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EXPRESSION OF BOTH *I-A* AND *I-E/C* SUBREGION ANTIGENS ON ACCESSORY CELLS REQUIRED FOR *IN VITRO* GENERATION OF CYTOTOXIC T LYMPHOCYTES AGAINST ALLOANTIGENS OR TNBS-MODIFIED SYNGENEIC CELLS

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We have previously reported that radioresistant, Thy 1-negative accessory cells (SAC) are required for the *in vitro* generation of cytotoxic T-effector cells to allogeneic or trinitrophenyl-modified syngeneic cells. These SAC were found to provide accessory functions irrespective of whether they were syngeneic, semi-syngeneic, or allogeneic to the responding cells. To further characterize the accessory cells active in CML, the expression of Ia antigens on this functional population was assessed by pretreated SAC with anti-Ia reagents and complement and by testing the accessory cell function of these treated populations. The results of these studies demonstrated that the relevant accessory cells for allogeneic and TNP-self CTL express Ia determinants encoded by genes mapping in the *I-A* and *I-E/C* subregions. For the TNP-self CTL the accessory function of both SAC syngeneic or allogeneic to the responding and stimulating cells was specifically abrogated by treatment with anti-Ia reagents and complement.

Cellular interactions have been shown to be important in several T cell-dependent immune responses (reviewed in 1). In each of these systems, Ia antigens appear to play a role in cell collaboration (2-5). Previous studies have demonstrated that the *in vitro* generation of cytotoxic T lymphocytes (CTL)[2] to allogeneic or trinitrophenyl-modified syngeneic cells (TNP-self) requires the interaction among different cell types of splenic origin, including responding, stimulating, and accessory cells (6). To clarify the role of Ia antigens in CTL, we have first investigated the expression of Ia antigens on the cells involved in *in vitro* cytotoxic responses. The present report is concerned with the expression of Ia antigens on accessory cells. The accessory cells (SAC) required for cell-mediated lympholysis (CML) have been shown to be radioresistant, θ-negative, and adherent to glass (6, 7). Therefore, they are similar to accessory cells required for *in vitro* antibody responses (8), antigen-induced cell proliferation (9), and concanavalin A (Con A) stimulation (10). Further analysis of the SAC required for antibody and proliferative responses indicated that the subpopulation that provided accessory function expressed Ia antigens (8-11). In order to characterize the antigens expressed on SAC required for CTL, the accessory function of SAC has been tested after treatment of this population with specific anti-*Ia* reagents. This was of particular interest since, in the TNP-self CTL, SAC are not required to be H-2 compatible with responding and stimulating cells for providing accessory function (6). Results of the present study indicate that the relevant accessory cell population required for allogeneic and TNP-self CTL expressed Ia determinants encoded by genes mapping in the *I-A* and *I-E/C* subregions. The accessory function of SAC that were syngeneic and those that were allogeneic to the responding and stimulating cells was eliminated by treatment with these anti-Ia reagents and complement (C).

MATERIALS AND METHODS

Mice. The strains of mice used and their respective H-2 haplotypes are listed in Table I. Animals of strains A/J, B10.A, C57BL/10(B10), B10.D2, B10.BR, B10.A(4R), and B10.A (5R) male mice, 5 to 8 weeks of age, obtained from the Jackson Laboratory, Bar Harbor, Maine, were used throughout the experiments. All other mice were bred in our own colonies.

Preparation of splenic adherent cells. The preparation of splenic adherent cells (SAC) has been described in detail elsewhere (6, 12). Briefly, spleen cells were adhered to glass for 2 to 3 hr, detached by exposure to Versene buffer (GIBCO, Grand Island, N. Y.), treated with rabbit anti-mouse brain serum (RAMB) and complement (C), irradiated with 1500 R, and cultured overnight on a roller drum at 37°C in Eagle's minimum essential medium (MEM) supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 20 mM HEPES[2] buffer (NIH Media Unit), and 20% fetal bovine serum (GIBCO) (MEM-20% FBS). The resulting cells were enriched for latex-ingesting cells (67 ± 13%) and contain less than 0.3% $RAMB^+$ cells (9).

