

# Allotypic Suppression of Adult Mouse Spleen Cells

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**Summary.** Adult mouse spleen cells were transferred into irradiated adult mice with xenogeneic erythrocytes. Antibody-producing cells were measured by the localized haemolysis in gel (LHG) assay. Anti-allotype serum directed against the  $\gamma G_{2a}$  allotype of the donor cells, given to the recipients at the same time as antigen and spleen cells, suppressed the plaque-forming cells (PFC) producing antibody of that allotype, and had little effect on the other classes investigated.

## INTRODUCTION

Since the original observation of Dray (1962) that the expression of L chain allotypes in foetal rabbits can be specifically suppressed by exposure to specific anti-allotype antibody of maternal origin, Dray and others have measured the duration, specificity and conditions of foetal and neonatal suppression in rabbits, of both L and H chain allotypes (Mage and Dray, 1965, 1966; Dubiski and Fradette, 1966; Dubiski, 1967; Mage, Young and Dray, 1967). In foetal and neonatal mice the expression of an allotype specificity has been suppressed by methods similar to those used successfully in rabbits (Herzenberg and Herzenberg, 1965; Herzenberg, Herzenberg, Goodlin and Rivera, 1967b; Herzenberg, Minna and Herzenberg, 1967a).

While rabbits compensate for the absence of immunoglobulin of one allotype by increasing the production of another allotype, this effect has not yet been shown in mice. Suppression in mice does not last as long as in rabbits, being measured in weeks rather than in months.

This paper describes experiments on the expression of an allotype specificity by antibody-producing spleen cells of adult mice. Suppression was obtained during the course of an immune response to sheep or horse erythrocytes (SRBC or HRBC), rather than being the result of a long-term developmental process in treated immature animals, as in previous studies. The LHG assay was used to enumerate cells producing antibody. These cells were taken from animals either homozygous or heterozygous at the Ig-1 locus, which controls the  $\gamma G_{2a}$  class of immunoglobulin molecule. Treatment of adult animals with anti-allotype serum was ineffective because all the antibody was absorbed by allotype in the serum. Accordingly, spleen cells from animals homozygous or heterozygous for the allelic specificity to be suppressed were transferred to irradiated recipients which did not carry the specificity under examination. Recipients were also immunized at the time of cell transfer, and they were assayed 8 days later for PFC. A cell transfer system provides a relatively short-term assay for studying allotype suppression of different cell populations from mice of any age.

## MATERIAL AND METHODS

*Animals*

Male mice, 3–6 months old, of the following strains were used in these experiments: BALB/c, CBA, C57BL/6, (CBA×C57BL/6) $F_1$ . In addition two coisogenic strains were used, C3H/SW Ig-1a (CSW) and CWB/5 Ig-1b, which were selected for histocompatibility (Klein and Herzenberg, 1967); the breeding nucleus for our stock was kindly supplied by Dr L. A. Herzenberg. These strains have been maintained as inbred mice by sib mating.

*Media*

Either of two media were used to prepare cell suspensions for transfer: (1) Gey's solution with 0.5 per cent gelatin pH 7.2, and (2) Parker 199 medium (Glaxo) with 0.05 M Tris and 10 per cent foetal calf serum (Flow Laboratories) heat treated at 56° for 30 minutes, final pH 7.4.

*Antisera*

Anti-allotype serum, for indirect plaque development or as a suppressant, was raised by coating *B. pertussis* with anti-pertussis antibody of one allotype and injecting the coated bacteria into a large group of mice of the other allotype. BALB/c or CBA, both Ig-1a, and C57BL/6 Ig-1b, were used to supply anti-pertussis and anti-allotype serum (see Table 1) (Dresser and Wortis, 1967). Antisera were heat inactivated at 56° for 30 minutes and absorbed with sheep or horse RBC as appropriate. Before injection the sera were spun at 48,000 g for 30 minutes. All anti-allotype sera were titrated for cytotoxicity against spleen and lymph node cells, and found to be negative. The cytotoxic assay utilizing  $^{51}\text{Cr}$  release from labelled killed cells were used as developed by Dr M. Ruzskiewicz (Lance, Ford and Ruzskiewicz, 1968). Polyspecific anti- $\gamma\text{G}$  developing sera were raised in rabbits as previously described (Wortis, Taylor and Dresser, 1966; Wortis, Dresser and Anderson, 1969).

*Irradiation*

Mice were irradiated the day before the cell transfer with whole body irradiation in rotating plastic boxes, using 125 kV, 5.5 mA, 1 mm Al filtration, target to mouse distance 32 cm, at a rate of 44 rads/min; they received a final dose of 750, 800, 830 or 1050 r.

