

CYTOTOXIC T CELLS SPECIFIC FOR ANTIGENS EXPRESSED ON SURFACE IMMUNOGLOBULIN-POSITIVE CELLS*

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The BCL₁ tumor is a B cell neoplasm that causes massive splenomegaly and leukemia in BALB/c (*Ig-1^a*) mice (1). The tumor cells bear IgM and IgD on their surface and are phenotypically and functionally analogous to immature B cells (2). When mice from an allotype-congenic strain, C.B-20 (*Ig-1^b*), are injected with 10⁶–10⁷ BCL₁ cells, they are resistant to tumor growth (3). These mice generate T cells that can adoptively transfer tumor immunity to sublethally irradiated C.B-20 recipients. Upon *in vitro* rechallenge of their spleen cells, cytotoxic effector cell activity is generated that is directed against antigens on BALB/c lymphoblast target cells as well as on BCL₁ cells (3).

Little information is available on the role of cytotoxic T cells in regulating B cell neoplasms. Abbas et al. (4) described effector cells that inhibited MOPC 315 secretion *in vitro*. These cells were phenotypically characteristic of cytotoxic T lymphocytes (CTL).¹ However, their specificity was directed against the myeloma's ligand, trinitrophenol, rather than against immunoglobulin (Ig) itself. Most CTL activity that has been described is directed against viral antigens, minor H antigens, or haptens covalently coupled to cell membranes (5, 6). With the exception of trinitrophenylated proteins (7–9), CTL apparently display specificity for integral membrane proteins or molecules that can fuse with the plasma membrane (10). Accordingly, CTL could potentially be generated against surface Ig (sIg) determinants. In this regard, Rolink et al. (11) generated CTL using allotype congenic strains. However, the antigen(s) recognized by their effector cells were detected on a T cell tumor as well as both T and B lymphoblasts and presumably represent minor H antigens.

In this report we have determined the specificity of C.B-20 anti-BALB/c (anti-Ig H) cytotoxic effector cells as well as the tissue distribution of the antigen(s) recognized by these effector cells. The simplest interpretation of our data is that the effector cells recognize either the constant portion of the μ and/or δ heavy chain on Ig or a molecule coordinately expressed on sIg⁺ cells.

Materials and Methods

Mice. Mice were initially obtained from Dr. Michael Potter at the National Institute of Health (National Cancer Institute contract N01-CB094326). Mice were subsequently bred in our laboratories at the University of Texas Health Science Center.

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¹ *Abbreviations used in this paper:* Con A, concanavalin A; CTL, cytotoxic T cells; FACS, fluorescence-activated cell sorter; FITC, fluoresceinated; LPS, lipopolysaccharide; NRIg, normal rabbit Ig; RAMIg, rabbit anti-mouse Ig; sIg, surface Ig.

Generation of CTL. Mice were inoculated with 3×10^7 spleen cells intraperitoneally. 1–6 mo later, spleens were removed from these injected mice and cultured in vitro as previously described (12) in order to generate cytotoxic effector cells.

Preparation of Target Cells. Concanavalin A (Con A) lymphoblast targets were prepared as previously described (12). Lipopolysaccharide (LPS) lymphoblast targets were generated by culturing spleen cells at a concentration of 4×10^6 cells/ml in RPMI 1640 with 10% fetal calf serum for 2 d, at which time 100 μ g of LPS was added. The cells were then cultured for an additional 3 d before use.

Splenic T cells were prepared by passing spleen cells over nylon wool columns. The T cells were then cultured for 3 d in the presence of 5 μ g/ml of Con A together with adherent cells. Splenic B cells were prepared by treating spleen cells with monoclonal anti-Thy-1.2 antibody (New England Nuclear, Boston, Mass.) and complement. The surviving cells were cultured for 3 d in the presence of LPS. On the day of assay, target cells were labeled with ⁵¹chromium and centrifuged through an Isolymp solution (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, N. Y.) to remove nonviable cells.

Analysis of Thy-1⁺ and Ig⁺ Cells. In some experiments, target cells were analyzed for the percentage of Ig⁺ cells by incubating the suspensions with fluoresceinated (FITC) rabbit anti-mouse Ig (F-RAMiG) (N. L. Cappel Laboratories, Cochranville, Pa.).