Fractionation of spleen cells over Sephadex G-10 columns. Spleen cells were passed through columns of Sephadex G-10 (Pharmacia Fine Chemicals, Piscataway, N. J.) prepared as described by Ly and Mishell (13). Spleen cells (1×10^8/ml, 3 to 4 ml) in MEM-20% FBS were added dropwise to G-10 columns, and 35 ml were collected by dropwise elution with MEM-20% FBS. The eluted cells were then passed through a second column. The range of cell recovery after double passage was 40

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[2] Abbreviations used in this paper: MHC, major histocompatibility complex; Ia, I region-associated antigens; CTL, cytotoxic T lymphocytes; SAC, spleen accessory cells; TNBS, trinitrobenzene sulfonate; TNP-self, TNBS-modified syngeneic spleen cells; RAMB, rabbit anti-mouse brain serum; CML, cell-mediated lympholysis; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid.

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TABLE I

Haplotype origin of H-2 regions of mice used

Strain	Haplotype Designation	Regions and Subregions								
			I							
		K	A	B	J	E	C	S	D	Tla
A, B10.A	a	k	k	k	k	k	d	d	d	a
C57BL/10	b	b	b	b	b	b	b	b	b	b
B10.D2	d	d	d	d	d	d	d	d	d	c
B10.A(4R)	h4	k	k	b	b	b	b	b	b	b
B10.A(5R)	i5	b	b	b	k	k	d	d	d	a
B10.BR	K	k	k	k	k	k	k	k	k	a
B10.S	s	s	s	s	s	s	s	s	s	b
A.TL	t1	s	k	k	k	k	k	k	d	c
A.TH	t2	s	s	s	s	s	s	s	d	a
B10.HTT	t3	s	s	s	s	k	k	k	d	c

to 70%. The G-10 column-eluted cells were centrifuged, treated with ammonium chloride-lysing buffer to remove erythrocytes, washed, and resuspended in culture medium. After G-10 fractionation, the remaining latex-ingesting cells ranged from 0.1 to 1.0%, whereas no change was detected in the proportion of T and B lymphocytes.

Antisera. Antisera and immune ascites preparation were generously provided by Dr. David H. Sachs and produced as previously described (14, 15). The reagents employed and their C-dependent cytotoxic titers on whole spleen cells were as follows: (B10.A × A)F_1 anti-B10 (1:64 on $H\text{-}2^d$); A.TH anti-A.TL (1:2048 on $H\text{-}2^k$); B10.HTT anti-A.TL (1:256 on $H\text{-}2^k$); and (B10.SxA.TH)F_1 anti-B10.HTT (1:128 on $H\text{-}2^k$).

Antiserum treatment of SAC. Treatment of SAC with anti-*Ia* reagents and C has been described in detail previously (15). SAC were incubated at 5×10^6/ml in MEM-10% FBS containing anti-*Ia* reagent at a 1:10 dilution (except for A.TH anti-A.TL, which was used at 1:30) at 4°C for 30 min. They were then washed and resuspended at the same concentration with screened rabbit C at a 1:10 dilution and incubated with agitation for an additional 30 min at 37°C. Cells were then washed twice for an additional 30 min with MEM-10% FBS, and viable cells were determined by trypan blue exclusion. Treatment of the SAC with anti-*Ia* reagents resulted in killing levels (above C background) of 50 to 70%.

Conditions for cell culture and cytotoxic assay. The conditions used for cell cultures and cytotoxic assays have been previously reported (16). Briefly, responding spleen cells were cultured with irradiated allogeneic or TNBS-modified syngeneic spleen cells for 5 days in FBS-supplemented RPMI 1640 culture medium (GIBCO) in the presence of 5×10^{-5} M 2-mercaptoethanol. The cultured cells were harvested, adjusted to equal numbers of viable cells, and assayed in a 4-hr ^{51}Cr-release assay on the appropriate unmodified or TNBS-modified targets.

