

CELLULAR AND HUMORAL RESPONSE TO  
TRANSPLANTATION ANTIGENS

I. DEVELOPMENT OF ALLOANTIBODY-FORMING CELLS AND CYTOTOXIC  
LYMPHOCYTES IN THE GRAFT-*VERSUS*-HOST REACTION\*

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It is generally accepted that the graft-*versus*-host (GVH)<sup>1</sup> reaction represents an immunological phenomenon called forth by the introduction of immunocompetent cells into a host carrying different transplantation antigens and incapable of rejecting the grafted cells (1, 2). Despite numerous studies, the actual mechanisms of the GVH reaction are poorly understood and, although there is ample evidence that some of the grafted cells proliferate and differentiate in the recipient lymphoid organs (3), no attempt has been made to assess their relative significance.

Previous *in vitro* studies have shown that transplantation immunity in mice is characterized by the development of at least two types of effector cells, namely cells which produce and release alloantibody, and cells which display a cytotoxic activity against target cells carrying the sensitizing alloantigens (4). The present study was undertaken to determine whether similar effector cells also appeared during the GVH reaction. Advantage was taken of the availability of two *in vitro* assay systems allowing the independent detection of lytic alloantibody plaque-forming cells (PFC) and of cytotoxic lymphocytes (CL) using the same target cells (5-7). It was found that both types of effector cells were present in spleens of heavily-irradiated mice injected 4-5 days earlier with allogeneic spleen cells. Specificity studies indicated that the effector cells were of the donor genotype and were specifically sensitized against the host alloantigens. Clear evidence for a different origin of alloantibody PFC and CL was obtained by studying their susceptibility to treatment with anti- $\theta$ -serum.

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<sup>1</sup> *Abbreviations used in this paper:* CL, cytotoxic lymphocytes; GVH, graft-*versus*-host; PFC, plaque-forming cells.

### Materials and Methods

*Mice.*—C57BL/6J, DBA/2J, A/J, C<sub>3</sub>H/J, BALB/cJ, and AKR/J mice were obtained from the colonies maintained in our institute or from the Jackson Laboratory, Bar Harbor, Maine. Mice of either sex were used at 2-4 months of age.

*Target Cells.*—Tumor cells syngeneic to various strains of mice were maintained in vitro by serial passages as described previously (5). They were used as target cells carrying alloantigens of the DBA/2, (P-815-X2 mastocytoma cells), C57BL/6 (EL-4 lymphoma cells), or A strains (YAC leukemia cells). Peritoneal macrophages were used as the source of target cells carrying C<sub>3</sub>H alloantigens.

*Cell Transfer.*—Spleen cells from normal mice were obtained as described previously (5). Recipient mice received 800 R whole body X-irradiation 2 hr before intravenous injection of 50-100 × 10<sup>6</sup> spleen cells. They were exsanguinated on days 4-5 after transfer and the spleens of each experimental group (5-8 mice/group) were pooled before preparation of cell suspensions as indicated above (5).

*Assay for Alloantibody Plaque-Forming Cells.*—Details of the method have been published elsewhere (7). In brief, spleen cell fractions (3-6 × 10<sup>6</sup> cells) were mixed in agarose with 2 × 10<sup>6</sup> target cells and the cell mixture was then evenly spread on microscope slides. After incubation for 1 hr at 37°C, the slides were washed and incubated for 1 hr with rabbit serum as a source of complement. After washing, the slides were incubated overnight with Eagle's medium containing 10% calf serum. The following day, the slides were washed, fixed with 95% ethanol, and stained with Giemsa.