*Plaque assay*

The method of Jerne, Nordin and Henry (1963) as modified and described by Wortis *et al.* (1966, 1968) and Dresser and Wortis (1967) was used. Plates for direct plaque-forming cells (PFC) and each developing serum were always made in duplicate. Developed PFC numbers were calculated after subtraction of direct PFC numbers. Numbers of PFC per spleen and per million white cells were calculated using means of  $\log(x+1)$  transformed data on all animals in a group whether or not they were positive for  $\gamma\text{G}$  PFC. Although the polyspecific serum used in these experiments to estimate total  $\gamma\text{G}$  PFC reacted against all  $\gamma\text{G}$  classes, the total number cannot be compared with numbers obtained using specific anti-allotype serum, because the polyspecific antiserum did not develop all classes fully and therefore gives only a relative measure of the contribution

of each class to the total count, and the true proportions also vary with each population. Anti-allotype serum was used to estimate Ig-1a and Ig-1b  $\gamma G_{2a}$ -PFC, henceforth abbreviated to Ig-1a PFC and Ig-1b PFC.

#### *Cell counts*

White cells were counted on a Model B Coulter Counter, set to count particles of the size of lymphocytes, and to exclude red blood cells.

#### *Spleen cell transfers*

Cells from a pool of donor spleens were prepared by homogenization in medium. A single cell suspension was obtained by the following procedure: the cells were washed twice by centrifuging at 600 *g* for 5 minutes using 15-ml aliquots of medium, resuspended, sieved through a 60 hole/cm stainless steel mesh sieve to remove connective tissues and drawn through a 26-g hypodermic needle. Viability counts were made using eosin dye exclusion as used previously by Dresser and Wortis (1967). Proportions of viable cells varied for different experiments between 70 and 80 per cent. The cell suspensions which were made up to  $1 \times 10^8$  viable cells per ml were kept at 0° in iced water. SRBC in Alsever's solution (Burroughs Wellcome & Co.) and HRBC were washed twice in citrate saline and twice in medium before dilution to 2 per cent of the packed cell volume. The suspension was corrected colorimetrically to  $4 \times 10^8$  cells/ml.

Where appropriate, spleen cells and antigen were mixed in equal volumes immediately before injection. Each mouse received  $1 \times 10^7$  spleen cells and/or  $4 \times 10^7$  SRBC or HRBC intravenously in 0.2 ml of medium. Anti-allotype serum was injected as an appropriate aliquot intraperitoneally within 1 hour of cell transfer, volumes below 0.1 ml were first diluted in medium. The spleens of recipients were assayed for PFC 8 days after cell transfer.

#### *Indirect agglutination assay to titrate anti-allotype sera*

Anti-allotype sera were titrated for their ability to agglutinate indirectly (passively) SRBC coated with strain specific immunoglobulin. A 0.75 per cent suspension of SRBC was made as described above in veronal saline buffer (VSB) (Kabat and Mayer, 1961) with 0.1 per cent gelatin (Difco). Anti-SRBC sera made in C57BL/6 and CBA mice bled 2 weeks after a primary injection of  $4 \times 10^7$  SRBC i.p. were heat inactivated. Equal volumes of VSB-gelatin diluted antiserum and SRBC were incubated at 37° for 2 hours, and agglutination titres were read as the last tube positive for agglutination. A large volume of SRBC suspension was then coated with either the C57BL/6 or CBA antiserum, by incubating the SRBC with a dilution of the antiserum at a concentration one-quarter of the end-point titre read previously.

The coated cells were centrifuged at 900 *g* for 6 minutes and again made up to a 0.75 per cent suspension. These SRBC which were now coated with antibody at a concentration insufficient to give direct agglutination, were used to titrate the anti-allotype serum. Anti-allotype serum was diluted in VSB-gelatin in a half-log<sub>10</sub> series, each coated SRBC suspension was also used to titrate a specific rabbit anti-mouse  $\gamma G_1$  and Fab, and a goat anti-mouse  $\gamma G_{2b}$ , to estimate total antibody on the cells, and proportions of different classes.

The coated cells had the same reactivity with anti-Fab, the CBA coated SRBC had three-fold more  $\gamma G_1$ , and  $\gamma G_{2b}$ . The anti-Ig-1b sera had three- to thirty-fold more antibody than the anti-Ig-1a.