RESULTS

Accessory cell function of SAC for allogeneic CTL is abolished by pretreatment with anti-Ia reagents and C. Spleen cells from B10.D2 ($H\text{-}2^d$) mice were fractionated over Sephadex G-10 and used as responding cells for the generation of an *in vitro* allogeneic CML response to G-10-fractionated B10.BR ($H\text{-}2^k$) spleen cells. Under these conditions, as previously reported (6) and as shown in Figure 1, no CTL activity was generated against alloantigens. However, accessory cell function can be provided by the addition of SAC syngeneic to either the responding or stimulating cells. In the experiment in Figure 1, B10.D2 SAC pretreated with normal (B10.A × A)F_1 ascites and C were as competent in providing accessory cell function as untreated SAC. However, pretreatment of the B10.D2 SAC with (B10.A × A)F_1 anti-B10 (a reagent that has been shown only to contain antibodies against products encoded by $I\text{-}A^d$) (14, 15) and C drastically reduced accessory cell function. These results indicated that the accessory function was provided by cells expressing determinants encoded by genes in *I-A* subregion. These observations were verified by absorption studies in which the activity of the (B10.A × A)F_1 anti-B10 reagent on B10.D2 SAC was removed by B10.A(5R) but not by B10.A(4R) spleen cells (unpublished observations). These results indicate that the SAC involved in the accessory function for allogeneic CTL express *Ia* antigen(s) encoded by gene(s) mapping in the *I-A* subregion.

Accessory cells function of SAC for TNP-self CML is abolished by pretreatment with anti-Ia reagents and C. Spleen cells from B10.BR ($H\text{-}2^k$) mice were fractionated over Sephadex G-10 and used as responding cells for the generation of an *in vitro* a) allogeneic CML response to G-10-fractionated B10 ($H\text{-}2^b$) spleen cells (to confirm the results of Fig. 1 and to provide a control for the TNP-self experiment), and b) TNP-self CML response to G-10-fractionated B10.BR spleen cells modified with TNBS. B10.BR SAC were pretreated with: 1) medium only; 2) normal (B10.A × A)F_1 ascites and C; 3) A.TH anti-A.TL alone; or 4) A.TH anti-A.TL and C before addition to the cultures. The addition of untreated SAC to the cultures of G-

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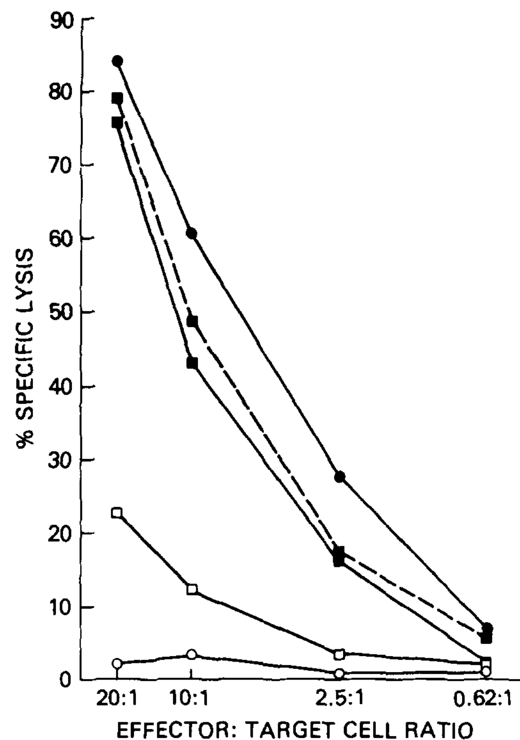

