INDUCTION OF LYMPHOID CELL CHIMERISM IN NONINBRED, HISTOCOMPATIBLE RABBITS

A New Model for Studying Allotype Suppression in the Rabbit*

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Considerable evidence now exists suggesting that chronic allotype suppression in the rabbit is associated with a mechanism of allotype-specific inhibition. Although suppression in the acute stage appears to be that of synthesis, chronic suppression seems to be due to a block in secretion, as shown by the presence in chronically suppressed rabbits of unexpectedly large numbers of lymphocytes able to synthesize membrane-bound immunoglobulin (Ig) of the suppressed type but with an apparent secretory block (1, 2). That this inhibition of secretion can be abrogated, at least in vitro, has been shown experimentally, and this finding has been interpreted in terms of interference with an active suppression mechanism (3, 4). Finally, deliberate immunization of thoroughly suppressed rabbits with their own suppressed type Ig has been shown to lead to auto-antibody formation, indicating that the potential for an auto-anti-allotypic response exists in such animals (5–7).

In investigating the mechanism of allotype suppression, we have sought to develop a suitable cell transfer model for studying lymphocytes from allotype-suppressed rabbits. In earlier publications we reported the results of preliminary trials using noninbred animals that were not defined with regard to the rabbit's major histocompatibility antigens (rabbit lymphocyte antigen [RLA][1] complex). The two most frequently observed consequences of transferring adult lymphoid cells to newborn recipients in these earlier experiments were (a) prompt rejection of donor cells, or (b) lethal graft vs. host disease. However, in a total of 77 recipients, 3 animals became stable chimeras exhibiting allotypic Ig determinants of both donor and recipient types throughout life (8). Another single animal's hemopoietic system was completely repopulated by cells from the donor's spleen and thereafter produced lymphocytes as well as erythrocytes with antigenic markers of the donor only (9). Furthermore, the allotype-suppressed state of the donor's cells was abrogated during the process, which led to benign takeover of the recipient's blood-forming system.

The attainment of stable chimerism in a small but significant number of randomly selected outbred rabbits has encouraged us to undertake the breeding of rabbits that

[1] *Abbreviations used in this paper:* BM, bone marrow; HA, hemagglutination; LN, lymph node; RLA, rabbit lymphocyte antigen.

are characterized with regard to RLA type and also are marked with suitable Ig allotypes for subsequent cell transfer experiments. We have found that when donors and recipients are matched and homozygous for RLA type, even though they are not related and are deliberately mismatched with regard to Ig allotypes, the transfer of lymphoid tissue at birth uniformly results in chimerism lasting for months or years. This is indicated by the continued synthesis of Ig products with allotypic determinants specified by the genotypes of both animals. The suitability of this cell transfer system for studying the lymphoid cells of allotype-suppressed rabbits is demonstrated in this report.

Materials and Methods

Rabbits. Breeding stock for these studies were raised and selected from the rabbit colony maintained at the University of Illinois Medical Center, Chicago, Ill. The major histocompatibility complex of the rabbit (RLA haplotype) includes two distinct classes of determinants (10, 11). The serologically defined components, specified by the RLA-A locus, are detected with panels of cytotoxic antisera, whereas the closely linked RLA-D locus controls determinants that are reactive in the mixed leukocyte reaction. Skin grafts between rabbits matched for RLA-A and RLA-D components do not survive longer than those between randomly selected or deliberately mismatched animals unless the matched pairs are also closely related (12). All of the breeders used to obtain animals for the studies to be described were homozygous for the RLA-A and RLA-D loci and, in accordance with the currently used system of nomenclature, were classified as RLA haplotype 3 (13). They were also characterized with regard to Ig allotypes specified by the *a* locus (V_H region) and *b* locus (kappa L chains) as shown in Table I. No evidence has thus far been obtained showing linkage between genes coding for histocompatibility antigens and allotypic determinants on Ig molecules. All efforts have been made to avoid inbreeding in expanding this colony, which now extends to five generations. Thus, no brother-sister or parent-offspring matings have been done.