Thy-1⁺ cells were detected by incubation with monoclonal anti-Thy-1.2, followed by the F-RAMiG reagent. Similarly, aliquots were stained with F-RAMiG alone. The percentage of anti-Thy-1⁺ cells was thus calculated by subtraction.

Tumor Cells. BCL₁ cells were originally obtained from Dr. Sam Strober, Stanford University Medical Center, Palo Alto, Calif. MOPC 315 was kindly provided by Dr. Richard Lynch, Washington University Medical School, St. Louis, Mo. MOPC 245, MOPC 300, TEPC 183, and P1798 were obtained from Dr. Michael Potter. MOPC 104E was provided for by Dr. Mel Bosma, Institute for Cancer Research, Philadelphia, Pa., and TEPC 15 was provided by Dr. John Cambier, Duke University, Durham, N. C. BCL₁ \times X63 is a hybridoma line resulting from the fusion of P₃ \times X63Ag8 (abbreviated X63) to BCL₁ (13).

Assay for CTL. The assay for cytotoxic effector cell activity has been described previously (12). Net release of isotope represents the percent release of isotope from target cells in the presence of immune cells minus the percent release of isotope from target cells in the presence of control cells. Control cells represent cells from cultures lacking stimulator cells. The release of isotope from target cells in the presence of control cells ranged from 20 to 35%.

Indirect Fluorescence Analysis Using the Fluorescence-activated Cell Sorter (FACS). The in vivo (1) and in vitro (14) lines of BCL₁ cells as well as the BCL₁ \times X63 hybridoma cells were stained with an affinity-purified polyvalent RAMiG or normal rabbit Ig (NRIg) control followed by FITC goat anti-rabbit Ig. Cells were analyzed on the FACS (B-D FACS Systems, Sunnyvale, Calif.) as described previously (15).

Results

Mice Challenged with Tumor Cells from an Ig H Chain Congenic Strain Generate Anti-Tumor CTL. We have previously reported (3) that BALB/c (*Ig-1^a*) mice inoculated with 10⁶ or 10⁷ BCL₁ tumor cells die from the tumor after ~8 wk. On the other hand, mice from an Ig H chain congenic strain, C.B-20 (*Ig-1^b*), are resistant to the same inoculum of BCL₁ cells. T cells from the spleens of these tumor-rejector mice adoptively transfer protection to irradiated C.B-20 animals.

When spleen cells from C.B-20 tumor-rejector mice were tested for their cytotoxic potential, they did not display activity against BCL₁ or BALB/c lymphoblasts (data not shown). However, their spleen cells could be sensitized against either irradiated BCL₁ or BALB/c cells in a secondary in vitro culture system so that cytotoxic activity was generated against both BCL₁ and BALB/c target cells (Table I). Because these effector cells could be responsible for adoptive transfer of tumor immunity against

TABLE I
Ability of C.B-20 Mice That Have Rejected BCL₁ Cells to Generate Effector Cell Activity

Cells			Net isotope release at E:T		
Effectors	Stimulators	Targets	100:1	50:1	10:1
C.B-20*	BCL ₁	BCL ₁	24.3	17.6	4.6
Anti-BCL ₁		BALB/c‡	19.7	18.7	5.4
	BALB/c	BCL ₁	9.6	7.0	1.5
		BALB/c	7.1	7.2	-3.8

* C.B-20 mice were inoculated with 10⁶ BCL₁ cells 46 d earlier. Their spleen cells were removed and cultured with irradiated stimulator cells for 5 d in vitro before testing.

‡ LPS-stimulated splenic lymphoblasts.