Figure 1. Effect of treatment with anti-*Ia* reagents and C on the accessory function of SAC for allogeneic CTL. (●——●) 7×10^6 unfractionated B10.D2-responding spleen cells were stimulated with 3×10^6 unfractionated, irradiated B10.BR spleen cells. (○——○) 7×10^6 G-10 nonadherent B10.D2 responding cells were stimulated with 3×10^6 G-10 nonadherent, irradiated B10.BR spleen cells. (■——■) 5×10^5 untreated B10.D2 SAC, (■----■) 5×10^5 B10.D2 SAC treated with normal (B10.A × A)F_1 ascites and C, or (□——□) 5×10^5 B10.D2 SAC treated with (B10.A × A)F_1 anti-B10, anti-$I\text{-}A^d$, plus C were added to the G-10-fractionated cell cultures. Effector cells were assayed on RDM-4 ($H\text{-}2^k$) target cells.

10-fractionated spleen cells restored both the allogeneic and TNP-self CML responses (Fig. 2), whereas treatment with the A.TH anti-A.TL reagent (which contains potential antibodies against products encoded by the entire *I* region) and C abrogated their ability to restore allogeneic or TNP-self CML. In contrast, treatment of the (B10.BR SAC with 1) A.TH anti-A.TL alone; 2) normal (B10.A × A)F_1 ascites and C; 3) (B10.A × A)F_1 anti-B10 (an anti-Ia reagent with no known activity against products of the $H\text{-}2^k$ haplotype but that did eliminate the accessory function of B10.D2 SAC [Fig. 1]) and C did not affect their accessory function. These results indicate that the SAC that provide accessory function for both TNP-self and allogeneic CML responses express Ia antigens.

The failure of SAC treated with anti-Ia and C to provide accessory function for TNP-self and allogeneic CML responses could result either from elimination of active accessory cells from the SAC population or from active suppression exerted by anti-Ia-treated SAC. To differentiate between these possibilities, untreated SAC were mixed with SAC treated with anti-Ia and C and the resulting mixture tested for its ability to restore the TNP-self or allogeneic response of G-10-fractionated spleen cells. The results (Fig. 3) demonstrate that the addition of treated SAC to untreated SAC had no effect on the accessory function of untreated SAC. Thus, the inability of SAC treated with anti-Ia and C to provide an accessory function for TNP-self and allogeneic CML probably represents the elimination of a subpopulation of Ia-positive SAC and is not merely due to an inhibitory effect exerted by treated cells.

Accessory cells for allogeneic and TNP-self CML express Ia determinants encoded by genes in I-A and I-E/C subregions. Determination of the *I* region-encoded determinants expressed on accessory cells for allogeneic and TNP-self CML was performed by treating SAC with reagents containing antibodies

