

INHIBITION OF SPECIFIC CELL-MEDIATED CYTOTOXICITY BY ANTI-T-CELL RECEPTOR ANTIBODY*

BY ARTHUR K. KIMURA‡

(From the Department of Biology, University of California,
Los Angeles, California 90024)

(Received for publication 17 December 1973)

Studies performed in a number of laboratories have clearly shown that alloantibodies directed at target cell antigens can prevent their recognition and subsequent destruction by effector cells specifically sensitized to them (1-3). This type of blocking has been taken as a partial explanation for enhancement phenomena of progressively growing tumors (4, 5) and for success of transplanted tissue in allogeneic recipients (6, 7). On the other hand, attempts at achieving a blockade of cellular recognition processes with alloantibodies against effector cell-surface components have met with less clear success. Ramseier and Lindenmann (8) have described a method for the production of an antireceptor site antiserum, which functions to prevent the release of a "product of antigen recognition" (PAR),¹ a leukotactic factor released when allogeneic cells are mixed in culture (9). This antiserum was tested in a number of different laboratories and reported by Lindahl (10) to have no apparent effect on proliferation in mixed leukocyte culture (MLC), survival of skin or heart allografts, expression of cell-mediated cytotoxicity in vitro, or graft-vs.-host (GvH) reactivity. The fact that this antiserum is ineffective in blocking such known T-cell functions raises some doubt as to its apparent specificity.

In the present study, a method for the production of specific anti-T-cell receptor antiserum is presented, together with experimental data demonstrating its ability to block specific cell-mediated cytotoxicity in vitro. The basis for the production of this antiserum involves the immunization of rabbits with C3H anti-BALB/c effector cells, and the subsequent adsorption of this antiserum with C3H anti-C57BL/6 effector cells. The resultant antiserum is able to discriminate C3H anti-BALB/c effector cells from other mouse lymphocytes.

Materials and Methods

Animals.—Inbred strains of mice (C3H, C57BL/6, BALB/c, and DBA/2) were obtained from either Jackson Laboratories (Bar Harbor, Me.) or Microbiological Associates, Inc.,

* Supported in part by NIH Research Grant HD-06071, American Cancer Society Research Grant IC-14, and Damon Runyon, DRE-1190 to Dr. W. R. Clark.

‡ Present address: Department of Immunology, University of Uppsala, Uppsala, Sweden.

¹ *Abbreviations used in this paper:* AS, rabbit anti-(C3H anti-BALB/c effector cell) antiserum; C', complement from reconstituted guinea pig serum; CMC, cell-mediated cytotoxicity; FCS, fetal calf serum; GvH, graft-vs.-host reactivity; MLC, mixed leukocyte culture; NRS, normal rabbit serum; PAR, product of antigen recognition; PBS, phosphate-buffered saline.

(Bethesda, Md.). Both male and female mice 6–10 wk of age were used in this study. Outbred white rabbits were obtained from local breeders.

Sera and Reagents.—The same lot of lyophilized guinea pig serum was used as the source of complement (C') for the entire study. C' was reconstituted just before each experiment and diluted to the appropriate concentration with cold phosphate-buffered saline (PBS). All test and control sera were C' inactivated by heating in a 56°C water bath for 30 min, and stored frozen at –10°C.

Source of Immune Cells.—In vivo-sensitized effector cells were obtained from spleens of lethally irradiated (850 R) mice which were injected i.v. 5–6 days previously with 5.0×10^7 allogeneic (C3H) lymphocytes (11). In vitro-sensitized effector cells were generated in MLC as described previously².

Cell-Mediated Cytotoxicity Testing.—Cell-mediated cytotoxicity (CMC) was determined against appropriate ⁵¹Cr-labeled tumor cells in suspension assay essentially according to Cauty and Wunderlich (12), modified for microassay as described previously (11). Effector cell preparations were suspended to a concentration of 10^7 cells/ml in Dulbecco's modified Eagle's minimal essential medium plus 5% heat-inactivated fetal calf serum (FCS). 50 μ l of this cell suspension was mixed with 25 μ l of the appropriate test serum (rabbit anti-[C3H anti-BALB/c effector cell] antiserum [AS] or normal rabbit serum [NRS]) at various dilutions. This mixture was then incubated in Falcon 3040 Microtest II wells (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) for 1–2 h at 4°C before the addition of the ⁵¹Cr-labeled target cells. The total volume of the assay mixture was 85 μ l with an effector to target cell ratio of 50:1. At the end of 4 h of incubation with rocking (12) at 37°C in a humidified atmosphere of 7.5% CO₂ in air, 0.1 ml of cold PBS was added to each well. This mixture plus additional wash (0.25 ml PBS) was transferred to Falcon tubes (no. 2051) containing 1.0 ml of PBS. The tubes were vortexed lightly and centrifuged at 600 g for 5 min. 1-ml aliquots of the supernates were removed and counted in a gamma scintillation spectrometer. Percent ⁵¹Cr released was determined according to Brunner et al. (1):

$$\frac{\text{Cpm released, exp.} - \text{cpm released, control}}{\text{Total cpm} - \text{cpm released, control}} \times 100.$$

Total ⁵¹Cr-released was determined by osmotic lysis of the target cells in distilled water, which was at least as effective as repeated freeze-thaw procedures.