TABLE II
Cytotoxic Activity of C.B-20 Anti-BALB/c Effector Cells

Cells		Net isotope release at E/T		
Effectors	Targets*	100:1	50:1	10:1
C.B-20 anti-BALB/c	BALB/c (<i>Ig-1^a</i>)‡	25.5	22.3	10.2
	C.B-20 (<i>Ig-1^b</i>)	1.3	0.4	2.5
	B10.D2 (<i>Ig-1^b</i>)	1.6	2.3	3.9
	(B6 × BALB/c)F ₁ (<i>Ig-1^a/Ig-1^b</i>)	25.4	21.6	9.7
	BAB-14 (<i>Ig-1^a(v):Ig-1^b(c)</i>)	0.2	1.0	1.3
BALB/c anti-C.B-20	C.B-20	21.9	17.7	10.5
	BALB/c	0.8	-0.3	-1.3
	B10.D2	16.9	10.6	4.5
	(B6 × BALB)F ₁	16.1	13.7	5.5
	B10 (<i>Ig-1^b</i>)	-1.4	2.0	3.0
BAB-14 anti-BALB/c	BALB/C	18.4	15.5	6.9

* LPS-stimulated splenic lymphoblasts.

‡ Genotype.

BCL₁, we further investigated the activity of effector cells generated against Ig heavy chain allotypic differences.

Specificity of CTL Generated from the Sensitization of Ig H Chain Congenic Mice. C.B-20 mice were inoculated with BALB/c splenocytes. 2-6 mo later, their spleen cells were removed and the cells were cultured in vitro with irradiated BALB/c splenocytes for 5 d, at which time the cultures were assayed for cytotoxic activity against LPS lymphoblasts. The data presented in Table II demonstrate that these cells display a cytotoxic effect against BALB/c and (BALB/c × B6)F₁ (C57BL/6 is abbreviated as B6) target cells. On the other hand, C.B-20 and B10.D2 (*Ig-1^b*) target cells were not lysed. This latter result was expected because B10.D2 has the same *Ig H* chain linkage group as C.B-20, and therefore should not be susceptible to lysis. Treatment of the effector cells with monoclonal anti-Thy-1.2 serum and complement abrogated their cytotoxic activity, indicating that the cytotoxic cells are T cells (data not shown).

BALB/c anti-C.B-20 effector cells were also treated for their activity against a similar panel of target cells. C.B-20, (BALB/c × B6)F₁ and B10.D2 (*H-2^d*) target cells were killed by these effector cells. The lysis of B10.D2 target cells presumably occurs because both C.B-20 and B10.D2 mice share the same alleles at their *Ig H* chain loci.

The fact that H-2 congenic C57BL/10 (B10) (*H-2^b*, *Ig-1^b*) target cells were not lysed indicates that the cytotoxic effector cells are *H-2* restricted.

C.B-20 anti-BALB/c effector cells did not lyse BAB-14 target cells. Because BAB-14 is a recombinant strain differing from C.B-20 at the *V* but not the *C* region of *Ig H* loci (16), this indicates that the specificity of the effector cells is directed against an antigen encoded for by a gene linked to the *C* region of the *Ig H* chain linkage group. Further evidence indicating that killer cells can be generated against an antigen controlled by an *IgH C* region-linked gene is the finding that BAB-14 animals sensitized against BALB/c spleen cells generate cytotoxic effector cells that lyse BALB/c targets (Table II). Thus, these data show that cytotoxic effector cells can be generated across the *Ig H* chain allotype barrier, and are in agreement with the previous report of Rolink et al. (11).

C.B-20 Anti-BALB/c Effector Cells Display Cross-reactive Lysis on Target Cells Carrying Different Ig H Alleles. The previous data indicate that C.B-20 and BALB/c mice can generate CTL with specificity for antigens controlled by genes that map to the *Ig H* chain linkage group. However, the specificity of these effector cells for other allotypes was not tested.

The data in Table III indicate that C.B-20 anti-BALB/c effector cells display cross-reactive lysis on C.AL-20 target cells, which carry the *Ig-1^d* allele. Further, these cross-reactive effector cells can be generated by in vitro stimulation with either BALB/c or C.AL-20 stimulator cells. In addition, C.B-20 effector cells also show cross-reactive lysis on *H-2*-matched DBA/2 (*Ig-1^c*) targets. These data suggest that either *Ig-1^a*, *Ig-1^c*, and *Ig-1^d* strains express identical target antigens; or alternatively, there is a cross-reactive determinant in these three strains in addition to unique specificities. Experiments are currently in progress to distinguish between these possibilities.

