. J. Old, and R. K. Gershon. 1983. Relation of cell surface antigens on methylcholanthrene-induced fibrosarcomas to immunoglobulin heavy chain complex variable region-linked T cell interaction molecules. *Proc. Natl. Acad. Sci. USA.* 80:1683.
26. Warner, N. L., J. F. Leary, and S. McLaughlin. 1979. Analysis of murine B cell lymphomas as models of B cell differentiation arrest. *In* B Lymphocytes in the Immune Response. M. Cooper, D. Mosier, I. Scher, and E. Vitetta, editors. Elsevier/North-Holland, New York. p. 371.
27. Gronowicz, E. S., C. A. Doss, F. D. Howard, D. C. Morrison, and S. Strober. 1980. An *in vitro* line of the B cell tumor BCL_1 can be activated by LPS to secrete IgM. *J. Immunol.* 125:976.

28. Tyler, J. D., D. Steinmuller, and W. J. Burlingham. 1982. Cell-mediated cytotoxicity to non-MHC alloantigens on murine epidermal cells. IV. An alloantigen shared with B cells but not T cells. *Cell. Immunol.* 68:93.
29. Eidels, L. 1979. IgD is present on the cell surface of murine lymphocytes in two forms: δ_2L_2 and δL_1. *J. Immunol.* 123:896.
30. Pollock, R. R., M. E. Dorf, and M. F. Mescher. 1980. Genetic control of murine IgD structural heterogeneity. *Proc. Natl. Acad. Sci. USA.* 77:4256.
31. Huber, B. T. 1982. B cell differentiation antigens as probes for functional B cell subsets. *Immunol. Rev.* 64:57.
32. Smith, K. 1984. Lymphokine regulation of T cell and B cell function. *In* Fundamental Immunology. W. Paul, editor. Raven Press, New York. p. 559.
33. Rosenthal, K. L., M. B. A. Oldstone, H. Hengartner, and R. M. Zinkernagel. 1983. Specificity of *in vitro* cytotoxic T cell clones directed against vesicular stomatitis virus. *J. Immunol.* 131:475.
34. Effros, R. B., M. E. Frankel, W. Gerhoard, and P. C. Doherty. 1979. Inhibition of influenza-immune T cell effector function by virus-specific hybridoma antibody. *J. Immunol.* 123:1343.
35. Forman, J., and L. Flaherty. 1978. Identification of a new CML target antigen controlled by a gene associated with the Qa-2 locus. *Immunogenetics.* 6:227.
36. Steinmuller, D., J. D. Tyler, and C. S. David. 1981. Cell-mediated cytotoxicity to non-MHC alloantigens on mouse epidermal cells. I. H-2 restricted reactions among strains sharing the $H\text{-}2^k$ haplotype. *J. Immunol.* 126:1747.
37. Carter, J., and T. G. Wegmann. 1973. Mendelian segregation of a tolerance-inducing self-antigen in the spleen. *Cell. Immunol.* 1:402.