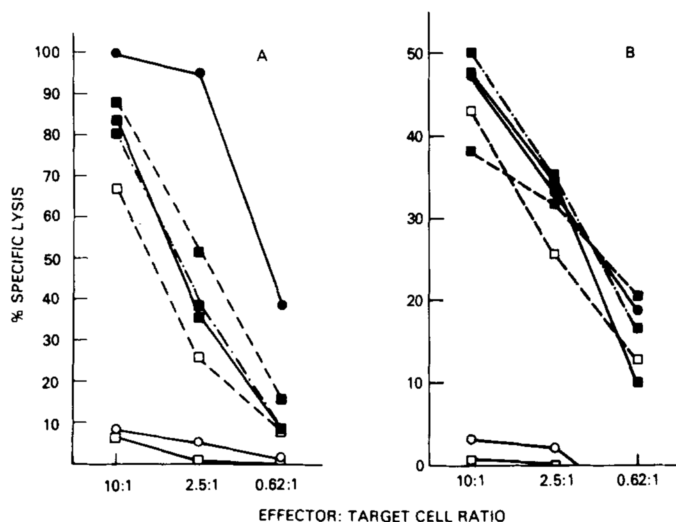

Figure 2. Effect of treatment with anti-*Ia* reagents and C on the accessory function of SAC for allogeneic (*A*) and TNP-self CTL (*B*). (●——●) 7 × 10^6 unfractionated B10.BR responding cells were stimulated with 3 × 10^6 unfractionated irradiated B10 cells (*A*) or TNBS-modified B10.BR cells (*B*). (○——○) 7 × 10^6 G-10 nonadherent B10.BR responding cells were stimulated with (*A*) 3 × 10^6 G-10 nonadherent B10 cells or (*B*) G-10 nonadherent TNBS-modified B10.BR cells. *A*, *B*, 5 × 10^5 B10.BR SAC were added to the G-10-fractionated cell cultures after treatment with: 1) (■——■) medium only; 2) (■-----■) normal (B10.A × A)F_1 ascites and C; 3) (■—·—■) (B10.A × A)F_1 anti-B10; 4) (□-----□) A.TH anti-A.TL alone; 5) (□——□) ATH anti-A.TL + C. Effectors were assayed on EL-4 ($H\text{-}2^b$) target (*A*) or TNBS-modified RDM-4 ($H\text{-}2^k$) (*B*).

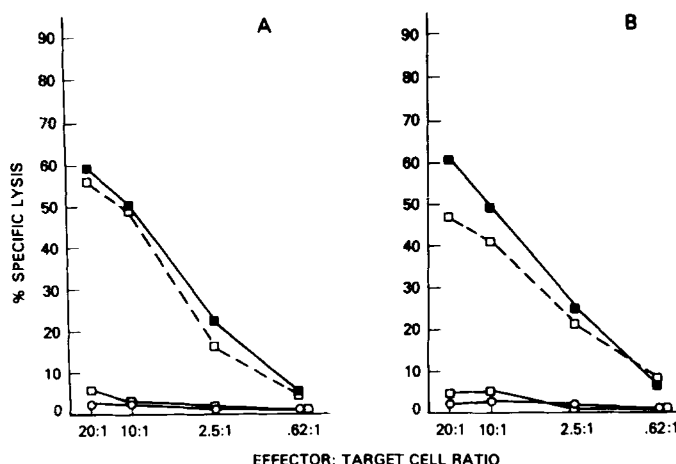

Figure 3. Lack of inhibitory effect of Ia^- SAC on CML. (○——○) 7 × 10^6 G-10 nonadherent B10.BR responding cells were stimulated with (*A*) 3 × 10^6 G-10 nonadherent B10 cells, or (*B*) 3 × 10^6 G-10 nonadherent TNBS-modified B10.BR cells. *A*, *B*, (■——■) 2.5 × 10^5 untreated B10.BR SAC, or (□——□) 5 × 10^5 B10.BR SAC treated with A.TH anti-A.TL plus C, or (□-----□) a mixture of untreated (2.5 × 10^5), and treated (5 × 10^5) B10.BR SAC was added to the G-10-fractionated cell cultures. The allogeneic effectors (*A*) were assayed on EL-4 target; the TNP-self effectors (*B*) were assayed on TNBS-modified RDM-4 targets.

specific for determinants encoded by *I-A* and *I-E/C*. Sephadex G-10-fractionated B10.BR spleen cells were stimulated with G-10-fractionated B10 spleen cells (Fig. 4*A*) or G-10-fractionated B10.BR-TNBS spleen cells (Fig. 4*B*), and accessory cells that had been treated with subregion specific anti-Ia reagents and C were added. The results indicated that both the allogeneic and TNP-self CML responses were abrogated when the SAC were treated with two reagents: B10.HTT anti-A.TL that contains known antibodies against products encoded by $I\text{-}A^k$ and (B10.SxA.TH)F_1 anti-B10.HTT that contains known antibodies against $I\text{-}E/C^k$. The complete abrogation of the accessory function of the B10.BR SAC was observed after treatment with either anti-Ia subregion specific reagents or with the broadly specific A.TH anti-A.TL reagent. Furthermore, the CML responses were not reconstituted when B10.BR SAC treated with the anti-$I\text{-}A^k$ reagent were mixed with the SAC treated with the anti-$I\text{-}E/C^k$ reagent and added to the cultures. This indicates that the SAC providing the accessory function express Ia determinants encoded by genes in the *I-A* as well as the *I-E/C* subregions and that these determinants are expressed on the same cell.