*Assay for Cytotoxic Lymphocytes.*—The in vitro cytotoxic activity of sensitized lymphocytes was determined according to the method described previously (6, 8). Cell mixtures containing a standard concentration of <sup>51</sup>Cr-labeled target cells (1 × 10<sup>5</sup> cells/ml) and varying concentrations of spleen cells (ranging from 10 × 10<sup>5</sup> to 10 × 10<sup>7</sup> cells/ml) were prepared in Eagle's medium with 10% inactivated calf serum. Volumes of 0.25 ml of the reaction mixtures were placed in flat-bottomed tubes and incubated at 37°C. After various incubation periods, 1.75 ml volumes of phosphate-buffered saline were added to each tube. After centrifugation at 700 g for 5 min, 1 ml volumes of the supernatant fluid were carefully removed and placed in glass tubes for assessment of radioactivity in a well-type gamma counter. Total <sup>51</sup>Cr incorporation was determined by measurement of radioactivity of 0.25 ml samples of the reaction mixtures. Maximal <sup>51</sup>Cr release was determined by incubating 0.25 ml samples of the reaction mixtures with 1.75 ml of distilled water at 37°C for 6 hr. After centrifugation at 700 g for 5 min, 1 ml volumes of the supernatant fluid were counted for radioactivity. Spontaneous <sup>51</sup>Cr release was determined after incubation of <sup>51</sup>Cr-labeled target cells with normal spleen cells instead of sensitized spleen cells. This release was identical to <sup>51</sup>Cr release from labeled target cells incubated without spleen cells. Results are expressed as per cent of specific cytotoxicity using the following formula:

$$\frac{\text{experimental } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release}}{\text{maximal } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release}} \times 100$$

*Preparation of Antisera.*—Rabbit antiserum against mouse  $\mu$ -chain was kindly provided by Dr. H. Jaquet (Institute of Biochemistry, University of Lausanne). Rabbit antiserum against mouse Fab fragment was obtained after repeated subcutaneous injections of 1 mg Fab incorporated in complete Freund's adjuvant. Mouse IgG obtained by Pevikon block electrophoresis (9) was digested with papain according to the method of Porter (10) using the conditions described previously (11). After digestion, the reaction mixture was applied to a diethylaminoethyl (DEAE)-cellulose column equilibrated with 0.01 M sodium phosphate buffer, pH 8.0. The protein fraction which was not retained on the column with these conditions was used as

the source of Fab fragment after elimination of undigested IgG by gel filtration on Sephadex G-100 (11).

Mouse alloantisera were obtained after four or more monthly intraperitoneal injections of  $30 \times 10^6$  tumor cells or  $60 \times 10^6$  spleen cells from the appropriate strain of mice. Anti- $\theta$ -C<sub>3</sub>H serum was prepared as described previously (12).

*Treatment with Antisera.*—To study the origin of cells in spleens of irradiated mice injected with allogeneic spleen cells, 0.1 ml volumes of spleen cells ( $2 \times 10^6$ /ml) were mixed with 0.1 ml of the corresponding alloantiserum diluted 1:10 in Eagle's medium and 0.1 ml of fresh rabbit serum as the source of complement. In the first series of experiments, unabsorbed rabbit serum was used at a dilution of 1:8, while rabbit serum diluted 1:4 after absorption with agar (13) was employed in subsequent studies. The reaction mixtures were incubated for 45 min at 37°C. The proportion of dye-excluding cells was determined by adding 0.1 ml of 0.16% trypan blue to each tube.

The effect of antisera against mouse  $\mu$ -chain and Fab fragment on plaque formation and cytotoxicity was tested by adding 1/10–1/20 (v/v) antiserum to the spleen cell-target cell mixtures. Before use, the rabbit antisera were heat-inactivated (30 min, 56°C) and absorbed with the tumor cells used as target cells in each assay system. Normal rabbit serum was used as control.

Susceptibility of alloantibody-forming cells and cytotoxic lymphocytes to treatment with anti- $\theta$ -serum and complement was tested as follows:  $50$ – $100 \times 10^6$  spleen cells obtained 5 days after transfer were mixed with AKR anti- $\theta$ -C<sub>3</sub>H serum (final dilution 1:20) and agar-absorbed rabbit serum (final dilution 1:12) in a total volume of 6.0 ml. After incubation for 15 min at 4°C and 45 min at 37°C, the cells were washed twice by centrifugation, adjusted to the appropriate concentration of living cells as determined by a dye-exclusion test, and tested in both in vitro assay systems as described above.