TABLE 1  
 DETAILS OF ANTI-ALLOTYPE SERA USED AND INDIRECT AGGLUTINATION ASSAY

Name	Anti-immunoglobulin donor strain	Anti-Ig directed against			Antibody titre against SRBC coated with:	
		Strain	Class	Allotype	C57B1/6 Ig	CBA Ig
A5	CBA	C57BL/6	$\gamma G_{2a}$	Ig-1b	$10^{-4}$	$< 10^{-1.5}$
A28	BALB/c	C57BL/6	$\gamma G_{2a}$	Ig-1b	$10^{-3}$	$< 10^{-1.5}$
A29	BALB/c	C57BL/6	$\gamma G_{2a}$	Ig-1b	$10^{-3.5}$	$< 10^{-1.5}$
A26	C57BL/6	CBA	$\gamma G_{2a}$	Ig-1a	$< 10^{-1.5}$	$10^{-2.5}$
A27	C57BL/6	CBA	$\gamma G_{2a}$	Ig-1a	$< 10^{-1.5}$	$10^{-2.5}$
	Rabbit	Mouse	Fab		$10^{-4}$	$10^{-4}$
	Rabbit	Mouse	$\gamma G_1$		$10^{-3}$	$10^{-3.5}$
	Goat	Mouse	$\gamma G_{2b}$		$10^{-3}$	$10^{-3.5}$
	Normal CBA serum	-	-		$< 10^0$	$< 10^0$
	Normal C57BL/6 serum	-	-		$< 10^0$	$< 10^0$

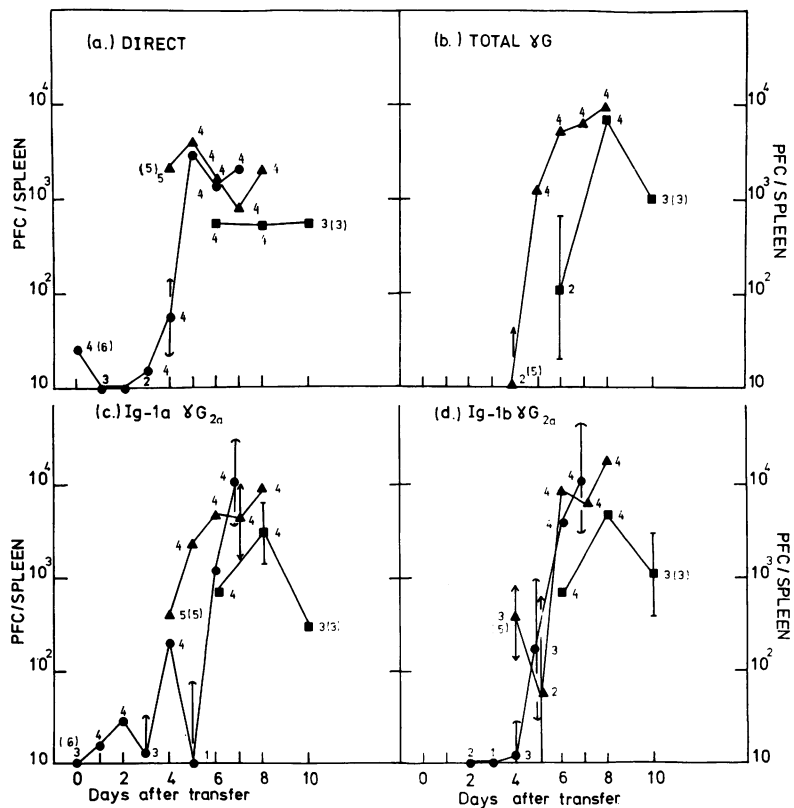


Fig. 1. Splenic response in three separate experiments after transfer of  $1 \times 10^7$  F spleen cells with  $4 \times 10^7$  SRBC i.v. into CBA mice irradiated 1 day before transfer. ●, Irradiated 1050 r; ▲, 830 r; ■, 800 r. Each point is calculated as geometric mean of four mice, unless indicated in parenthesis. Figures are number of positive mice in group. Vertical bars are  $\pm$  SE where SE factor is  $s$  and mean is  $x$ . One SE is  $xs$  and  $x/s$ ; they are only indicated where  $s \geq 2.0$ .

## RESULTS

## PRELIMINARY EXPERIMENTS

Mice irradiated with 830 r or more, and receiving SRBC or HRBC, produce less than 10 PFC spleen of any class on any day up to day 8. This is less than 1 per cent of the response in mice receiving antigen and syngeneic or allogeneic spleen cells from unprimed donors (see Tables 3 and 4, and Fig. 2). Mortality in this transfer system was less than 15 per cent over 8 days. Limiting dilution experiments show that  $1 \times 10^7$  spleen cells is the minimum dose for use in this system to ensure that all recipients have  $\gamma G_{2a}$  PFC (Wortis, Dresser and Anderson, unpublished). Similar experiments show that  $4 \times 10^7$  SRBC gives maximum  $\gamma G_{2a}$  antibody production.