Immunization Protocols.—Rabbits were immunized i.v. with 6.5×10^8 C3H anti-BALB/c spleen cells in PBS. Red blood cells were removed before injection by treating the spleen cell suspensions with 0.85% Tris-buffered NH₄Cl according to Boyle (13) and washed twice with PBS. 10 days following the primary injection, the rabbits were boosted with 4.3×10^8 immune cells. The rabbits were bled 1 wk after the second challenge. The serum was collected, sterilized by filtration, and stored in aliquots at –10°C.

Adsorption Protocols.—All adsorptions of undiluted, heat-inactivated antiserum were carried out at a ratio of 1:1 (antiserum:packed cells) for 45 min on ice. Either NH₄Cl-treated BALB/c spleen cells or the DBA/2 (*H-2^b*) mastocytoma, P815-X2, were equally effective in removing anti-BALB/c C'-fixing antibodies, and were both used as adsorbing cells during the course of these studies. Likewise, C57BL/6 spleen cells or the syngeneic leukosis, EL-4, could be used interchangeably as adsorbing cells. Adsorptions with immune cells (C3H anti-C57BL/6) were carried out as with the normal or tumor cells. Immune cells were obtained from spleens of GVH recipients, treated with NH₄Cl, and washed twice with PBS before the adsorption.

² Kimura, A. K., and W. Clark. 1974. Functional characteristics of T-cell receptors during sensitization against histocompatibility antigens in vitro. *Cell Immunol.* In press.

RESULTS

The high degree of immunological specificity seen in the destructive phase of cellular immune responses (14, 15) suggests possible qualitative differences in the macromolecular architecture of cell-surface recognition units present on effector cell populations. It has been demonstrated² that C3H lymphocytes undergoing sensitization to either BALB/c (*H-2^d*) or C57BL/6 (*H-2^b*) exhibit strain-specific recognition and target cell destruction of the sensitizing strain, from the beginning of measurable effector cell function till the generation of full cytotoxicity.

The rationale employed in raising a specific rabbit anti (C3H receptors for BALB/c) antiserum is schematically depicted in Fig. 1. If antiserum could be

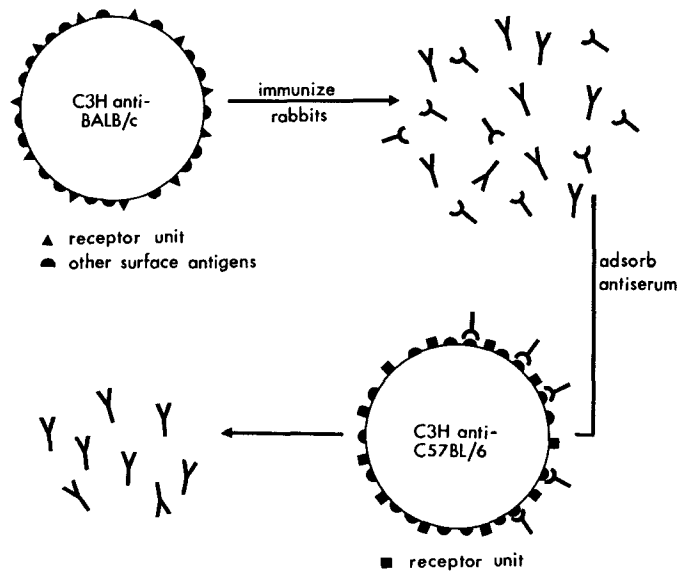


FIG. 1. Protocol for the production of rabbit anti-(C3H receptors for BALB/c) antiserum.

successfully raised against the cell-surface components of C3H effector cells immune to BALB/c, antibodies directed against recognition structures would hopefully be represented within this antiserum. Removal of other species, strain, effector or blast cell antigen-directed antibodies could be accomplished by adsorbing the antiserum with C3H anti-C57BL/6 effector cells. The resulting antiserum should be predominantly if not exclusively directed against the receptor units which distinguish the immunological reactivity of these two syngeneic effector cell populations.

Since the C3H effector cells used for immunization of the rabbits were derived from spleens of lethally irradiated BALB/c hosts undergoing GvH sensitization, some BALB/c cells were likely included in the original sensitizing inocu-

lum. Thus, antibodies against BALB/c antigens would undoubtedly also be present in this antiserum. Adsorptions of the resulting antiserum with spleen or tumor cells of the target cell genotype ($H-2^d$) would thus be necessary, since in part of the experiments the DBA/2 mastocytoma ($H-2^d$) cells are used as targets.