Immunizations. Methods of obtaining anti-allotype sera have been described in detail elsewhere (3, 4).

Measurements of Allotypes and Anti-Allotypes. Serum samples were analyzed for anti-allotypic antibodies or for Ig of known allotypes using the techniques of passive hemagglutination (HA) or hemagglutination inhibition, respectively, as described in earlier publications (3, 14).

Induction of Allotype Suppression. Newborn rabbits heterozygous with regard to allotypes on kappa light chains (*b* group) were treated with high-titered anti-allotype serum specific for the paternal kappa chain determinant. Each rabbit received a total of 8–15 ml of antiserum intraperitoneally during the 1st wk of life. These animals were bled starting at age 3 wk and their sera were analyzed for the presence of passive anti-allotypic antibody and also for Ig with *a* and *b* group allotypes specified by both parental genotypes. Passively obtained antibody was routinely detected by passive HA up to age 6–8 wk, whereas Ig with the suppressed (paternal) allotypic marker was undetectable (<2 µg per ml serum) until age 12 wk or more (15).

Immunoadsorbents. Pooled normal rabbit sera of the desired allotypic specificities were insolubilized using ethylchloroformate (16) and the resulting gels were used to obtain purified

TABLE I
Ig Allotype Characterization of Breeding Stock

Buck number	*a* locus	*b* locus	Doe number	*a* locus	*b* locus
2949	1, 2	4	3179	3	6
3109	3	5	3271	3	4
3107	3	5	3595	3	5
3950	1	4	4177	2	4
3951	1	4	3135	3	6

anti-allotype antibodies as described previously (9). In addition, an anti-b4 immunoadsorbent was prepared in the same manner by using serum from a rabbit of allotype a^2a^2/b^9b^9, which had been extensively immunized against a2/b4 IgG. This antiserum had no demonstrable cross-reactivity for allotypes b5 or b6 when tested by HA.

Cell Transfers. Spleen cells were prepared in Eagle's minimal essential medium by mincing the spleens and gently pressing the pieces against wire gauze using a rubber policeman. Lymph node (LN) cells were obtained from both mesenteric and popliteal nodes by using essentially the same technique except that the tissue was gently teased apart using two probes. Bone marrow (BM) was obtained from both femurs using lightly heparinized medium, followed by mincing and teasing of the tissue. After removal of debris and clumped cells by gravitation and filtration through gauze, all tissues were washed twice with Eagle's minimal essential medium and resuspended to contain the transfer inoculum (1 × 10^8–3 × 10^8 nucleated cells) in a volume of 1 ml. The mean viability, as determined by trypan blue exclusion, was 75% for spleen cells, 85% for LN cells, and 90% for BM cells. Each 1-2-d-old recipient received 1 ml of cell suspension divided into two intraperitoneal sites.

Enumeration of Lymphocytes with Membrane Ig of Specific Allotypes by Rosetting. Sheep erythrocytes to which purified anti-allotype antibody had been chemically linked were used to detect lymphocytes bearing the corresponding allotypic membrane marker, as described in detail in an earlier publication (9). Cells in spleen, LN, or BM populations that formed rosettes with antibody-linked sheep erythrocytes of relevant allotypic specificities were enumerated by scoring at least 300 cells, and the results were expressed as the precentage of surface Ig^+ cells of that allotype. Blood lymphocytes were obtained as described previously (9) and scored for surface Ig^+ cells in the same manner.

Results

Survey of Results Obtained by Injecting Adult Lymphoid Cells into Histocompatible Newborn Recipients. Each of 38 newborn rabbits from 6 litters received from 1 × 10^8 to 3 × 10^8 spleen, LN, or BM cells from allotype-suppressed adult donors. Donors and recipients were all of RLA haplotype 3 as determined by pedigree. 28 recipients survived to adulthood; most of the others died within the 1st wk of life due to maternal neglect, trauma, or undetermined causes. None of the dead rabbits showed any signs of GVHD previously noted in other groups of rabbits injected with lymphoid cells of undetermined RLA type (9). Table II presents a summary of six cell transfer experiments to be described subsequently in greater detail. In experiments A–D, the objective was to test the hypothesis that allotype-specific suppression could be transferred to normal newborn recipients by means of lymphoid cells from allotype-suppressed, RLA-matched donors. Recipients in these experiments were chosen so that their maternal or paternal allotype would be the target for the donor's hypothetical suppressor cells. In addition, the donors and recipients differed by at least one other *a* or *b* group determinant, thus providing controls on the specificity of possible suppression and the success or failure of donor cell engraftment. Experiments E and F were designed to reveal whether allotype suppression is maintained in a cell population transferred to a histocompatible host not capable of synthesizing the target allotype.