The allogeneic SAC that provide accessory function of TNP-self CML express Ia determinants. Allogeneic SAC have been shown to provide accessory function for generating TNP-specific, H-2-restricted CML responses (6). It is not clear whether accessory function provided by syngeneic or allogeneic SAC is mediated by a functionally similar or distinct SAC subpopulation. The finding that syngeneic accessory cells express Ia antigens provides a useful marker for accessory cells in CML and therefore permits an analysis of the allogeneic cell type involved in the accessory activity for TNP-self CML. Therefore, we have compared the accessory function for generating a TNP-self CML response by G-10-fractionated B10.BR responding spleen cells using syngeneic B10.BR or allogeneic B10.D2 SAC treated with anti-Ia reagents specific for these two haplotypes, respectively. The accessory function for TNP-self CML with B10.BR-responding cells was provided either by syngeneic B10.BR or allogeneic B10.D2 SAC (compare lines 5 and 6 with

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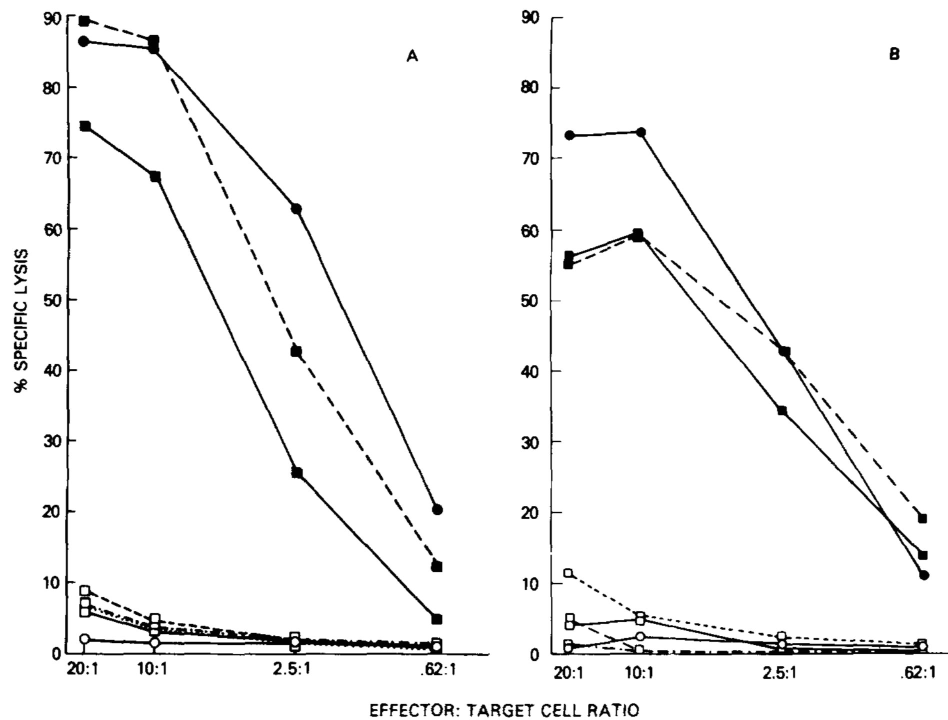

Figure 4. Accessory cells for allogeneic (*A*) and TNP-self (*B*) CML express Ia determinants encoded by genes in *I-A* and *I-E/C* subregions. (●——●) 7 × 10^{6} B10.BR cells were stimulated with 3 × 10^{6} B10 spleen cells (*A*), or 3 × 10^{6} B10.BR-TNBS spleen cells (*B*). (○——○) 7 × 10^{6} G-10 nonadherent B10.BR cells were stimulated with 3 × 10^{6} G-10 nonadherent B10 spleen cells (*A*), or 3 × 10^{6} G-10 nonadherent B10.BR-TNBS spleen cells. *A*, *B*, 5 × 10^{5} B10.BR SAC were added to the G-10-fractionated cell cultures after treatment with: 1) (■——■) medium alone; 2) (■----■) (B10.A × A)F1 anti-B10 + C; 3) (□——□) A.TH anti-A.TL + C; 4) (□----□) B10.HTT anti-A.TL + C; 5) (□—···—□) (B10.SxA.TH)F1 anti-B10.HTT, and (□····□) a mixture of 4) and 5). The allogeneic effectors were assayed on EL-4 targets (*A*); the TNP-self effectors were assayed on TNBS-modified RDM-4 targets (*B*).