Titration of the Antiserum.—The initial studies in the characterization of this antiserum were involved in determining titers and kinetics of antibody-induced cytolysis. Normal C3H lymph node cells were labeled with ^{51}Cr according to Berke's method of labeling tumor cells (16). $10\ \mu\text{l}$ of undiluted serum (AS or NRS) were dispensed into $17 \times 100\text{-mm}$ Falcon tubes (no. 2051) on ice along with $25\ \mu\text{l}$ of C' diluted 1:1 with PBS. 1×10^5 ^{51}Cr -labeled cells in a volume of $25\ \mu\text{l}$ were then added to the tubes and incubated in a water bath at 37°C . At given time points, $1.5\ \text{ml}$ of ice-cold PBS was added to triplicate tubes of both AS and NRS groups. The tubes were vortexed lightly and centrifuged at $600 \times g$ for 5 minutes at 10°C . 1-ml aliquots of the supernates were removed and counted in a gamma scintillation spectrometer. The results of these experiments are shown in Fig. 2. Maximal release of ^{51}Cr from target cells under these conditions occurred within 15 min.

Antiserum titrations were performed exactly as described above using dilutions of AS and NRS in a volume of $10\ \mu\text{l}$. Incubations were carried out for 20 min in a 37°C water bath. The results of these experiments are shown in Fig. 3. It can be seen that final AS dilutions of up to 1:200 were effective in destroying greater than 90% of the labeled target cells under these conditions.

Removal of Antitarget Cell Antibodies by Adsorption.—It was necessary to remove antibodies directed against BALB/c antigens, since these antigens ($H-2^d$) would also be present on target cells used in the next step of the protocol. BALB/c spleen cell suspensions were treated with NH_4Cl to remove red blood

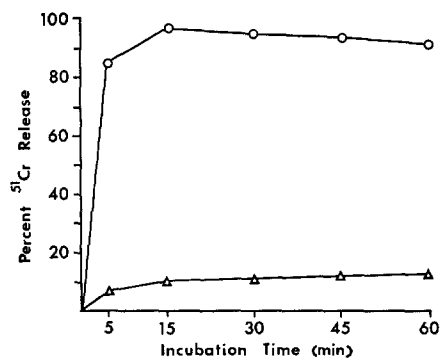


FIG. 2. Kinetics of antibody-induced cytolysis. 10^5 ^{51}Cr -labeled C3H lymph node cells were incubated with unadsorbed AS (\circ — \circ), or NRS (\triangle — \triangle), for various lengths of time in a 37°C water bath, and percent ^{51}Cr released was determined. Points represent the means of triplicate samples in which the SE was less than 10% of the mean.

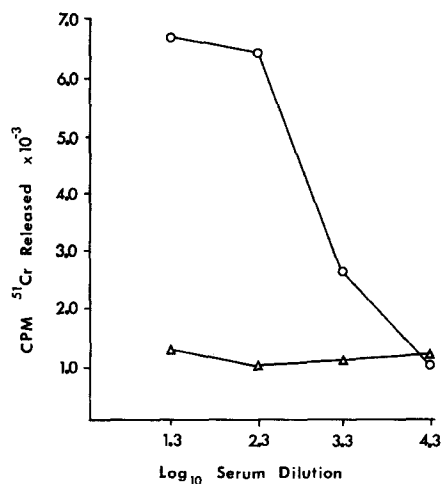


FIG. 3. Titration of AS. Various dilutions of unadsorbed AS (O—O) or NRS (Δ—Δ), plus complement were tested for their ability to release ⁵¹Cr from 10⁵ labeled C3H lymph node cells. Incubations were carried out for 20 min in a 37°C water bath. Points represent means of quadruplicate samples in which the SE was less than 10% of the mean.

cells and washed twice in PBS. Undiluted AS was then adsorbed with these cells (1 ml AS: 1 ml packed cells) for 45 min on ice. The resulting serum was then compared with unadsorbed AS and NRS in the presence of C' for their ability to release ⁵¹Cr from labeled BALB/c lymphocytes. The results of these experiments are seen in Fig. 4. After a single 45-min adsorption procedure, essentially all of the C'-fixing antitarget cell reactivity was removed. In all subsequent experiments, this adsorption procedure was performed twice to ensure removal of other noncomplement fixing classes of immunoglobulin which might be present and serve to cover target cell antigens. This adsorption should also function to remove antibodies against most mouse species-specific antigens.

Blocking Activity of AS on Cell-Mediated Cytotoxicity.—Unadsorbed and BALB/c cell adsorbed AS were then tested for their ability to block the in vitro lysis of DBA/2 target cells by C3H anti-BALB/c effector cells (Table I). Four independent experiments were performed utilizing a fresh aliquot of C'-inactivated AS, either unadsorbed or adsorbed against BALB/c spleen cells just before the experiment. The conditions for cytotoxicity testing were as described in the Materials and Methods section. In experiment 1, it can be seen that unadsorbed antiserum completely inhibited cell-mediated cytotoxicity. However, it is possible that this inhibition is due to covering of antigens on the target cells, rather than receptor sites on the effector cells. Experiments 2–4 clearly demonstrate a dose-dependent inhibition using BALB/c adsorbed antiserum in which greater than 95% inhibition can be achieved with a total AS dilution of 1:3 present during the cytotoxicity assay.