Susceptibility of BCL₁ Cells to C.B-20 Anti-BALB/c Effector Cells. We previously demonstrated (3) that C.B-20 animals that had rejected a large inocula of BCL₁ cells generated cytotoxic effector cells that lysed BCL₁ and BALB/c target cells (Table I). In the following experiments, we ascertained whether effector cells produced by in vivo immunization of C.B-20 mice with BALB/c spleen cells would also generate effector cells that could recognize BCL₁ target cells.

The data in Table IV demonstrate that C.B-20 anti-BALB/c effector cells are able to lyse BCL₁ target cells. In this experiment, the in vivo BCL₁ cells were obtained from the spleen, an organ that (in tumor-bearing animals) contains ~70% tumor cells (2). Because it is possible that BALB/c host cells rather than BCL₁ cells are responsible

TABLE III
Specificity of C.B-20 Anti-BALB/c Effector Cells

Effectors	Cells		Net release at E:T		
	Stimulators	Targets*	100:1	50:1	10:1
C.B-20 anti-BALB/c	BALB/c	BALB/c (<i>Ig-1^a</i>)‡	13.9	8.5	7.9
		C.AL-20 (<i>Ig-1^d</i>)	16.8	11.2	4.7
	C.AL-20	BALB/c (<i>Ig-1^a</i>)	17.5	11.0	1.4
		C.AL-20 (<i>Ig-1^d</i>)	23.6	17.4	4.9
	BALB/c	DBA/2 (<i>Ig-1^c</i>)	28.1	26.5	10.0

* LPS-stimulated splenic lymphoblasts.

‡ Genotype.

TABLE IV
Susceptibility of BCL₁ Cells to Anti-Ig H Effector Cells

Cells		Net isotope release at E:T		
Effectors	Targets	100:1	50:1	10:1
C.B-20 anti-BALB/c	BALB/c*	25.9	20.8	12.0
	C.B-20*	-3.5	-2.7	-1.5
	BCL ₁ in vivo‡	20.9	19.0	9.0
	BCL ₁ in vitro§	22.7	26.4	21.5
	BCL ₁ × X63	-4.3	-3.8	-2.9

* LPS-stimulated splenic lymphoblasts.

‡ Spleen cells from a tumor-bearing animal.

§ Line maintained by in vitro passage.

|| Hybridoma line (see text).

for the lytic effect observed, we also tested an in vitro-adapted BCL₁ cell line (14). The data (Table IV) demonstrate that these in vitro-cultured cells are sensitive to lysis. Thus, the anti-Ig H region CTL do recognize antigenic determinants expressed by BCL₁.

BCL₁ cells have been fused to the myeloma line, X63. This hybridoma secretes IgM, which bears the same idiotypic determinants expressed on the IgM and IgD of the parental BCL₁ tumor cell, as well as the IgG_{1k} from the X63 cells (13). However, when this hybridoma line was tested for its sensitivity to lysis, no cytotoxic effect was observed (Table IV).

These three BCL₁ lines were also analyzed for cell surface Ig expression using the FACS. The data in Fig. 1 reveal that the two BCL₁ lines contain a large percentage of sIg⁺ cells (>50%), with a relatively heterogenous distribution of staining intensity. On the other hand, the BCL₁ hybridoma line contains very few sIg⁺ cells, and these cells stain with a relatively low intensity.

Therefore, these results suggest that the expression of sIg is necessary for a target cell to be sensitive to lysis by anti-Ig H chain effector cells.

Susceptibility of Tumor Lines to Lysis by Anti-Ig H Effector Cells. Because the BCL₁ (sIg⁺) B cell leukemia was killed by C.B-20 anti-BALB/c effector cells, we next determined whether other BALB/c tumor cells also expressed the target antigen. Target cells included IgA-, IgG₁-, and IgD-secreting myeloma cells and a thymic lymphoma (P1798).