## RESULTS

*Origin of Cells in Spleens of Irradiated Mice Injected with Allogeneic Spleen Cells.*—Groups of DBA/2 or C57BL/6 mice were irradiated (800 R) and injected intravenously with  $50 \times 10^6$  spleen cells from C57BL/6 or DBA/2 mice respectively. 4 days later, the genotype of the lymphoid cells present in the recipient spleens was determined individually using cytotoxic antisera against the alloantigens of the donor and the recipient strain. Susceptibility to the cytotoxic activity of anti- $\theta$ -serum was tested in parallel. As shown in Table I, most, if not all, lymphoid cells carried the alloantigens of the donor strain. In addition, about 65–70% of these cells were susceptible to treatment with anti- $\theta$ -serum, a percentage which is much higher than that of normal spleen cells before transfer into irradiated allogeneic recipients (about 30–40%)<sup>2</sup> (14). Similar experiments performed on mice 5 days after irradiation and transfer gave essentially the same results.

*Detection of Alloantibody PFC.*—When tumor cells bearing alloantigens of the DBA/2 strain were mixed in agarose with lymphoid cells obtained from spleens of lethally irradiated DBA/2 mice injected 5 days earlier with  $100 \times 10^6$  C57BL/6 spleen cells, it was found that circular areas of lysis appeared after the

<sup>2</sup> Cerottini, J.-C. Unpublished data.

addition of complement (7). As compared to the plaque assay described by Jerne and Nordin (15) for the detection of cells producing lytic antibody against heterologous erythrocytes, two modifications were found to be crucial for the detection of alloantibody PFC. First, rabbit serum had to be used as the source of complement, while guinea pig serum was ineffective; second, areas of lysis were not detectable at the end of the incubation period with complement, but

TABLE I  
*Origin of Cells in Splens of Irradiated Mice Injected with Allogeneic Spleen Cells\**

Transferred spleen cells	Irradiated recipient	Per cent reduction in cell number after treatment with complement and antiserum†		
		C57BL/6 Anti-DBA/2	DBA/2 Anti-C57BL/6	AKR Anti- $\theta$ -C <sub>3</sub> H
C57BL/6	DBA/2	0 (0-9)	81 (70-89)	68 (64-78)
DBA/2	C57BL/6	80 (70-89)	0 (0-3)	64 (61-84)

\*  $50 \times 10^6$  spleen cells were injected into 800 R-irradiated mice (five animals/group). Lymphoid cells present in the individual recipient spleen were collected 4 days after transfer.

† As compared with cells treated with complement alone. Numbers in parentheses indicate the range of values.

TABLE II  
*Specificity of the Alloantibody PFC*

Transferred spleen cells*	Irradiated recipients	Indicator cells	PFC/10 <sup>6</sup>	PFC/spleen
C57BL/6	DBA/2	C57BL/6	0	0
		DBA/2	9.0	260
DBA/2	C57BL/6	C57BL/6	9.7	160
		DBA/2	0	0

\*  $100 \times 10^6$  spleen cells were injected intravenously into 800 R-irradiated allogeneic mice. Lymphoid cells present in the recipient splens were collected 5 days after transfer.

appeared after an additional 24 hr incubation in normal medium. These findings reflect (a) the superior activity of rabbit serum as the source of complement in immune lysis of nucleated cells as compared to guinea pig serum (16), and (b) the longer incubation time required for visible disintegration of lysed tumor cells incorporated in agarose as compared to red cells. Moreover, tumor cells could not be replaced by red cells as the source of alloantigen-bearing target cells, probably because of the much lower alloantigen content of the latter cells.

The specificity of the plaque assay system was assessed by using target cells specific for the DBA/2 or C57BL/6 strain, and C57BL/6 or DBA/2 spleen cells transferred into DBA/2 or C57BL/6 animals. As shown in Table II,

plaque formation occurred only with target cells syngeneic to the spleen cell recipient strain, but not with target cells syngeneic to the spleen cell donor strain. Since normal spleens contained no naturally occurring alloantibody PFC, these results indicated that alloantibody PFC were formed after transfer of spleen cells into allogeneic recipients.