Fig. 1 illustrates the results of several experiments designed to establish the best time to assay spleens for  $\gamma G_{2a}$  PFC in irradiated mice repopulated by spleen cells from normal donors. The direct PFC/spleen reach a peak on day 5 and then their response plateaus at a lower level until day 10. The  $\gamma G$  response continues to increase until day 8, and then decreases rapidly over the next week. Day 8 was chosen for subsequent experiments because the ratio of  $\gamma G$  to direct PFC was greatest on that day.

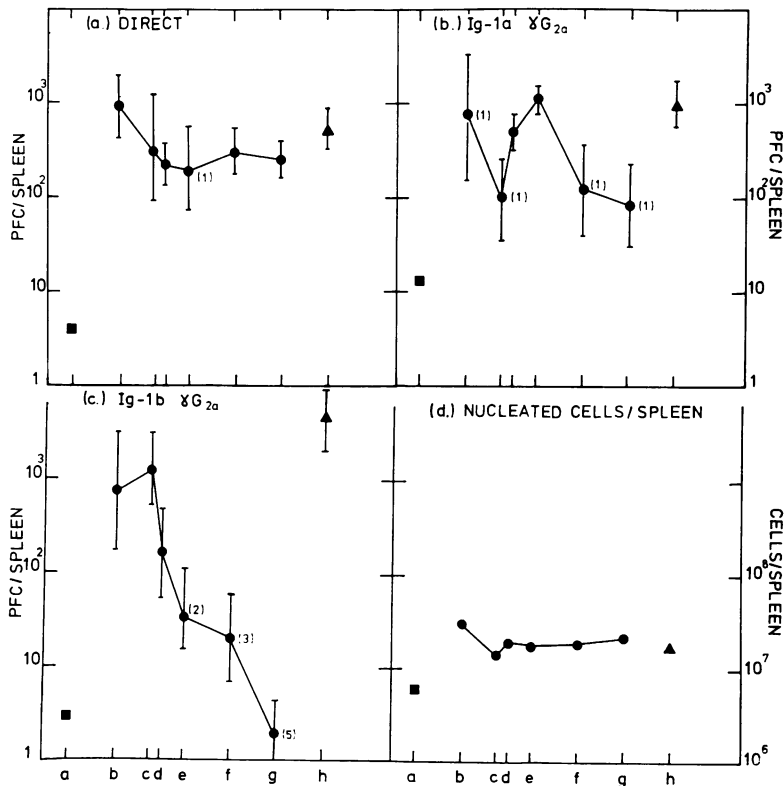


FIG. 2. The effect of various doses of anti-Ig-1b serum (A29) on different classes and allotypes of the splenic PFC response 8 days after transfer of  $1 \times 10^7$   $F_1$  spleen cells with  $4 \times 10^7$  SRBC i.v., and BALB/c anti-allotype serum or BALB/c anti-pertussis serum i.p., into CBA mice irradiated with 830 r 1 day before transfer. Each point represents geometric means of six mice. Vertical bars indicate  $\pm$  1 SE as in Fig. 1. Figures in parenthesis are number of negative animals in group. Group (a) no spleen cells, only SRBC; (b-h) spleen cells + SRBC, (b) control, (c) 0.025 ml anti-Ig-1b i.p., (d) 0.05 ml, (e) 0.1 ml, (f) 0.2 ml, (g) 0.3 ml, (h) 0.3 ml anti-pertussis i.p.

TABLE 2  
 PFC IN CBA MICE 9 DAYS AFTER IRRADIATION AND 8 DAYS AFTER RECEIVING  $1 \times 10^7$  F<sub>1</sub> SPLEEN CELLS WITH  $4 \times 10^7$  RBC i.v. AND ANTI-Ig-1b SERUM i.p.

Experiment	Group No.	X-ray dose (r)	S or H RBC	Antiserum		Cells $\times 10^6$ /spleen	Direct	Total $\gamma$ G	PFC/spleen		Per cent Ig-1b of controls
				Name	Dose (ml)				Ig-1a	$\gamma$ G <sub>2a</sub>	
Y	1 (7)*	1050	S	-	-	18.7	828	9734 (1.4)	873 (3.1)	13412 (1.2)	100
	2 (7)	1050	S	A28	0.3	20.8	†(1.3) 925 (1.4)	1104 [1] (3.4)	303 (3.2)	7 [5] (2.9)	<1
Z	1 (4)	1050	H	-	-	34.3	242 (1.7)	1522 (1.7)	1265 (1.4)	14915 (1.5)	100
	2 (4)	1050	H	A28	0.3	27.1	121 (1.5)	150 [1] (5.4)	321 (2.4)	95 (1.6)	<1
2D	1 (6)	1050	S	-	-	28.4	3700 (1.3)	53211 (1.4)	4980 (4.8)	33145 (1.5)	100
	2 (5)	1050	S	A28	0.2	19.0	1542 (1.4)	11694 (1.7)	12375 (2.0)	4 [4] (2.9)	<1
2N	1 (4)	830	S	-	-	18.8	1907 (1.8)	9428 (1.8)	9744 (1.3)	17414 (1.7)	100
	2 (4)	830	S	A29	0.2	10.7	265 (1.8)	859 (2.5)	1498 (2.8)	15 [3] (3.5)	<1