All 28 rabbits listed became lymphoid cell chimeras, as shown by the presence of serum Ig with allotypic determinants associated with cells of both donor and recipient. This was evident at age 3 wk when they were first tested, and has lasted for at least 10–20 mo. Chimerism followed the injection of spleen cells in 17 of these animals, while 7 were recipients of LN cells and 4 of BM cells. No attempts have been made so far to relate the degree of chimerism to the number of cells transferred, but the

TABLE II

Summary of Cell Transfer Experiments in Rabbits of RLA Haplotype 3

	Allotype-suppressed donor						Newborn recipients		
			Cells trans-ferred source	Cells/ recipient × 10^8	State of suppression			Allotype of parents	
Experiment	Allotype	Age			Serum (SI)*	Cells	Number	Dam	Sire
		mo							
A	$a^3a^3/(b^4)b^5$‡	3	LN	1.5	>1,000	<1% b4⁺	1	a^3a^3/b^6b^6	a^1a^2/b^4b^4
			Spleen	2.4		<1% b4⁺	3		
B	$a^1a^3/(b^4)b^6$	6	LN	1.5	600	2.3% b4⁺	2	a^2a^2/b^4b^4	a^3a^3/b^5b^5
			Spleen	2.0		2.8% b4⁺	3		
C	$a^1a^3/(b^4)b^6$	4	LN	2.0		4.2% b4⁺	1	a^2a^2/b^4b^4	a^3a^3/b^5b^6
			Spleen	2.3	67	3.9 % b4⁺	3		
			BM	1.8		1.2% b4⁺	2		
D	$a^3a^3/(b^5)b^6$	6	Spleen	3.0	70	4.4% b5⁺	1	a^3a^3/b^4b^5	a^3a^3/b^5b^6
			BM	1.5		1.1% b5⁺	1		
E (1)	$a^2a^3/(b^5)b^6$	3	Spleen	2.0	>1,000	3.0% b5⁺	2	a^1a^3/b^4b^6	a^1a^1/b^4b^4
(2)	$a^2a^3/(b^5)b^6$	3	LN	1.5	>1,000	1.7% b5⁺	1		
			Spleen	1.7		2.0% b5⁺	2		
F	$a^2a^3/b^4(b^5)$	4	LN	1.7		<1% b5⁺	2	a^1a^3/b^4b^6	a^1a^1/b^4b^4
			Spleen	1.8	>1,000	<1% b5⁺	3		
			BM	0.7		<1% b5⁺	1		

* Suppression index: ratio of concentrations of nonsuppressed/suppressed allotypic Ig in serum on the day donor was killed.

‡ The convention of placing the suppressed allotype in parentheses is used throughout this paper.

overall impression is that lymphoid cells from the three types of tissues tested are of comparable efficacy in colonizing the host animals.

Attempts to Transfer Suppression with Lymphoid Cells of Allotype-suppressed Donors. Four litters of newborn rabbits were the recipients of cell suspensions made from spleens, LN, or BM of allotype-suppressed rabbits, as outlined in the first four experiments of Table II. A representative protocol for preparation of donor and recipients is presented in greater detail in Fig. 1 and refers specifically to experiment A in Table II. At the time of cell transfer, no b4 Ig was detectable in the donor's serum, nor were lymphocytes with membrane Ig of the b4 type present in the cell populations used.