*Detection of Cytotoxic Lymphocytes.*—The transferred spleen cell populations were also tested for in vitro cytotoxicity using the assay system described previously (6). For that purpose, the DBA/2 tumor cells used as target cells in the plaque assay system were labeled with  $^{51}\text{Cr}$  and incubated in suspension

TABLE III  
 *$^{51}\text{Cr}$  Release from Labeled DBA/2 Target Cells Incubated with Syngeneic or Allogeneic Spleen Cells\**

Lymphoid cells	Percent $^{51}\text{Cr}$ release†		
	2hr	4hr	6hr
None	8.1	12.9	16.7
Normal C57BL spleen cells	8.3	13.2	16.0
DBA/2 spleen cells transferred into irradiated DBA/2 mice§	8.2	12.7	15.1
C57BL spleen cells transferred into irradiated DBA/2 mice§	28.0	48.1	63.7

\* Lymphoid cell:target cell ratio of 100:1

† 86% of incorporated  $^{51}\text{Cr}$  released by treatment with distilled water (= 100% lysis).

§ 800 R-irradiated mice were injected intravenously with  $50 \times 10^6$  spleen cells from DBA/2 or C57BL/6 mice. Lymphoid cells present in the recipient spleens were collected 4 days after transfer.

with C57BL/6 spleen cells transferred into irradiated DBA/2 mice.  $^{51}\text{Cr}$  release from the target cells was measured after incubation for 2, 4, and 6 hr and compared with that observed in suspensions containing target cells alone or target cells and normal C57BL/6 or DBA/2 spleen cells. As indicated in Table III, the amount of  $^{51}\text{Cr}$  released from target cells in the presence of transferred C57BL/6 spleen cells increased rapidly during the incubation period, reaching 64% of the incorporated isotope after 6 hr. On the other hand, there was no difference between  $^{51}\text{Cr}$  release from target cells incubated alone or with normal spleen cells. In the subsequent experiments, the percentage of specific lysis was calculated using the formula given in Materials and Methods.

To substantiate these findings, spleen cells from various mouse strains were injected into irradiated allogeneic mice and tested for in vitro cytotoxicity using  $^{51}\text{Cr}$ -labeled target cells syngeneic to the recipient strain. Table IV illustrates the strain combinations investigated and the percentage of target cell-specific lysis observed. It can be seen that high levels of cytotoxicity were always found in spleen cells transferred into mice of a different H-2 genotype.

Non-H-2 differences between the cell donor and the recipient strains resulted in smaller but definite cytotoxicity as shown by the activity of BALB/c (H-2<sup>d</sup>)

TABLE IV  
*Cytotoxicity of Spleen Cells Sensitized by Transfer into Heavily Irradiated  
Allogeneic Recipients\**

Lymphocyte donor	Lymphocyte recipient and target cell donor	Per cent specific lysis in 6 hr		
		ratio:‡10:1	30:1	100:1
C57BL/6	DBA/2	55	75	87
C57BL/6	A	7	14	30
C57BL/6	C <sub>3</sub> H	ND	ND	53
A	DBA/2	43	62	ND
A	C57BL/6	40	64	75
DBA/2	C57BL/6	25	48	66
C <sub>3</sub> H	DBA/2	35	66	ND
AKR	DBA/2	34	61	ND
BALB/c	DBA/2	ND	9	17

ND: not done.

\*  $50 \times 10^6$  spleen cells were injected intravenously into 800 R-irradiated mice. Recipient spleen cells were collected 4 days after transfer

‡ Lymphocyte: target cell ratio.

TABLE V  
*Specificity of the Cytotoxic Lymphoid Cells*

Transferred spleen cells*	Irradiated recipients	Target cell mixture		Per cent specific lysis of labeled target cell
		Labeled cell	Unlabeled cell	
C57BL/6	DBA/2	DBA/2	—	71
		C57BL/6	—	0
DBA/2	C57BL/6	DBA/2	—	0
		C57BL/6	—	76
C57BL/6	DBA/2	DBA/2	C57BL/6	73
		C57BL/6	DBA/2	0
DBA/2	C57BL/6	DBA/2	C57BL/6	0
		C57BL/6	DBA/2	40

\* 800 R-irradiated mice were injected intravenously with  $50 \times 10^6$  spleen cells. Lymphoid cells present in the recipient spleens were collected 4 days later and incubated with target cells for 6 hr (lymphoid cell:target cell ratio, 100:1).