Notes: A28 and A29 are two different preparations of anti-Ig-1b serum. Mice received sheep (S) or horse (H) RBC. Total  $\gamma$ G PFC were developed by a poly-specific anti- $\gamma$ C serum,  $\gamma$ G<sub>2a</sub> PFC by an anti-allotype serum (A5) or (A26). Total nucleated cells per spleen and PFC/spleen were calculated as geometric means on  $\log (x+1)$  transformed data.

\* Number of animals.

† Number of mice in group negative for this class of PFC.

‡ Numbers in parenthesis are factors of the standard error (SE), if factor =  $s$ , mean =  $x$ , then 1 SE is ( $x/s$ ) and ( $x/s$ ).

SUPPRESSION OF NUMBERS OF PFC PRODUCING Ig-1b ANTIBODY IN MICE RECEIVING HETEROZYGOUS F<sub>1</sub> SPLEEN CELLS

The results of one such experiment are shown in Fig. 2. In this experiment F<sub>1</sub> spleen cells were transferred with SRBC into CBA mice 1 day after the recipients had received 830 r; at the same time the recipients also received various doses of anti-Ig-1b serum. The body weights of mice in experimental groups were slightly lower than the control; this was accompanied by lower spleen weights and cell numbers. Direct PFC were reduced by more than one standard error of the controls in groups receiving anti-allotype serum (c-g), and Ig-1a PFC were variably reduced, but neither was depressed proportionally to anti-serum dose. The results of other similar experiments are shown in Table 2. A dramatic suppression of Ig-1b PFC to less than 1 per cent was obtained. Specific suppression of Ig-1b PFC is shown here to be a dose-dependent phenomenon, and suppression reached 1 per cent and less of the controls. Table 2 shows that direct, total  $\gamma$ G and Ig-1a PFC, and cell numbers in experimental groups were generally lower than the controls, but were not suppressed to the same extent as the Ig-1b PFC. Suppression of an anti-HRBC response was also demonstrated.

## SUPPRESSION OF Ig-1b PFC IN HOMOZYGOUS SPLEEN CELLS

CBA mice irradiated with 1050 r failed to survive for 8 days after receiving C57BL/6 spleen cells. Because of this CWB/5 spleen cells were used in CSW mice. A dose-dependent suppression was obtained but with a less dramatic slope over a similar dose range as in the heterozygotes (see Table 3). Table 3 also shows the results of other similar experiments. The highest doses of antiserum suppressed the Ig-1b PFC to 1-3 per cent of the controls.

## EFFECT OF ANTI-Ig-1a SERUM ON HOMOZYGOUS AND HETEROZYGOUS SPLEEN CELLS

Spleen cells from CSW or F<sub>1</sub> mice were transferred into CWB/5 or C57BL/6 (Ig-1b) recipients, respectively. The results are shown in Table 4. In contrast to the results with anti-Ig-1b serum, there was no evidence of suppression, PFC of all classes were within one standard error of the controls. This experiment will have to be repeated with a higher dose of antiserum, since the anti-Ig-1a serum had three- to ten-fold less antibody than the anti-Ig-1b serum (Table 1).

## EFFECT OF NORMAL MOUSE SERUM AND ANTI-PERTUSSIS SERUM ON THE PFC RESPONSE

Normal mouse serum (NMS) was obtained from the same strain used to supply anti-allotype serum. Results are shown in Tables 3 and 4. Values for PFC/spleen were within 95 per cent confidence limits of the controls without NMS. Anti-pertussis serum was also injected as a control in the experiment illustrated in Fig. 2 since the anti-allotype sera contain high anti-pertussis titres. Except for a possible increase in Ig-1b PFC it had no visible effect.

## DISCUSSION

Previously, allotype suppression has been studied using young intact animals which have an immature immune system, and are not yet producing circulating antibodies. In these

TABLE 3  
 PFC IN CSW MICE 9 DAYS AFTER IRRADIATION AND 8 DAYS AFTER RECEIVING  $1 \times 10^7$  CWB/5 SPLEEN CELLS WITH  $4 \times 10^7$  SRBC i.v. AND ANTI-Ig-1b SERUM i.p.