Concentrations of Ig of recipient types (b4 and b6) and of Igb5 (the expressed donor type) in sera of the recipients at age 3 wk are shown in Table III, and compared with allotypic Ig levels in a litter of age-matched controls similarly derived from a b6 dam and a b4 sire. At this age, most of the Ig present is passively obtained from the maternal circulation, as indicated by the predominance of b6 Ig in both litters and by low levels of the endogenously synthesized, paternally derived product, b4, in both normal and treated litters. In the injected animals, the donor cell product accounted for 17–43% of total allotypic Ig at age 3 wk.

The data presented in Fig. 2 show the mean serum concentrations of Ig made by cells of the donor and recipient over a 24-wk period. Ig attributed to cells of donor origin (b5) remained at a constant level of about 1 mg/ml serum, whereas b4 and b6 levels rose steadily. Thus, the proportion of donor allotype declined from about 50% of total at age 4 wk to 10% at 24 wk, concomitant with increased synthesis of Ig by the recipient's own cells. This proportion was maintained with minor variations for the

DERIVATION

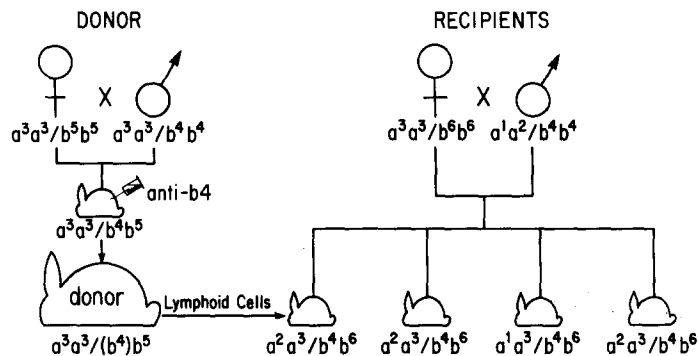DONOR   RECIPIENTS

FIG. 1. Protocol for transfer of cells from an allotype-suppressed donor to normal newborn recipients (Table II, experiment A). Allotype b5 provided a convenient marker for the survival of donor cells in the recipients. Synthesis of b6 by the recipients served as a control on the specificity of possible b4 suppression.

TABLE III

Allotypic Ig of Donor and Recipient Types in Serum of b^4b^6 Rabbits at Age 3 Wk

Rabbit	Donor cells injected		Allotypic Ig in recipient's serum			Total (b4 + b5 + b6)	b5 (donor), percent of total
	Source	Number × 10⁸	b4 (paternal)	b5 (donor)	b6 (maternal)		
			mg/ml				
T404	LN	1.5	0.03	0.61	1.48	2.12	29
T405	Spleen	2.4	0.03	0.31	1.11	1.45	21
T406	Spleen	2.4	0.03	0.31	1.48	1.82	17
T407	Spleen	2.4	0.06	0.61	0.74	1.41	43
T400	—		0.09	—	2.96	3.05	—
T401	—		0.12	—	1.48	1.60	—
T402	—		0.06	—	1.48	1.54	—
T403	—		0.06	—	2.22	2.28	—

Rabbits T404–T407 were the recipients of cells from a donor of allotype b^4b^5 suppressed for b4. Litter T400–T403 were uninjected controls, also derived from a b^6b^6 dam and a b^4b^4 sire.

lifetime of these animals (up to age 20 mo). At age 16–24 wk, the characteristic "pecking order" found in b^4b^6 rabbits had established itself, resulting in the synthesis of somewhat more b4 than b6 Ig (17).

A comparison of the additive concentrations of kappa chain-bearing Ig types (b4 + b5 + b6) in the injected and control litters is presented in Fig. 3. These data demonstrate that the total of donor and recipient Ig products was somewhat elevated at 4 wk of age in the chimeric rabbits as compared with controls of the same allotype. Subsequently, however, accommodation was made for the Ig contribution made by the transplanted cells, resulting in Ig totals that were not distinguishable from those of the uninjected b^4b^6 rabbits.