spleen cells transferred into irradiated DBA/2 (H-2<sup>d</sup>) mice. The specificity of the cytotoxic assay system was assessed by testing the same combination of target cells and transferred spleen cells as used for the plaque assay system. As shown in Table V, target cell lysis occurred only with target cells syngeneic

to the spleen cell recipient strain. Moreover, nonspecific cytotoxicity was not observed when target cells syngeneic to the spleen cell donor strain were added to a mixture of transferred spleen cells and target cells syngeneic to the cell recipient strain. This absence of nonspecific cytotoxicity was demonstrated by incubating C57BL/6 spleen cells transferred into irradiated DBA/2 mice, and

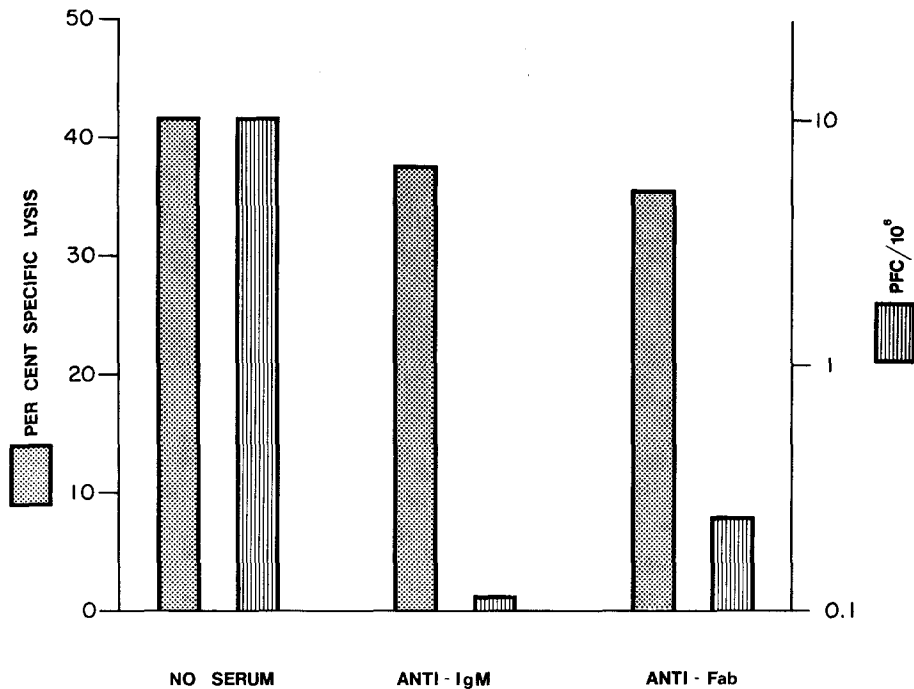


FIG. 1. Effect of anti-immunoglobulin antisera on alloantibody plaque formation and cytotoxicity. C57BL/6 spleen cells obtained 5 days after transfer into irradiated (800 R) DBA/2 mice were assayed for alloantibody plaque formation and cytotoxicity using DBA/2 target cells. Rabbit antisera to mouse  $\mu$ -chain or Fab fragment were added to the spleen cell-target cell reaction mixtures at a final dilution of 1:10 (cytotoxic assay) or 1:20 (plaque assay).

vice versa, with either a mixture of  $^{51}\text{Cr}$ -labeled DBA/2 target cells and unlabeled C57BL/6 target cells, or  $^{51}\text{Cr}$ -labeled C57BL/6 target cells and unlabeled DBA/2 target cells (Table V). The results confirmed previous studies (4) demonstrating that  $^{51}\text{Cr}$  release in the presence of transferred spleen cells was restricted to target cells carrying the sensitizing alloantigens, namely the alloantigens of the recipient strain.