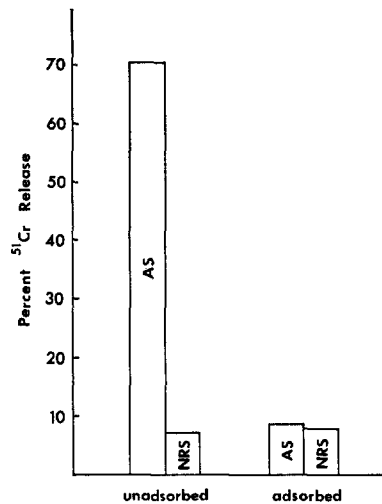


FIG. 4. Removal of C'-fixing antitarget cell reactivity by adsorption. Unadsorbed rabbit AS and NRS were compared with antitarget cell adsorbed AS and NRS, for their ability to release ⁵¹Cr from 10⁵ BALB/c target cells. Antiserum adsorptions were done with BALB/c spleen cells at an adsorbing ratio of 1:1 (AS:packed cells). The results represent the means of two separate experiments in which the SE was less than 10% of the mean.

TABLE I
Rabbit Anti-(C3H Anti-BALB/c) Dose-Dependent Inhibition of Cell-Mediated Cytotoxicity of C3H Anti-BALB/c Effector Cells

Exp.	Effector cell	Antiserum adsorbed against:*	Total serum dilution‡	% ⁵¹ Cr released in the presence of:§	
				NRS	AS
1	C3H anti-BALB/c	—	1:26	22.3	0
2	C3H anti-BALB/c	BALB/c	1:16	43.4	28.5
3	C3H anti-BALB/c	BALB/c	1:10	52.3	20.1
4	C3H anti-BALB/c	BALB/c	1:3	19.2	0.9

* Undiluted, C'-inactivated, AS was adsorbed with BALB/c spleen cells and the P815-X2 mastocytoma, at a ratio of 1:1 (antiserum:packed cells) for 45 min on ice to remove anti-target cell antibodies.

‡ Final serum dilution present during the cytotoxicity assay.

§ 5×10^5 C3H anti-BALB/c effector cells were preincubated with various dilutions of NRS or AS for 1.5 h on ice before to the addition of 10^4 ⁵¹Cr-labeled P815-X2 target cells. After the 4-h cytotoxicity assay, at an effector to target cell ratio of 50:1, percent ⁵¹Cr release was determined. Values represent the means of triplicate samples in which the SE was always less than 10% of the mean.

Specificity of Antibody-Induced Inhibition of Cell-Mediated Cytotoxicity.—It was then of interest to examine the specificity of this antibody dependent inhibition of cell-mediated cytotoxicity. The ability of this AS to block the in vitro cytotoxicity of C3H anti-C57BL/6 effector cells against EL-4 target cells

TABLE II
Specificity of Inhibition of Cell-Mediated Cytotoxicity by Rabbit Anti-(C3H Receptors for BALB/c) Antiserum

Exp.	Effector cell	Antiserum adsorbed against:*			Target cell‡	% ⁵¹ Cr released in the presence of:§	
		BALB/c	C57BL/6	C3H anti-C57BL/6		NRS	AS
1	C3H anti-C57BL/6	+	+	-	EL-4	18.9	0
2	C3H anti-BALB/c	+	+	+	P815-X2	10.5	0.6
	C3H anti-C57BL/6	+	+	+	EL-4	7.4	5.7
3¶	C3H anti-BALB/c	+	+	+	P815-X2	12.3	1.8
	C3H anti-C57BL/6	+	+	+	EL-4	37.0	33.0

* Undiluted AS was adsorbed with the indicated cell types at an adsorbing ratio of 1:1 (antiserum:packed cells). All antiserum was adsorbed twice to remove antitarget cell antibodies against the *H-2^d* target, P815-X2, (once with BALB/c spleen cells and once with the P815-X2 tumor target). The antiserum was similarly adsorbed twice against the *H-2^b* antigens with C57BL/6 spleen cells and the EL-4 tumor target cells. Adsorptions with immune cells were performed once under identical conditions.

‡ ⁵¹Cr-labeled EL-4 and P815-X2 tumor targets were used to measure specific cytotoxicity of C3H anti-C57BL/6 and C3H anti-BALB/c effector cells, respectively.

§ AS or NRS, adsorbed against the indicated cell types were preincubated with the effector cells for 2 h on ice before the addition of the ⁵¹Cr-labeled target cells. Percent ⁵¹Cr released was determined after the 4-h assay with an effector to target cell ratio of 50:1. Values represent the means of triplicate samples in which the SE was less than 10% of the mean.

|| Effector cells were generated in vitro in MLC.

¶ Effector cells were sensitized in vivo.

was thus tested (Exp. 1, Table II). In this experiment, the AS was also adsorbed against C57BL/6 spleen cells and the EL-4 target cells to remove any possible antitarget cell antibodies. The degree of inhibition of this reaction was essentially 100% at an antiserum dilution of 1:3. This nonspecific inhibition may be related to the *K_a* antigen of Sullivan et al. (17), which has been shown to be present on many strains of effector cells but not on normal lymphocytes. The antigen(s) may be present at a high density on effector cells and/or sufficiently close to the receptor units to cause the observed inhibition. Alternatively, C3H anti-BALB/c and C3H anti-C57BL/6 effector cells may share common regions in their recognition units, and antibodies directed to these areas could be responsible for this inhibition.