The data presented in Fig. 4 compare serum b4 and b6 concentrations in the treated and untreated litters. It can be seen that b4 levels tended to be somewhat lower in the cell recipients than in the controls except at the 4-wk period. However,
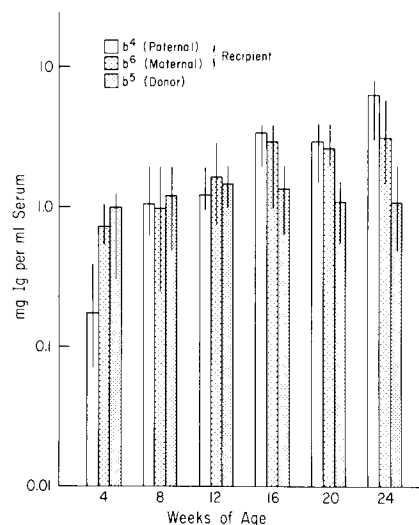
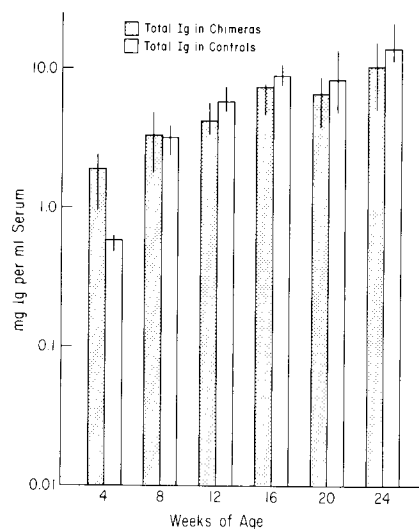FIG. 2.   Distribution of allotypes in b^4b^6 recipients of (b4)b5 lymphoid cells.

FIG. 3.   Comparison of total allotypic Ig (b4 + b5 + b6) in a litter of b^4b^6 rabbits chimeric with (b4)b5 cells and total Ig levels of uninjected b^4b^6 controls.

b6 levels also showed the same tendency. This slight degree of nonspecific suppression of recipient types corresponds with the previous observation of total Ig levels in the chimeric rabbits, which were not abnormally high, except at the 4-wk period (Fig. 3).

We considered the possibility that b4 Ig present in the sera of the chimeric rabbits could be wholly or in part a product of cells of the b4-suppressed donor, which were released from suppression as a result of transplantation and that such an outcome could obscure interpretation of the results. To test this, we took advantage of the fact that the recipients were heterozygous with regard to a group allotypes (a^1a^2 or a^2a^3), whereas the donor was homozygous (a^3a^3). Igb4 from two of the chimeric rabbits (both of genotype a^2a^3/b^4b^6) was isolated by attachment to an anti-b4 immunoadsorbent.
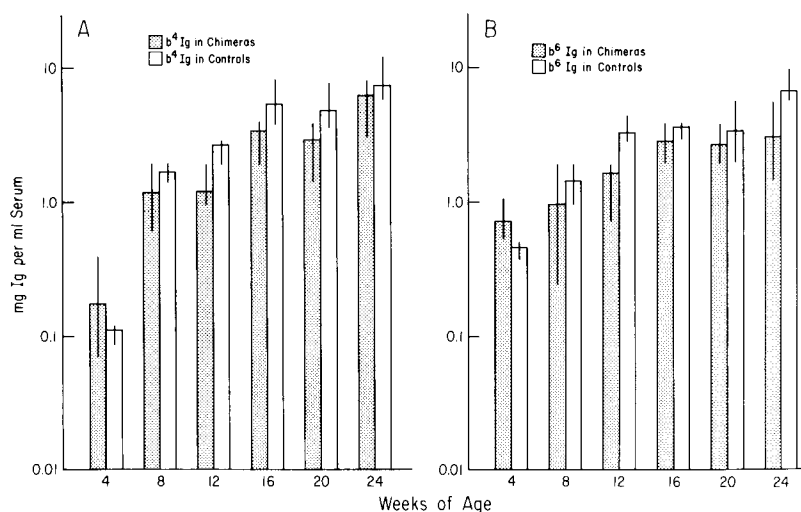
Fig. 4.   Comparative concentrations of b4 (panel A) and b6 Ig (panel B) in chimeric b^4b^6 rabbits with those in an age-matched litter of untreated b^4b^6 animals.