*Effect of Anti-Immunoglobulin Antisera on Alloantibody Plaque Formation and Cytotoxicity.*—Since both in vitro assays are based on target cell lysis, it was of importance to establish that two different effector cells were involved in

each assay system. Previous studies have shown that the *in vitro* cytotoxicity of spleen cells from mice immunized with allografts was not inhibited by antisera against mouse immunoglobulins (17-18), while alloantibody plaque formation was completely abolished in the presence of antibody against mouse  $\mu$ -chain (4). The results of five similar experiments performed on spleen cells sensitized by transfer into irradiated allogeneic recipients are summarized in Fig. 1. It can be seen that the addition of rabbit antiserum to mouse  $\mu$ -chain or Fab fragment caused, respectively, 99, or 95% reduction in PFC number, but had no significant effect on cytotoxicity.

TABLE VI  
*Inhibitory Activity of Alloantibody on Cytotoxicity of Sensitized Lymphocytes*

Lymphocyte donor*	Irradiated recipient and target cell donor	Antiserum†	Lymphocyte: target cell ratio	Per cent specific lysis
C57BL/6	DBA/2	—	3:1	19
		—	10:1	44
		—	30:1	63
		C57BL/6 anti-DBA/2	30:1	17
		DBA/2 anti-C57BL/6	30:1	68
DBA/2	C57BL/6	—	10:1	13
		—	30:1	23
		—	100:1	31
		C57BL/6 anti-DBA/2	100:1	28
		DBA/2 anti-C57BL/6	100:1	12

\*  $50 \times 10^6$  spleen cells injected intravenously into 800 R-irradiated mice. Recipient spleen cells were collected 4 days later.

† Added to the reaction mixture at a final concentration of 1:10.

§ Incubation time, 6 hr.

*Effect on Cytotoxicity of Alloantisera to Target Cells or to Sensitized Lymphocytes.*—Another indication that alloantibody was not involved in the cytotoxic assay system was obtained from studies in which hyperimmune alloantiserum to target cells was added to the lymphoid cell-target cell mixture (Table VI). Cytotoxicity of sensitized lymphocytes was strongly inhibited in the presence of alloantiserum to target cell but was unaffected by the addition of alloantiserum against the lymphoid cells. These results, as well as previous studies (5, 19), speak against a role for alloantibody in the cytotoxic effect of sensitized lymphocytes.

*Effect of Anti- $\theta$ -Antiserum on Alloantibody Plaque Formation and Cytotoxicity.*—Further evidence that cytotoxic lymphocytes were quite distinct from alloantibody-forming cells was obtained by studying the susceptibility of both cell types to treatment with anti- $\theta$ -serum. Spleen cells obtained 5 days after



transfer into irradiated allogeneic recipients were treated in vitro with anti- $\theta$ -serum and complement before the tests for alloantibody plaque formation and cytotoxicity. As indicated in Table VII, pretreatment with anti- $\theta$ -serum and complement completely abolished the cytotoxic activity, but had no effect on alloantibody plaque formation. In fact, on a living cell basis, the anti- $\theta$ -treated spleen cell population was enriched in alloantibody PFC as compared with controls.

TABLE VII  
*Effect of Pretreatment of Transferred Spleen Cells with anti- $\theta$ -Serum and Complement on In Vitro Cytotoxicity and Alloantibody Plaque Formation\**

Pretreatment of spleen cells†	Per cent specific lysis in 6 hr‡	PFC/10 <sup>6</sup> spleen cells§
None	45	11.3
NMS + C	47	10.5
Anti- $\theta$ + C	0	42.0

\*  $100 \times 10^6$  C57BL/6 spleen cells were injected into 800 R-irradiated DBA/2 mice. The lymphoid cells present in the recipient spleens were collected 5 days later, pretreated in vitro with anti- $\theta$ -serum, and tested using DBA/2 target cells.

† Cell samples ( $50 \times 10^6$  cells) were incubated with normal AKR serum (NMS), or AKR anti- $\theta$ -C<sub>3</sub>H serum (anti- $\theta$ ), and rabbit serum as the source of complement (C) for 15 min at 4°C and 45 min at 37°C.

§ Lymphoid cell:target cell ratio (30:1) and PFC/10<sup>6</sup> spleen cells based on cell counts performed after pretreatment.

#### DISCUSSION

The present studies demonstrate the development of two types of effector cells after transfer of mouse spleen cells into lethally irradiated allogeneic recipients, namely alloantibody plaque-forming cells and cytotoxic lymphocytes. These effector cells have been shown to derive from precursor cells present in the transferred spleen cells and to be specifically sensitized against the recipient alloantigens. In this respect, the immune process taking place in the transferred spleen cells is similar to that occurring in spleens of mice immunized with allografts (4).