TABLE II

The effect of pretreatment with anti-Ia reagents and C upon the accessory function of allogeneic SAC for TNP-self CTL

B10.BR Responding Cells (7 × 10⁶)	Stimulating Cells (3 × 10⁶)		SAC Added (5 × 10⁵)	Pretreatment of SAC	% Lysis on Target Cells Shown: RDM-4-TNBS (1 mM) 40:1	10:1	25:1	RDM-4 40:1
Unfractionated spleen cells	Unfractionated	B10.BR	None		0.2	4.4	−4.4	−13.6
		B10.BR-TNBS			68.9	53.2	27.8	−9.3
G-10-fractionated spleens cells	G-10-fractionated	B10.BR	None		0.8	−7.7	−13.6	−13.4
		B10.BR-TNBS			−5.2	−5.4	−5.7	−5.4
↓	↓	B10.BR	B10.BR	None	23.2	7.5	2.4	−0.5
		B10.BR-TNBS			74.8	79.7	53.4	−0.1
		B10.BR	B10.BR	A.TH. anti-A.TL + C	−11.6	−12.4	−1.8	−16.0
		B10.BR-TNBS			13.5	−1.3	N.T.	−8.3
		B10.BR	B10.D2	None	35.1	19.4	5.6	−1.0
		B10.BR-TNBS			73.4	72.6	31.2	−3.4
		B10.BR	B10.D2	(B10.A)F1 + C	44.2	27.7	4.9	−0.6
		B10.BR-TNBS			77.9	70.4	25.2	−3.1
		B10.BR	B10.D2	(B10.A × A)F1 anti-B10 + C	7.6	1.9	−1.7	−3.6
		B10.BR-TNBS			12.8	1.8	−3.8	−4.2

9 and 10 of Table II). The accessory function provided by either syngeneic or allogeneic SAC was susceptible to treatment with specific anti-Ia reagents, since cytotoxicity was abolished when the B10.BR (syngeneic) SAC were treated with the A.TH anti-A.TL reagent and C (compare lines 6 and 8), or when the B10.D2 (allogeneic) SAC were treated with the (B10.A × A)F1 anti-B10 reagent and C (compare lines 12 and 14). As previously shown (1), low but significant cytotoxic activity can be detected on RDM-4-TNBS targets when the only stimulating cells were the allogeneic B10.D2 SAC. This is possibly due to the observation that allogeneically stimulated CTL lyse TNBS-modified target cells (17).

DISCUSSION

Cell surface determinants encoded by genes in the *I* region of the murine major histocompatibility complex (MHC) appear to play an important role in antigen presentation and/or in cell interaction (2–5). However, the requirement for the presence of Ia antigens on responder, stimulator, antigen-presenting, or effector cells for *in vitro* T cell-mediated cytotoxicity has not yet been clarified (18). The splenic adherent cell could be a useful candidate for Ia antigen analysis in CML function, since a) a high percentage of SAC express Ia antigens (8); b) SAC have been shown to provide accessory function and to be

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stimulator cells in allogeneic as well as TNP-self CML (1); c) SAC functionally involved in other immunologic systems, e.g., *in vitro* antibody responses (11), antigen-induced cell proliferation (9), Con A stimulation (10), express Ia antigens.