After extensive washing of the gel, the b4 molecules were eluted with glycine-HCl buffer at pH 2.2. After neutralization the extracts were dialyzed, concentrated, and analyzed for Ig of the relevant allotypes. Although the unfractionated serum of the chimeric rabbits contained a preponderance of allotype a3, a portion of which would be associated with the expressed donor b group allotype, b5, the b4 eluates contained approximately equal amounts of a2 and a3, suggesting that most or all of the b4 was a product of the recipient's cells. Other experiments to test directly the possibility that transfer of allotype-suppressed cells to compatible recipients might trigger release from suppression will be presented in a later section.

Three more cell transfers were done using donors exhibiting varying degrees of suppression, as outlined in Table II. Table IV presents data relevant to experiment B of Table II, in which a litter of five neonates of allotype b^4b^5 received spleen or LN cells from a highly suppressed $(b^4)b^6$ donor, aged 6 mo. As with the previously described litter, the proportion of donor allotype (in this case, b6) was highest when the recipients were 4 wk old, although the concentation of b6 remained fairly constant for the 24-wk period shown. As before, total Ig levels in the chimeras were comparable to those of the age-matched rabbits with the same allotypic parentage, and this resulted in slightly lower levels of both b4 and b5 in the chimeras.

Spleen, BM, and LN cells from a $(b^4)b^6$ donor were tested for suppressive activity by adoptive transfer in experiment C of Table II. Experiment D involved recipients from a b^4b^5 dam and a b^5b^5 sire, and only the marrow recipient proved to have the b^4b^5 genotype. Results of cell transfers in these seven rabbits were similar to those just recorded. The proportion of serum Ig of donor origin at age 4 wk was 36-67% in recipients of spleen and LN cells and accounted for 9, 20, and 36% of total Ig in the three rabbits that received BM cell inocula. All of these rabbits have remained chimeric for the entire period of observation (10 mo). No evidence for specific suppression of the b4 allotype in experiment C or of the b5 allotype in the single heterozygous recipient of experiment D was noted.

TABLE IV

Ig of Donor and Recipient Types in Serum of Chimeric Rabbits and Age-matched Controls

Group	Age	Allotypic Ig in serum				
		b4 (maternal)	b5 (paternal)	b6 (donor)	Total	b6 (donor), percent of total
	wk	*mg/ml*	*mg/ml*	*mg/ml*	*mg/ml*	
Chimeric	4	0.53 (0.48–0.60)*	0.33 (0.08–0.76)	1.68 (0.96–2.88)	2.54 (1.52–4.24)	66
	8	1.54 (0.96–2.64)	1.03 (0.36–1.92)	2.47 (0.84–3.84)	5.04 (3.60–7.20)	49
	12	1.78 (1.33–2.66)	2.07 (0.98–2.96)	1.90 (0.36–2.88)	5.75 (4.62–7.17)	33
	16	3.41 (2.66–5.32)	2.16 (0.96–2.88)	1.93 (0.30–2.88)	7.51 (5.08–10.60)	26
	20	4.80 (2.88–7.68)	2.78 (0.96–5.76)	2.21 (0.23–5.76)	9.79 (5.52–10.56)	23
	24	6.91 (5.34–9.68)	2.78 (1.92–3.84)	1.37 (0.11–2.40)	11.06 (7.86–13.63)	13
Normal	4	0.80 (0.48–0.96)	0.64 (0.48–0.96)	—	1.44 (0.96–1.92)	—
	8	3.20 (2.88–3.84)	2.40 (1.44–3.84)	—	5.60 (4.32–6.72)	—
	12	3.20 (2.88–3.84)	1.92 (1.92–1.92)	—	5.12 (4.80–5.76)	—
	16	5.12 (3.84–5.76)	2.40 (1.92–2.88)	—	7.52 (5.76–8.64)	—
	20	8.32 (5.76–11.52)	5.44 (2.88–7.68)	—	13.76 (8.64–19.20)	—
	24	8.00 (4.80–9,60)	5.60 (3.36–6.72)	—	13.60 (8.16–16.32)	—

Both groups of rabbits were genotypically b^4b^5. The chimeric group were recipients of (b4)b6 lymphoid cells and are further characterized in experiment B of Table II.