The final step in the preparation of this AS was its adsorption with C3H anti-C57BL/6 effector cells, to see if antibodies against this cell type could be removed independently of the blocking activity towards C3H anti-BALB/c effector cells. The AS was adsorbed against C3H anti-C57BL/6 immune cells at an adsorbing ratio of 1:1 (AS:packed cells) for 45 min on ice. The results of such adsorptions on blocking activities are seen in the remaining experiments of

Table II. In each of the experiments, the removal of antibody-induced blocking of C3H anti-C57BL/6 effector cells was achieved without seriously altering the specific inhibition against C3H anti-BALB/c. Each of these experiments represents totally separate protocols, from unadsorbed AS through the final adsorption with C3H anti-C57BL/6 cells. These experiments demonstrate that antibodies present in this AS can discriminate idiotypic differences between the structure of the recognition units present on C3H lymphocytes immune to two different strains of mouse cells.

Evidence for Effector Cell Blockade.—Other experiments were performed to demonstrate conclusively that the blocking of CMC was due to antibodies directed against the effector cell. As shown in Fig. 4, a single adsorption of the AS with lymphoid cells of the target cell genotype was sufficient to remove essentially all of the C'-fixing antitarget cell antibodies. This adsorption procedure was routinely performed twice on all batches of AS used in the inhibition studies. It was further shown that adsorptions with C3H anti-C57BL/6 effector cells would remove nonspecific blocking reactivity without affecting the inhibition imposed on C3H anti-BALB/c effector cells. A necessary corollary of this would be that if the inhibition were due only to antibodies directed at effector T lymphocytes, a further adsorption of the AS against C3H anti-BALB/c effector cells should remove the inhibition. Experiments of this type were performed and the results of such an adsorption are shown in Table III.

Further evidence against covering of target cell antigens as an alternative

TABLE III
Complete Removal of Antiserum Inhibition by Adsorption with C3H Anti-BALB/c Effector Cells

Effector cell*	Antiserum adsorbed against:†				Targets cell§	% ⁵¹ Cr released in the presence of:	
	BALB/c	C57BL/6	C3H anti-C57BL/6	C3H anti-BALB/c		NRS	AS
C3H anti-C57BL/6	+	+	+	—	EL-4	51.2	43.0
C3H anti-BALB/c	+	+	+	—	P815-X2	19.4	7.9
C3H anti-C57BL/6	+	+	+	+	EL-4	56.9	53.8
C3H anti-BALB/c	+	+	+	+	P815-X2	26.1	27.5

* Effector cells were sensitized in vivo.

† Antiserum adsorptions were performed at an adsorbing ratio of 1:1 (undiluted antiserum: packed cells) for 45 min on ice with the indicated cell types. All antiserum was adsorbed twice to remove anti-*H-2^d* antibodies, once with BALB/c spleen cells and once with P815-X2 tumor cells. The resulting antiserum was then twice adsorbed to remove any anti-*H-2^b* antibodies, once with C57BL/6 spleen cells and once with EL-4 tumor cells.

§ EL-4 is the syngeneic leukemia of C57BL/6 (*H-2^b*) and P815-X2 is the DBA/2 mastocytoma (*H-2^d*), which represent the specific targets for C3H anti-C57BL/6 and C3H anti-BALB/c effector cells, respectively.

|| AS or NRS were present at a final dilution of 1:3 during the cytotoxicity assay.

explanation for the observed inhibition comes from the following experiment. ^{51}Cr -labeled target cells (P815-X2) were preincubated with fully adsorbed-undiluted AS, NRS, or FCS for 1 h on ice before cytotoxicity testing. The target cell suspensions were diluted to 2 ml with PBS (total AS dilution of 1:40), counted using trypan blue, centrifuged and resuspended to the appropriate concentration ($10^6/\text{ml}$) in PBS plus 2% C'-inactivated FCS. These target cell suspensions were then added to the microtest wells containing the C3H anti-BALB/c effector cells and taken through the cytotoxicity assay. If the site of blockade was even slightly involved at the target cell, one would expect to see some degree of inhibition in the AS-treated cells. The results of such an experiment (Table IV) fail to show such an inhibition. It can be argued,

TABLE IV
Effect of Serum Pretreatment of Target Cells on Cell-Mediated Cytotoxicity

Effector cell	Target cells pretreated with:*			Percent cytotoxicity resulting†
	AS	NRS	FCS	
C3H anti BALB/c§	+	—	—	31.2
	—	+	—	30.8
	—	—	+	32.4

* ^{51}Cr -labeled P815-X2 target cells (*H-2^d*) were preincubated with BALB/c-, C3H anti-C57BL/6-adsorbed AS, NRS, or FCS for 1 h on ice. The cells were washed once with PBS + 2% C'-inactivated FCS, counted with trypan blue, and suspended to a concentration of $10^6/\text{ml}$. $10\ \mu\text{l}$ of the various serum pretreated target cells were then mixed with 5×10^5 C3H anti-BALB/c effector cells and taken through the cytotoxicity assay.

† Percent cytotoxicity = percent ^{51}Cr released.