The present study was performed to determine whether Ia-bearing SAC are required as accessory cells for the *in vitro* generation of T cell-mediated cytotoxic activity to alloantigens and TNP-self. Our results indicate that the accessory function for cytotoxicity is provided by SAC that express Ia antigens, irrespective of whether the CTL are directed against allogeneic or modified-self determinants. The specific Ia determinants expressed by the SAC appear to be products of the *I-A* and *I-E/C* subregions, both of which are expressed on a single population of SAC. Abolition of accessory function by anti-Ia reagents and C appears to be due to a loss of accessory function rather than the activation of suppression as demonstrated by cell mixing experiments.

The requirement for accessory cells in the *in vitro* generation of CTL against alloantigens has been reported by several other investigators (7, 18–21). A previous study of the expression of Ia antigens on accessory cells has shown that the relevant accessory cells for CTL against alloantigens do not appear to express Ia antigens (18). It should be noted, however, that spleen cells that had not been fractionated were treated with anti-Ia reagents and C and used as a source of accessory cells. Under such conditions, the selective depletion of SAC subpopulation(s) that bear Ia antigens could be less effective. In contrast with the above study, but in agreement with our results, is the observation that accessory cells required for the *in vitro* differentiation of CTL against syngeneic UV-induced tumors (22), as well as for a secondary cytotoxic response by MSV-immune spleen cells (23), express Ia antigens.

We have previously reported (6) that allogeneic SAC can provide accessory function for TNP-self CTL, and that the H-2 restriction is not determined by the haplotype of the SAC. Allogeneic cells have been shown to be able to enhance the T cell immune response in several *in vitro* systems (24). An allogeneic effect can be also mediated by factors such as AEF (allogeneic effect factor), which contain components of Ia antigens (25, 26). Therefore, it was of interest to determine whether the allogeneic SAC responsible for accessory function involved in generating TNP-self CML would express Ia antigens. The results indicate that treatment of SAC allogeneic to the responding and modified stimulating cells with an anti-Ia reagent specific for the SAC abolished accessory function. The mode of action of these syngeneic and allogeneic Ia-bearing accessory cells is not yet clear. It should be noted, however, that allogeneic SAC depleted of Ia-bearing cells do not stimulate proliferative T cell responses in mixed lymphocyte reactions (27); whereas, other recent studies indicate that the same allogeneic SAC depleted of Ia bearing cells can stimulate the generation of allogeneic CTL (Pettinelli *et al.*, unpublished observations).

Similarities have been observed between accessory cells required for CTL and those necessary in T cell proliferative and antibody responses for the parameters investigated thus far. These include radioresistance, lack of expression of Thy-1 antigens, and expression of the same Ia specificities. Although these common characteristics have been shown for accessory cells required in these different immune systems, it has not yet been demonstrated that accessory function is provided by the same cell population in these immune responses.

Accessory cells involved in primary cytotoxic response to alloantigens or TNBS-modified syngeneic cells do not show the same histocompatibility requirements observed for the interactions of accessory cells and T-lymphocytes in antibody production or antigen specific proliferation (28–30). It is not clear that such a lack of genetic restriction is due to a different function of accessory cells in CTL compared to the other immune responses. The recent observation that allogeneic macrophages are unable to restore an *in vitro* secondary cytotoxic response to syngeneic tumor cells (23) suggests that only primary cytotoxic responses show such a lack of genetic restrictions between accessory cells and T lymphocytes. It is possible that specific helper cells for CML responses from primed mice will be restricted to interact with only MHC-matched accessory cells. Further analysis of the function(s) of accessory cells in CTL is necessary in order to explain these discordant results.

The studies presented here demonstrate that only SAC that express Ia antigens can provide accessory function for modified-self (as well as allogeneic) CML, despite the fact that these SAC appear not to determine H-2 restriction. It remains to be established whether the Ia antigens expressed on SAC are themselves involved in the accessory function. Studies are in progress to analyze further the functional significance of Ia antigens expressed on accessory cells by attempting to block accessory function with anti-Ia reagents, to replace accessory function with a supernatant from cultures of SAC, and to determine if such a supernatant contains *Ia* antigens.

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