Experiment	Group	X-ray dose (r)	Antiserum		Cells $\times 10^6$ /spleen	PFC/spleen		Per cent Ig-1b of controls
			Name	Dose (ml)		Total $\gamma$ G	Ig-1b $\gamma$ G <sub>2a</sub>	
P	1 (5)*	1050	-	-	31.2	3195 (1.6)†	8871 (1.8)	100
	2 (4)	1050	A28	0.4	21.3	1682 (1.4)	164 (4.4)	2
	3 (6)	1050	A28	0.1	20.2	947 (1.4)	2165 (1.5)	24
	4 (6)	1050	A28	0.025	19.6	1388 (1.4)	5098 (1.3)	57
	5 (6)	1050	A28	0.006	18.4	1832 (1.6)	14,790 (1.6)	109
	6 (4)	1050	A5	0.1	15.0	1271 (1.3)	4,931 (1.8)	1
	7 (5)	1050	BALB/c	0.3	12.5	1377 (1.6)	14,900 (1.6)	22
V	1 (4)	1050	-	-	17.8	457 (1.3)	ND	100
	2 (4)	1050	A28	0.4	14.4	864 (1.9)	78 (4.5)	3
2Q	1 (6)	830	-	-	28.0	503 (2.1)	ND	100
	2 (6)	830	A29	0.3	16.0	377 (2.0)	ND	1
	3+(6)	830	-	-	9.0	2 (2.6)	ND	-

Notes: A5, A28, A29 are three different preparations of anti-Ig-1b serum; BALB/c NMS is pooled normal mouse serum, heat inactivated and absorbed with SRBC.

\* Group 3 of Experiment 2Q received antigen but no spleen cells. ND, not done. Other notes as for Table 2.



TABLE 4  
PFC IN Ig-1b MICE 9 DAYS AFTER IRRADIATION AND 8 DAYS AFTER RECEIVING  $1 \times 10^7$  Ig-1a SPLEEN CELLS WITH  $4 \times 10^7$  SRBC i.v. AND ANTI-Ig-1a SERUM i.p.

Experiment	Group	Mouse strain		Antiserum		Cells $\times 10^6$ / spleen	Direct	Total $\gamma$ C	PFC/spleen		
		Donor	Recipient X-ray dose (r)	Name	Dose (ml)				Ig-1a	Ig-1b	
O	1 (5)*	CSW	CWB/5 1050	-	-	31.3	1865 (2.1)†	4076	(8.0)	1689	(4.5)
	2 (5)	CSW	CWB/5 1050	A26	0.1	16.3	860 (1.3)	2792	(1.9)	9872	(1.9)
	3 (4)	CSW	CWB/5 1050	A27	0.1	36.7	4651 (1.4)	9849 [1]†	(1.6)	993 [1]	(7.3)
	4 (3)	CSW	CWB/5 1050	C57BL/6 NMS	0.1	24.7	3818 (1.3)	11774	(1.7)	363 [1]	(11.3)
Q	1 (5)	CSW	CWB/5 1050	-	-	10.4	416 (1.6)	129 [2]	(4.3)	135 [1]	(5.3)
	2 (3)	CSW	CWB/5 1050	A27	0.2	33.2	984 (1.3)	106 [1]	(10.5)	207 [1]	(9.5)
Y	1 (6)	F <sub>1</sub>	C57BL/6 750	-	-	23.1	570 (1.6)	ND	ND	79 [1]	(2.6)
	2 (5)	F <sub>1</sub>	C57BL/6 750	A27	0.3	10.6	307 (1.4)	ND	ND	66 [1]	(2.9)
	3+ (6)	-	C57BL/6 750	-	-	2.9	1	ND	ND	2	(1.8)

Notes: A26 and A27 are two different anti-Ig-1a sera; C57BL/6 NMS is pooled normal mouse serum, heat inactivated and SRBC absorbed.  
 † Group 3 of Expt. Y received antigen but no spleen cells. ND, Not done. Other notes as for Table 2.

experiments adult spleen cells have been specifically and totally suppressed in terms of their ability to produce one class of antibody in an immune response.

A transfer system has been described here which enables cells to be cultured with anti-allotype serum in another environment. The cells have been tested for their ability to respond to xenogeneic red blood cells. When they are assayed 8 days after transfer, cells producing the target Ig-1b allotype were strongly suppressed in all experiments. Heterozygous cells were suppressed to less than 1 per cent of the controls, whilst homozygous cells were suppressed to 1–3 per cent of the controls.