§ Effector cells were sensitized in vivo.

however, that such blocking antibodies are removed by the single wash and therefore are of no consequence to the cytotoxicity assay. Although this criticism cannot be ruled out, it seems unlikely that antitarget antibodies, if present at all, and of such low affinity for target cell antigens could function to achieve the degree of inhibition seen in Table II.

DISCUSSION

That B lymphocytes have antigen-binding receptors has now been demonstrated by a variety of experiments, including binding to antigen-coated columns (18, 19), direct antigen binding visualization studies (20, 21) and antigen-induced selective suicide (22, 23). It is generally conceded that the antigen receptor on B cells is a form of immunoglobulin (24, 26).

On the other hand, the nature of the antigen-recognition unit on T cells is less well understood (27, 29). Immunoglobulins are certainly very much more sparse on T-cell surfaces than on B cells (30, 31); nonetheless, some recent

evidence has been obtained in support of the notion that at least the helper T-cell receptor may be immunoglobulin (32, 33).

Attempts at blocking T-cell activity by various antibodies to immunoglobulins have met with apparently contradictory results in different laboratories. Brondz et al. (34) were unable to block *in vitro* cell-mediated cytotoxicity of immune lymphocytes on target cells by rabbit antimouse IgG, IgM, and IgA. Similar attempts at inhibiting the transfer of delayed hypersensitivity or GvH reactivity could not be demonstrated by such antisera (34, 35). Greaves et al. (36) found that anti-light-chain serum or its Fab fragment could suppress the mitogenic response to tuberculin as well as the mixed leukocyte reaction, implying that the receptors involved in these reactions may have an immunoglobulin-like structure. In a similar system, however, Koch and Nielsen (37) could not corroborate the results of Greaves et al. Mason and Warner (35) have reported partial inhibition of GvH reactions and delayed hypersensitivity by antibodies specific for light chains of immunoglobulins but could not demonstrate a removal of inhibition with the addition of light chains. In view of recent evidence for B-cell proliferation in MLC (38), the observed inhibition due to anti-light-chain antibodies may represent B-cell inactivation. Evidence related to the size and specificity of the determinant recognized by T cells in allograft reactions stands in contrast to the immunoglobulin receptors of B cells (39–43).

One approach to the identification and characterization of the T-cell receptor would be the production of a specific antireceptor antiserum. The only systematic attempt at the production of such an antiserum that has been reported in the literature has been carried out by Ramseier and Lindenmann (8). The protocol for the production of their antiserum involved injection of parental lymphocytes into semiallogeneic F₁ hybrids. The rationale was that while the parental lymphocytes would have receptors for the alloantigens on the F₁ cells, the F₁ lymphocytes should not possess such receptors, and thus respond by producing an antireceptor antiserum. While this idea seems conceptually sound, the evidence obtained so far regarding the function of the supposed antiserum is not compelling. The assay routinely used to measure the activity of this antiserum, the PAR assay (9), suggests that the antiserum may be specific for some T-cell determinant. On the other hand, antiserum raised in this manner and titered in the PAR assay was tested in a number of laboratories and reported by Lindahl (10) to have no effect on T-cell reactivity in MLC, on allograft survival, cell-mediated cytotoxicity *in vitro*, or GvH reactivity. Antibodies specifically directed against the T-cell antigen receptor might reasonably be expected to affect all of these known T-cell functions.

The approach taken in the present study was to develop immunization and antiserum adsorption protocols selective for idiotypic differences in the recognition units of syngeneic effector lymphocytes immune to two different strains of mouse cells. Antiserum was successfully raised against the cell-surface elements of one set of effector cells, including the specific recognition units. Anti-

bodies to all other surface elements except the receptor units were then removed by adsorption with a second set of effector cells syngeneic to the first, but immune to a different set of alloantigens. An antiserum raised according to such a protocol can thus be directed to, and only to, structural differences in the receptor units which distinguish the immunoreactivity of two such effector cell populations. All other surface elements are by definition identical. The ability of antiserum raised in this manner to detect idiotypic differences in receptor structure confirms the validity of such an approach. Rabbit anti-(C3H receptors for BALB/c) antiserum can specifically block the *in vitro* cytotoxicity of C3H anti-BALB/c effector cells without inhibiting C3H anti-C57BL/6 effector cells. The demonstration of these specific antireceptor antibodies is dependent upon proper adsorption procedures as seen in Table II.

Since the antiserum described here was raised in rabbit against mouse cells, it is extremely important to show that the blocking effect obtained is against the effector cell and not against the mouse target cells, since antibodies against the latter are known to block cell-mediated cytotoxicity (1-3). That the antiserum described here does not block sites on the target cell is affirmed by the experiments shown in Fig. 4 and Tables III and IV. A single adsorption of the original antiserum was sufficient to remove all C'-fixing cytotoxic antibodies (Fig. 4). Pretreatment of target cells with antiserum twice adsorbed in this manner showed no inhibitory effect on their subsequent cytolysis by effector cells. Finally, the inhibitory effect of the adsorbed antiserum is essentially completely abolished by a final adsorption with just those effector cells used for raising the original antiserum (Table III). These experiments demonstrate conclusively that the site of blocking is on the effector cell, and not on the target cell.