The data indicate that none of these tumor cells were sensitive to lysis by either C.B-20 anti-BALB/c or BALB/c anti-C.B-20 effector cells (Table V). The latter effector cells were used because three of the myelomas tested, MOPC 315, MOPC 300, and MOPC 245, are genotypically heterozygous at *Ig-1*; i.e., they were obtained from mice during backcrossing to generate the C.B-20 congenic line (17, 18) and could be sensitive to lysis as were (BALB/c × B6)F₁ target cells (see Table II). The data also show that although the MOPC 315, MOPC 300, and P1798 cell lines are insensitive to lysis by C.B-20 anti-BALB/c effector cells, they are all sensitive to lysis by anti-H-2 CTL (B10.Q anti-BALB/c).

If Ig is the target antigen, then it is possible that secreted Ig from myeloma cells could adsorb onto unlabeled cells in the effector cell population and block or inhibit lysis. However, this alternative is unlikely. Thus, LPS induced B lymphoblasts and

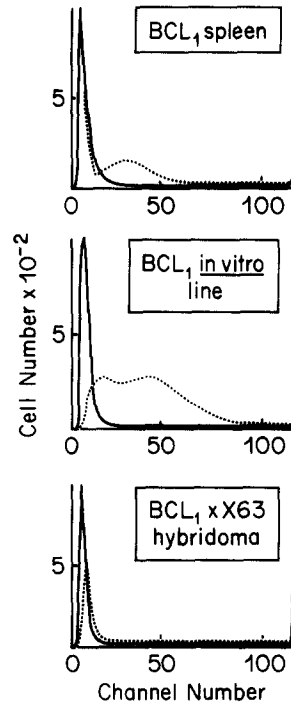


FIG. 1. Analysis of sIg on BCL₁ and BCL₁ × X63 hybridoma cells. Cells were exposed to NR Ig (solid lines) or RAM Ig (dashed lines) followed by FITC goat anti-rabbit Ig. The cells were then analyzed using the FACS.

TABLE V
Susceptibility of Lysis of BALB/c Tumor Cells to Anti-Ig H Effector Cells

Cells		Cell genotype	Myeloma protein allotype	Net isotope release at E:T		
Effectors	Targets			100:1	50:1	10:1
C.B-20 anti-BALB/c	BALB/c*	<i>Ig-1^a</i>		24.8	20.7	16.4
	(BALB/c × B6)F ₁ *	<i>Ig-1^a/Ig-1^b</i>		20.8	17.9	11.9
	C.B-20*	<i>Ig-1^b</i>		-0.8	-0.6	-0.2
	MOPC 315	<i>Ig-1^a/Ig-1^b</i>	<i>Ig-2^a</i>	1.6	0.5	0.1
	MOPC 300	<i>Ig-1^a/Ig-1^b</i>	<i>Ig-4^b</i>	5.7	4.3	1.4
	MOPC 245	<i>Ig-1^a/Ig-1^b</i>	<i>Ig-4^b</i>	3.7	2.5	0.5
	P ₃ × X63Ag8	<i>Ig-1^a</i>	<i>Ig-4^a</i>	-0.8	-2.6	-4.3
	MOPC 315			1.2 (25.7)‡	0.4 (24.3)	0.8 (14.4)
	MOPC 300			-0.8 (31.8)	-0.7 (27.5)	-0.5 (9.1)
	P1798	<i>Ig-1^a</i>		0.3 (36.1)	-0.5 (35.7)	-4.5 (22.6)
BALB/c*			25.3 (42.1)	25.9 (40.5)	17.1 (21.3)	
BALB/c anti-C.B-20	BALB/c*					xt
	(BALB/c × B6)F ₁ *			0.8	-0.3	-1.3
	C.B-20*			16.1	13.7	5.5
	MOPC 300			21.9	17.7	10.5
	MOPC 245			-2.6	-4.6	-7.0
	MOPC 315			-2.1	-3.7	-6.3
			-1.4	-5.9	-10.0	

* LPS-stimulated splenic lymphoblasts.

‡ Values in parentheses represent isotope release from target cells in the presence of anti-H-2 CTL (B10.Q anti-BALB/c).

the in vitro BCL₁ cell line secretes Ig (19; and unpublished observations), but both are sensitive to lysis. Furthermore, this type of mechanism for inhibition of lysis has not been observed in CTL assays. For example, anti-viral CTL lyse virus-infected target cells even though such targets release large quantities of virus into the assay media (20). This inability of soluble antigen to inhibit effector CTL activity is most likely due to the H-2 restriction of T cell specificity where shed antigen does not apparently reassociate with H-2 class I antigens in in vitro assays (21).