Both types of effector cells could be detected in vitro since lysis of target cells carrying the host alloantigens occurred as an end result of their activity. Despite the fact that the in vitro assay systems for PFC and CL rely on the same phenomenon, i.e. target cell lysis, ample evidence has been obtained that they detect two separate types of effector cells which differ not only in their physicochemical properties, but also in their origin. Plaque formation depends upon the release of IgM alloantibody from PFC, requires the presence of an appropriate source of complement (7), and is inhibited by the addition of antisera to mouse  $\mu$ -chain or Fab fragment. The actual mechanism

by which CL destroy target cells is less well understood (20). The lytic event has been shown to depend on contact between viable CL and target cells (21), is independent of added complement or serum (5, 17), and is not affected by antisera against the various mouse immunoglobulins (17-18), L chains (17), or Fab fragment.

Previous studies have shown that the lytic activity of CL obtained from mice immunized with allografts was inhibited by the addition of antibody against target cell alloantigens (5, 19). These findings, confirmed by the present results obtained with CL sensitized by transfer, speak against the role of alloantibody in the *in vitro* activity of CL and suggest, on the contrary, a mechanism of competition between alloantibody and CL for the same determinants on the target cell surface (19).

Further indication that different cells are involved in plaque formation and *in vitro* cytotoxicity has been obtained from fractionation studies of spleen cells sensitized to alloantigens by differential flotation in discontinuous albumin density gradients (22). Most of the alloantibody PFC were found to collect in fractions containing cells of low density, while CL accumulated in fractions of intermediate and higher density.

Recent studies concerning the origin of effector cells have clearly demonstrated that CL belong to the thymus-derived cells, while alloantibody PFC derive from thymus-independent precursors. First, CL, but not PFC, are formed upon transfer of thymus cells into irradiated allogeneic recipients (23). Second, PFC, but not CL, appear after transfer of spleen cells pretreated with anti- $\theta$ -serum and complement.<sup>3</sup> Third, treatment of spleen cells sensitized to alloantigens with anti- $\theta$  and complement before *in vitro* assays for PFC and CL completely abolished the *in vitro* cytotoxic activity, but had no effect on plaque formation (reference 12 and this paper). It is therefore evident that CL, contrary to alloantibody PFC, are thymus-derived cells, and as such represent effector cells involved in cell-mediated immunity.

The possibility of differentiating CL and their precursors from PFC and their precursors by virtue of their susceptibility to anti- $\theta$ -serum and complement makes it feasible to study their relative pathological role in GVH reactions. It has been found that transfer of normal or sensitized parental spleen cells pretreated with anti- $\theta$ -serum and complement into F<sub>1</sub> hybrids was not followed by a GVH reaction.<sup>3</sup> These data, together with the results presented in this paper, suggest that CL play the major role in the GVH disease.

#### SUMMARY

After transfer into heavily-irradiated allogeneic mice, spleen cells were found to produce two types of effector cells directed against the recipient

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<sup>3</sup> Cerottini, J.-C., and K. T. Brunner. Manuscript in preparation.

alloantigens, namely alloantibody plaque-forming cells (PFC) and cytotoxic lymphocytes (CL). Both types of effector cells were detectable *in vitro* by virtue of their lytic effect on target cells carrying the recipient alloantigens.

Alloantibody PFC activity was dependent on the presence of an exogenous source of complement and could be inhibited by the addition of heterologous antisera to mouse  $\mu$ -chain or Fab fragment in the assay system. CL activity was independent of added complement, was not affected by anti-immunoglobulin antisera, but was inhibited by the addition of antibody against target cell alloantigens. Treatment of the transferred spleen cells with anti- $\theta$ -serum and complement before *in vitro* assays for PFC and CL completely abolished the CL activity but had no effect on alloantibody-plaque formation. These results indicate that the two types of effector cells can be differentiated *in vitro* by virtue of their susceptibility to anti- $\theta$ -serum and the mechanisms by which they cause cell lysis.

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