It seems entirely likely that the conditions described here for production of this antiserum may not be optimal. Particularly troublesome has been the necessity to use the antiserum at 1:3 dilutions after adsorption, which has led to its very rapid consumption. It is anticipated that continuing experience will lead to protocols yielding higher initial titers.

The experiments reported here are concerned only with the production and characterization of the antireceptor unit antiserum. The potential application of such a method for the production of a specific antilymphocyte serum for transplantation must be evaluated. It will also be of particular interest to look for cross-reactivity of the fully adsorbed antiserum with various suggested candidates for the receptor unit itself, such as different classes of immunoglobulin, light and heavy chains. Such studies are currently underway and will provide the basis for future communications.

SUMMARY

The present study describes a method for the production of a specific anti-T-cell receptor antiserum, and characteristics of its ability to block specific cell-

mediated cytotoxicity *in vitro*. Immunization and antiserum adsorption procedures were designed to select for idiotypic differences in the recognition units of C3H lymphocytes immune to two different strains of mouse cells, such that the reactivity of only one population of effector cells is inhibited by this antiserum. Both *in vivo* and *in vitro* sensitized effector T cells are subject to this inhibition. That the site of the antiserum blockade is clearly on the effector cell and not on the target cell is demonstrated.

The author wishes to thank Doctors W. R. Clark, E. E. Sercarz, P. I. Terasaki, and B. Bonavida for helpful suggestions in the preparation of the manuscript.

BIBLIOGRAPHY

1. Brunner, K. T., J. Mauel, J. Cerottini, and B. Chapuis. 1968. Quantitative assay of the lytic action of immune lymphoid cells on ^{51}Cr -labelled antigenic target cells *in vitro*. Inhibition by isoantibody and drugs. *Immunol.* **14**:181.
2. Möller, E. 1965. Antagonistic effects of humoral isoantibodies on the *in vitro* cytotoxicity of immune lymphoid cells. *J. Exp. Med.* **122**:11.
3. MacDonald, H. R., R. A. Phillips, and R. G. Miller. 1973. Allograft immunity in the mouse. I. Quantitation and specificity of cytotoxic effector cells after *in vitro* sensitization. *J. Immunol.* **111**:565.
4. Hellström, K. E., I. Hellström, C. A. Evans, G. Heppner, G. E. Pierce, and J. P. S. Yang. 1969. Serum mediated protection of neoplastic cells from inhibition by lymphocytes immune to their tumor specific antigens. *Proc. Natl. Acad. Sci. (U.S.A.)* **62**:362.
5. Hellström, K. E. and I. Hellström. 1970. Immunological enhancement as studied by cell culture techniques. *Annu. Rev. Microbiol.* **24**:373.
6. Hildemann, W. H., and Y. Mullen. 1973. The weaker the histoincompatibility, the greater the effectiveness of specific immunoblocking antibodies. *Transplantation.* **15**:231.
7. Bildsøe, P., W. L. Ford, O. Pettirossi, and M. Simonsen. 1971. GVH analysis of organ-grafted rats which defy the normal rules for rejection. *Transplantation.* **12**:189.
8. Ramseier, H., and J. Lindenmann. 1969. F₁ hybrid animals: reactivity against recognition structures of parental strain cells. *Pathol. Microbiol.* **34**:379.
9. Ramseier, H., and J. Lindenmann. 1971. Cellular receptors. Effects of antiallo-antiserum on the recognition of transplantation antigens. *J. Exp. Med.* **134**:1083.
10. Lindahl, K. F. 1972. Antisera against recognition sites. Lack of effect on the mixed leukocyte culture interaction. *Eur. J. Immunol.* **2**:501.
11. Clark, W., and A. Kimura. 1973. Effect of monolayer fractionation of lymphocytes on graft-versus-host reactivity. *Transplantation.* **16**:110.
12. Canty, T. G., and J. R. Wunderlich. 1970. Quantitative *in vitro* assay of cytotoxic cellular immunity. *J. Natl. Canc. Inst.* **45**:731.
13. Boyle, W. 1968. An extension of the ^{51}Cr -release assay for the quantitation of mouse cytotoxins. *Transplantation.* **6**:761.
14. Brunner, K. T., and J. C. Cerottini. 1971. Cytotoxic lymphocytes as effector cells of cell-mediated immunity. *Prog. Immunol.* **1**:385.
15. Wagner, H., and M. Feldmann. 1972. Cell-mediated immune response *in vitro*. I.