Table VI summarizes experiments that include all of the tumor lines that were tested for the presence of the target antigen. The data indicate that only sIg⁺ BCL₁ cells are sensitive to lysis, whereas IgG-, IgM-, IgD-, and IgA-secreting myeloma cells, which have been reported to express low amounts of sIg (22), are negative.

Sensitivity of Splenic B and T Cell Lymphoblasts to Anti-Ig H Effector Cells. The previous data indicate that not all tissues of BALB/c origin express detectable quantities of the antigenic determinants recognized by C.B-20 anti-BALB/c effector cells. Therefore, experiments were performed to ascertain whether splenic T and B cell lymphoblasts were equally sensitive to lysis.

The data in Table VII indicate that both splenic LPS and Con A lymphoblasts were sensitive to lysis by anti-Ig H region effector cells, although LPS blasts were usually more sensitive than Con A blasts. Because splenic Con A blasts contain a considerable portion of Ig⁺ cells and splenic LPS blasts contain a considerable portion of Ig⁻ cells, we purified T and B cells from spleens and then cultured each with either Con A or LPS, respectively. These populations of T and B cell lymphoblasts were enriched for the appropriate cells and showed relatively little cross contamination (see footnotes to Table VI). When these cells were tested for their sensitivity to lysis, it was noted that the LPS-stimulated B lymphoblasts were usually more sensitive to lysis than the LPS-stimulated splenic lymphoblasts, whereas the Con A-stimulated T lymphoblasts displayed little or no sensitivity to lysis. It should also be noted that the four types of target cells tested were equally sensitive to lysis by anti-H-2 effector cells (B10.Q anti-BALB/c).

Therefore, these data indicate that the target antigen(s) recognized by anti-Ig H region effector cells have a restricted tissue distribution that appears to be coincident with the expression of sIg.

TABLE VI
Tumor Cell Typing for Target Antigen

Tumor	Genotype	Myeloma protein allotype	Target antigen
BCL ₁	<i>Ig-l^a</i>	Ig6 ^a	+
BCL ₁ X63	<i>Ig-l^a</i>	Ig6 ^a , Ig4 ^a	-
MOPC 315	<i>Ig-l^a/Ig-l^b</i>	Ig2 ^a	-
MOPC 300	<i>Ig-l^a/Ig-l^b</i>	Ig4 ^b	-
MOPC 245	<i>Ig-l^a/Ig-l^b</i>	Ig4 ^b	-
MOPC 104E	<i>Ig-l^a</i>	Ig6 ^a	-
TEPC 183	<i>Ig-l^a</i>	Ig6 ^a	-
TEPC 15	<i>Ig-l^a</i>	Ig2 ^a	-
TEPC 1033	<i>Ig-l^a</i>	Ig5 ^a	-
X63	<i>Ig-l^a</i>	Ig4 ^a	-
P1798	<i>Ig-l^a</i>	-	-

TABLE VII
Sensitivity of Splenic Lymphoblasts to Anti-Ig H Effector Cells

Cells		Net release at E:T = 100:1					
Effectors	Targets	Mitogen	Net release at E:T = 100:1				
			Experi- ment 1	Experi- ment 2	Experi- ment 3	Experi- ment 4	
C.B-20 anti-BALB/c	BALB/c	WS	LPS	16.1*	25.3 (42.1)‡	—	—
		WS	Con A	14.8§	7.6 (41.9)	—	—
	BALB/c	B cells	LPS	20.2	17.8 (42.7)	35.8	—
		T cells	Con A	8.8¶	7.3 (39.0)	0.8	—
BALB/c anti-C.B-20	C.B-20	WS	LPS	—	—	—	20.0*
		WS	Con A	—	—	—	15.0§
	C.B-20	B cells	LPS	—	—	—	29.5
		T cells	Con A	—	—	—	8.7¶

* WS, whole spleen; whole spleen LPS lymphoblasts contained 68–77% Ig⁺ cells and 15–18% Thy-1.2⁺ cells.