Direct PFC do not compensate for suppressed  $\gamma G_{2a}$  immunoglobulin. Although total  $\gamma G$  PFC are usually reduced in experimental groups it is not possible to test for compensation by  $\gamma G$  classes without specific antisera and with data from an assay of only one day of the PFC response. In heterozygous cells there was no evidence for compensation by Ig-1a PFC in animals with suppressed Ig-1b PFC, in fact they were usually slightly depressed.

Information on suppression of linked Ig alleles controlling other classes could be obtained by using anti-allotype sera for plaque development directed against other allotypes (Herzenberg, 1964; Warner, Herzenberg and Goldstein, 1966). Unfortunately these anti-Ig sera are difficult to raise.

One hypothesis suggests that when allelic exclusion is operating in immunoglobulin producing cells, a representative molecule of the product of the active locus for one class on the active chromosome is expressed on the cell surface, and directly or indirectly represses the other loci; and the other homologous chromosome is irreversibly inactivated at least in this region (Herzenberg *et al.*, 1967a, b). The effect of anti-allotype serum on adult rabbit peripheral blood lymphocytes shows that adult cells can recognize and respond to antibody directed against them by transformation to blast cells, and that there is little anti-allotype induced transformation in cells from allotypically suppressed animals (Sell, 1968a, b). The total suppression obtained here supports the view that immunoglobulin-producing cells or their precursors are inactivated by contact with antibody directed against their surface receptor.

The slight concomitant depression of Ig-1a PFC by anti-Ig-1b treatment has also to be accounted for, either by a specific or non-specific effect. If precursor cells are uncommitted with respect to allele and bear receptors representing both allotypes on their surface, then those cells which carry a sufficient number of Ig-1b receptors will be inactivated by anti-Ig-1b treatment, although they would have proceeded into Ig-1a production (Gell, personal communication). Two non-specific possibilities are: (1) That the introduction of circulating Ig-1a immunoglobulin, as anti-allotype serum acts by feedback inhibition on the regulation of subsequent Ig-1a production. This possibility is supported by experiments of Dubiski and Fradette (1966) in rabbits recovering from neonatal suppression whose recovery was delayed by the introduction of normal rabbit serum of the suppressed allotype. The increase in circulating Ig-1a in Ig-1a mice would however only be minimal. (2) That  $\gamma G_{2a}$  PFC or precursors are inhibited or damaged by injection of mouse serum, the effect on Ig-1b production being masked by the greater specific suppression. The depressive effect of NMS on  $\gamma G_{2a}$  production supports both these non-specific explanations. It is not possible to draw any conclusions from the attempts to suppress with anti-Ig-1a, since no suppression was demonstrated. Presumably larger doses will be required.

Several authors have reported the effect of immunization on the maintenance of suppression. Mage and Dray (1966) found that the immunization of heterozygous adult suppressed rabbits with bovine serum albumin azobenzene arsonic acid, failed to break

the suppression, but David and Todd (1969) found homozygous animals responded to antigen (ovalbumin) by producing antibodies of the allotype totally suppressed in the serum at the time of injection.

The experiments described here show that the response to antigen is suppressed 8 days after transfer, by anti-allotype serum given at the same time as antigen. Since it is possible to obtain suppression of an immune response, experiments with young animals may be considered to involve suppression over a long developmental period in animals continuously exposed to and responding to a series of new antigens. These animals have to be suppressed at this particular stage of development before they start to produce antibody themselves. The system allows an easy approach to a study of the role antigen plays in the induction and maintenance of suppression, and to the stage in differentiation when commitment to class, allele and antigen occurs.