- A new in vitro system for the generation of cell-mediated cytotoxic activity. *Cell. Immunol.* **3**:405.
16. Berke, G., K. A. Sullivan, and D. B. Amos. 1972. Rejection of ascites tumor allografts. I. Isolation, characterization, and in vitro reactivity of peritoneal lymphoid effector cells from BALB/c mice immune to EL4 leukemia. *J. Exp. Med.* **135**:1334.
 17. Sullivan, K. A., G. Berke, and D. B. Amos. 1973. An antigenic determinant of cytotoxic lymphocytes. *Transplantation.* **16**:388.
 18. Wigzell, H. 1970. Specific fractionation of immunocompetent cells. *Transpl. Rev.* **5**:76.
 19. Truffa-Bachi, P., and L. Wofsy. 1970. Specific separation of cells on affinity columns. *Proc. Natl. Acad. Sci. (U.S.A.)* **66**:685.
 20. Naor, D., and D. Sulitzeanu. 1967. Binding of radioiodinated bovine serum albumin to mouse spleen cells. *Nature (Lond.)* **214**:687.
 21. Humphrey, J. H., G. Roelants and N. Willcox. 1971. Specific lethal radioactive antigens. *In Cell Interactions and Receptor Antibodies in Immune Responses.* O. Mäkelä, A. M. Cross, and T. Kosunen, editors. Academic Press, Inc., New York. 123.
 22. Ada, G. L. and P. Byrt. 1969. Specific inactivation of antigen-reactive cells with ¹²⁵I-labelled antigen. *Nature (Lond.)* **222**:1291.
 23. Basten, A., J. F. A. P. Miller, N. L. Warner, and J. Pye. 1971. Specific inactivation of thymus-derived (T) and non-thymus derived (B) lymphocytes by ¹²⁵I-labelled antigen. *Nature (Lond.)* **231**:104.
 24. Warner, N. L., P. Byrt, and G. L. Ada. 1970. Blocking of the lymphocyte antigen receptor site with anti-immunoglobulin sera in vitro. *Nature (Lond.)* **226**:942.
 25. Raff, M. C. 1970. Two distinct populations of peripheral lymphocytes distinguishable by immunofluorescence. *Immunology.* **19**:637.
 26. Mäkelä, O. 1970. Analogies between lymphocyte receptors and the resulting humoral antibodies. *Transpl. Rev.* **5**:3.
 27. Crone, M., C. Koch, and M. Simonsen. 1972. The elusive T cell receptor. *Transpl. Rev.* **10**:105.
 28. Roelants, G. E. 1972. Antigen recognition by B and T lymphocytes. *Curr. Top. Microbiol. Immunol.* **59**:135.
 29. Brondz, B. D. 1972. Lymphocyte receptors and mechanisms of in vitro cell-mediated immune reactions. *Transpl. Rev.* **10**:112.
 30. Raff, M. C., M. Sternberg, and R. B. Taylor. 1970. Immunoglobulin determinants on the surface of mouse lymphoid cells. *Nature (Lond.)* **225**:553.
 31. Rabellino, E., S. Colon, H. M. Grey, and E. R. Unanue. 1971. Immunoglobulins on the surface of lymphocytes. I. Distribution and quantitation. *J. Exp. Med.* **133**:156.
 32. Marchalonis, J. J., J. L. Atwell, and R. E. Cone. 1972. Isolation of surface immunoglobulins from lymphocytes from human and murine thymus. *Nature (Lond.)* **235**:240.
 33. Feldmann, M., R. E. Cone, and J. J. Marchalonis. 1973. Cell interactions in the immune response in vitro. VI. Mediation by T cell monomeric IgM. *Cell. Immunol.* **9**:1.
 34. Brondz, B. D., E. S. Ievleva, and G. N. Vornakova. 1968. Effect of antiglobulin

- antibodies on the cytotoxic action of immune lymphocytes in allogeneic cell culture. *Byull. Eks. Biol. Med.* **66**:73.
35. Mason, S., and N. L. Warner. 1970. The immunoglobulin nature of the antigen recognition site on cells mediating transplantation immunity and delayed hypersensitivity. *J. Immunol.* **104**:762.
 36. Greaves, H. F., G. Torrigiani, and I. M. Roitt. 1969. Blocking of the lymphocyte receptor site for cell-mediated hypersensitivity and transplantation reactions by anti-light chain sera. *Nature (Lond.)* **222**:885.
 37. Koch, C., and H. E. Nielsen. 1973. Effect of anti-light chain antibodies on rat leukocytes in vitro. *Scand. J. Immunol.* **2**:1.
 38. Wagner, H. 1972. The correlation between the proliferative and the cytotoxic responses of mouse lymphocytes to allogeneic cells in vitro. *J. Immunol.* **109**:630.
 39. Brondz, B. D. 1968. Complex specificity of immune lymphocytes in allogeneic cell cultures. *Folia Biol. (Prague)* **14**:115.
 40. Brondz, B. D., and N. E. Golberg. 1970. Further in vitro evidence for polyvalent specificity of immune lymphocytes. *Folia Biol. (Prague)*. **16**:20.
 41. Brondz, B. D. and A. E. Snegirova. 1971. Interaction of immune lymphocytes with the mixtures of target cells possessing selected specificities on the H-2 immunizing allele. *Immunol.* **20**:457.
 42. Schlossman, S. F., J. Hermon, and A. Yaron. 1969. Antigen recognition: in vitro studies on the specificity of the cellular immune response. *J. Exp. Med.* **130**:1031.
 43. Davie, J. M., and W. E. Paul. 1970. Receptors on immunocompetent cells I. Receptor specificity of cells participating in a cellular immune response. *Cell. Immunol.* **1**:404.