‡ Numbers in parentheses represent lysis from target cells by B10.Q anti-BALB/c effector cells.

§ Whole spleen Con A lymphoblasts contained 19–24% Ig⁺ cells and 55–64% Thy-1.2⁺ cells.

|| B cell lymphoblasts contained 85–95% Ig⁺ cells and 2–7% Thy-1.2⁺ cells.

¶ T cell lymphoblasts contained 12–14% Ig⁺ cells and 75–78% Thy-1.2⁺ cells.

Discussion

The data in this report provide the first direct evidence for the existence of CTL specific for antigenic determinants expressed on sIg⁺ cells. The target antigens recognized by these CTL are encoded for by genes either linked to or identical with *Ig H* chain loci. Furthermore, because C.B-20 anti-BALB/c effector cells do not lyse BAB-14 lymphoblasts, the antigen recognized on BALB/c targets is encoded for by a gene linked to the *C* rather than the *V* region of the *Ig-1* locus. Thus, our data demonstrate that the target antigens detected by the cytotoxic effector cells generated in this system are expressed on LPS-induced B cell lymphoblasts but not Con A-induced T cell lymphoblasts or a T cell leukemia. Although this indicates that the target antigens are expressed on cells of the B and not the T cell lineage, these antigens are not expressed on all cells of B cell origin. We were unable to detect target antigens on several myeloma cell lines that have been reported to have low levels of sIg (reviewed in 22). On the other hand, BCL₁, an sIg⁺ B cell leukemia was sensitive to lysis. Furthermore, a BCL₁ × X63 hybridoma line that secretes the same IgM as is expressed on the surface of the parental BCL₁ line, but displays almost no sIg, was not lysed by the anti-Ig H effector cells. Taken together, these data suggest that the target antigen being detected by the CTL is either sIg itself or a determinant that is coordinately expressed on Ig⁺ B cells.

These data provide a possible mechanism whereby CTL could regulate B cells expressing cell membrane allotypic (Ig) determinants. This type of activity would be consistent with previous studies indicating that other subpopulations of T lymphocytes, *viz.*, helper and suppressor T cells, can regulate the growth and differentiation of B cells possessing idiotypic or allotypic determinants on their membrane Ig. Thus, Herzenberg et al. (23, 24) have demonstrated allotype specific helper and suppressor T cells. Eichmann (25), Woodland and Cantor (26), and Bottomly and Maurer (27), have described helper cells that are idio-type specific. Rosenberg and Chiller (28) have evidence for an allotype (C region)-specific helper cell involved in the regulation of IgG responses. Both L'age-Stein (29) and Nutt et al. (30) have data to indicate that

antigen-primed B cells induce the expansion of helper T cell populations that recognize allotypes or idiotypes on B cells.

Rolink et al. (11) reported that CTL could be generated using allotype congenic strains that were similar but not identical to those used in this study. The effector cells they described detected an antigen expressed on a T cell tumor as well as both T and B cell lymphoblasts. In contrast, the antigen recognized by the cytotoxic cells generated here were restricted to sIg⁺ B cells. This discrepancy between the two sets of data could be due to the fact that the BALB/c animals used by Rolink et al. (11) may differ at an *Ig*-linked H-antigen locus from the BALB/c line used to derive the allotype congenic strains. Accordingly, the CTL generated by Rolink et al. (11) might be specific for such an H antigen(s). Alternatively, their culture system may allow for the generation of clones of effector cells that recognize additional non-Ig antigenic determinants on lymphoid cells. In this regard, we have noted that in some experiments where CTL from individual animals were tested on P815 (*Ig-1^c, H-2^d*) target cells, that some C.B-20 anti-BALB/c effector cells lysed this cell line. The activity of the CTL from these mice may be similar to that described by Rolink et al. (11) and is also consistent with the data of Riblet et al. (31), who demonstrated that allotype congenic strains reject skin grafts. Thus, those antigens might be analogous to minor H antigens that have been shown to elicit both skin graft rejection and to induce cytotoxic effector T cell activity (32). However, it is also possible that P815 cells express the same antigen that is detected on the sIg⁺ cells. In this case, the target antigen would most likely be a differentiation antigen with coordinate expression on sIg⁺ cells and P815 cells.