## REFERENCES

- DAVID, G. S. and TODD, C. W. (1969). 'Suppression of heavy and light chain allotypic expression in homozygous rabbits through embryo transfer.' *Proc. nat. Acad. Sci. (Wash.)*, **62**, 860.
- DRAY, S. (1962). 'Effect of maternal antibodies on the quantitative expression of 2 allelic genes controlling  $\gamma$  globulin allotype specificities.' *Nature (Lond.)*, **195**, 677.
- DRESSER, D. W. and WORTIS, H. H. (1967). 'Localised haemolysis in gel.' *Handbook of Experimental Immunology* (Ed. by D. M. Weir), p. 1054. Blackwell Scientific Publications, Oxford and Edinburgh.
- DUBISKI, S. (1967). 'Suppression of synthesis of allotypically defined immunoglobulins and compensation by another sub-class of immunoglobulins.' *Nature (Lond.)*, **214**, 1365.
- DUBISKI, S. and FRADETTE, K. (1966). 'The feedback mechanism in immunoglobulin synthesis.' *Proc. Soc. exp. Biol. (N.Y.)*, **122**, 126.
- HERZENBERG, L. A. (1964). 'A chromosome region for gamma<sub>2a</sub> and beta<sub>2a</sub> globulin H chain isoantigens in the mouse.' *Cold Spring Harb. Symp. quant. Biol.*, **29**, 455.
- HERZENBERG, L. A. and HERZENBERG, L. A. (1965). 'Suppression of a  $\gamma$ G-globulin allotype in mice by anti-allotype antibodies.' *Symposium on the Mutational Process: Genetic Variations in Somatic Cells* (Ed. by J. Klein), p. 227. Academia.
- HERZENBERG, L. A., MINNA, J. D. and HERZENBERG, L. A. (1967a). 'The chromosome region for immunoglobulin heavy chains in the mouse: allelic electrophoretic mobility differences and allotype suppression.' *Cold Spring Harb. Symp. quant. Biol.*, **32**, 181.
- HERZENBERG, L. A., HERZENBERG, L. A., GOODLIN, R. C. and RIVERA, E. (1967b). 'Immunoglobulin synthesis in mice. Suppression by anti-allotype antibody.' *J. exp. Med.*, **126**, 701.
- JERNE, N. K., NORDIN, A. A. and HENRY, C. (1963). 'The agar plaque technique for recognising antibody producing cells.' *Cell Bound Antibodies* (Ed. by B. Amos and H. Koprowski), p. 109. Wistar Institute Press, Philadelphia.
- KABAT, E. A. and MAYER, M. M. (1961). *Experimental Immunochimistry*, p. 149. Thomas, Springfield, Illinois.
- KLEIN, J. and HERZENBERG, L. A. (1967). 'Congenic mouse strains with different immunoglobulin allotypes. I. Breeding scheme, histocompatibility tests and kinetics of  $\gamma$ G<sub>2a</sub>-globulin production by transferred cells for C3H.SW and its congenic partner CWB/5.' *Transplantation*, **5**, 1484.
- LANCE, E. M., FORD, P. J. and RUSZKIEWICZ, M. (1968). 'The use of subcellular fractions to raise antilymphocyte serum.' *Immunology*, **15**, 571.
- MAGE, R. G. and DRAY, S. (1965). 'Persistent altered phenotypic expression of allelic  $\gamma$ G immunoglobulin allotypes in heterozygous rabbits exposed to isoantibodies in foetal and neonatal life.' *J. Immunol.*, **95**, 525.
- MAGE, R. G. and DRAY, S. (1966). 'Persistence of altered expression of allelic  $\gamma$ G immunoglobulin allotypes in an allotype suppressed rabbit after immunization.' *Nature (Lond.)*, **212**, 699.
- MAGE, R. G., YOUNG, G. and DRAY, S. (1967). 'An effect upon the regulation of gene expression: allotype suppression at the  $\alpha$  locus in heterozygous offspring of immunized rabbits.' *J. Immunol.*, **98**, 502.
- SELL, S. (1968a). 'Studies on rabbit lymphocytes *in vitro*. VIII. The relationship between heterozygosity and homozygosity of lymphocyte donor and percentage blast transformation induced by anti-allotype sera.' *J. exp. Med.*, **127**, 1139.
- SELL, S. (1968b). 'Studies on rabbit lymphocytes *in vitro*. IX. The suppression of anti-allotype induced blast transformation in lymphocyte cultures from allotypically suppressed donors.' *J. exp. Med.*, **128**, 341.
- WARNER, N. L., HERZENBERG, L. A. and GOLDSTEIN, G. (1966). 'Immunoglobulin isoantigens (allotypes) in the mouse. II. Allotypic analysis of three  $\gamma$ G<sub>2</sub> myeloma proteins from (NZB  $\times$  BALB/C)F<sub>1</sub> hybrids and of normal  $\gamma$ G<sub>2</sub> globulin.' *J. exp. Med.*, **123**, 707.
- WORTIS, H. H., TAYLOR, R. B. and DRESSER, D. W. (1966). 'Antibody production studied by means of the LHG assay. I. The splenic response of CBA mice to sheep erythrocytes.' *Immunology*, **11**, 603.
- WORTIS, H. H., TAYLOR, R. B. and DRESSER, D. W. (1968). 'Antibody production studied by means of the LHG assay. II. Assay procedure.' *Immunology*, **14**, 69.
- WORTIS, H. H., DRESSER, D. W. and ANDERSON, H. R. (1969). 'Antibody production studied by means of the LHG assay. III. Mouse cells producing five different classes of antibody.' *Immunology*, **17**, 93.