There are several molecules in addition to CTL target antigens that are controlled by *Ig-1* linked loci. For example, Owen et al. (33) have immunized BALB/c animals with cells from allotype congenic cells C.AL-20 mice and were able to produce an antiserum that recognized a molecule found on Lyt-2⁺ suppressor cells. A hybridoma antibody that recognizes a second *Ig-H*-encoded antigen expressed on Lyt-1⁺ suppressor inducer cells has been described (G. M. Spurril and F. L. Owen, personal communication). A. Finnegan and F. L. Owen (personal communication) have produced a C.B-20 anti-BALB/c antiserum that detects an antigen controlled by a gene linked to the *V* region of *Ig-H* and is expressed on CTL.

The role of cytotoxic and suppressor T cells in controlling growth of B cell neoplasms is not clear. Bosma and Bosma (34) have shown that anti-allotype suppressor cells can regulate the secretion of Ig by myeloma cells from *Ig-1^b* mice. Rohrer and Lynch (35) have demonstrated that suppression or enhancement of MOPC 315 tumor cell growth, myeloma stem cell production, and IgA secretion can be influenced by carrier-specific helper or suppressor T cells. Flood et al. (36) have demonstrated that mice immunized with purified myeloma proteins generate idiotypic-specific T cells that regulate MOPC 315 growth.

Abbas et al. (4) have reported that Lyt-2⁺, cyclophosphamide- and radiation-resistant T cells can regulate Ig secretion by MOPC 315 cells in vitro. The criterion for defining these cytotoxic cells was based on these phenotypic characteristics and by their ability to reduce the viability of the tumor cells over a 2-3-d culture period. However, these effector cells did not cause direct lysis of target cells in a 4-h chromium release assay. Further, unlike the specificity of the CTL described in this report, these

cells were specific for the epitope which the MOPC 315 cells bind (trinitrophenol) rather than the Ig itself.

Although CTL could play a role in preventing B cell tumors, they may not be relevant in regulating myeloma cell growth because the level of surface Ig on these cells is relatively small (22). On the other hand, tumor cells such as BCL₁ may be more amenable to regulation by CTL because these cells generally have a high density of sIg (2), which could be recognized as a tumor-specific antigen.

It is not known whether the ability of C.B-20 mice to resist BCL₁ is due to the activity of CTL. We previously reported (3) that the tumor rejection antigen maps to the V region rather than the C region of the Ig gene complex because BAB-14 mice were tumor susceptible. However, recent data (R. Ciavarra and J. Forman, unpublished data) suggest that for tumor rejection to occur, mice need to recognize two determinants, one in the V region and the other in the C region. A similar conclusion has been drawn in the anti-Qa-1 CTL system (37). If this interpretation is correct, then the anti-allotype CTL described here could be relevant anti-tumor effector cells.²

Summary

C.B-20 mice were immunized with splenocytes or B leukemia cells (BCL₁) from Ig H chain allotype congenic strains. Spleen cells from these immunized mice were rechallenged in vitro to generate H-2-restricted cytotoxic T cells that were specific for target antigens controlled by genes linked to the Ig H chain locus.

The anti-Ig H cytotoxic T cells detected an antigen(s) expressed only on surface Ig⁺ cells. Thus, T cell lymphoblasts, eight BALB/c myeloma cell lines, and a T cell lymphoma were not lysed by the effector cells. In contrast, B cell lymphoblasts and the surface Ig⁺ BCL₁ cells were sensitive to lysis. A surface Ig⁻ hybridoma (which secretes the IgM from the BCL₁ cells) generated by fusing BCL₁ cells to X63 myeloma cells was not killed by the effector cells.

These data indicate that cytotoxic T cells specific for antigenic determinants on either surface IgM or IgD or on a molecule that is coordinately expressed on IgM⁺ or IgD⁺ cells can be generated and that such cells might play a role in regulating the growth of normal B cells or surface Ig⁺ tumor cells in vivo.

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