* Numbers in parentheses express the range of concentrations seen in sera of individual rabbits.

Chimeric Drift after Lymphoid Cell Engraftment.   Although the chimeric state has been maintained to date in all of the 28 rabbits that received histocompatible lymphoid cells, a significant number have exhibited "chimeric drift" (18); that is, a gradual diminution in levels of donor type Ig when observed over a prolonged period. This did not occur in the rabbits included in experiment A (Table II), but was apparent in the second group described above (experiment B). This phenomenon is documented in Fig. 5, which plots the concentrations of donor type (b6) over an 80-wk period in the latter group. Fig. 5 shows that in three of these animals, concentrations of donor cell products were highest during the 1st 20 wk of life and thereafter declined gradually but steadily to the time of writing (80 wk). Ig with donor allotypes reached peak levels at age 4 wk in two members of the litter, which showed a more accelerated downward drift than the others, but still continued to synthesize small amounts of b6 for the entire period. The rate of decline does not seem to be related to the origin (spleen or LN) of the cells transplanted.

Maintenance of Allotype Suppression in Transferred Cells.   Experiments E and F in Table II were designed to test the possibility that transfer of lymphoid cells from allotype-suppressed donors with subsequent engraftment could lead to abrogation of suppression in the transplanted cells. It can be seen from Table II that the three donors used possessed the gene of the *a* group allotype, a2, which was lacking in the recipients. This provided an indicator other than the suppressed *b* group allotype, b5, for the success or failure of engraftment. A total of six transfers of spleen cells, three of LN cells, and one of BM cells, were carried out. The data presented in Table V demonstrate that Ig-forming cells of donor origin became established in all of the 10 recipients, as judged by the presence of Ig with the a2 determinant in their sera. Concentrations of a2 reached peaks of a few milligrams per milliliter in some
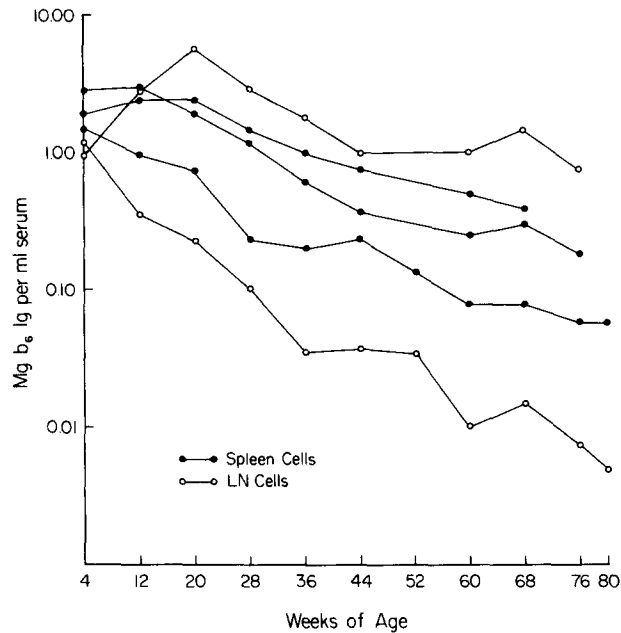
FIG. 5. Concentrations of b6Ig in serum of a litter of b^4b^5 rabbits transplanted with (b4)b6 spleen or LN cells neonatally. (●), spleen cells; (○), LN cells.

TABLE V

Maintenance of Allotype Suppression by Cells Transplanted to Histocompatible Hosts

	Cell source of injected cells			Ig with donor allotype (a2) in serum (mg/ml)						
	Spleen	LN	BM							
				4 wk	*8 wk*	*12 wk*	*16 wk*	*20 wk*	*24 wk*	*52 wk*
Recipient	1			0.40	2.44	0.72	0.47	0.17	0.27	0.03
	2			0.40	1.22	0.36	0.27	0.12	0.10	0.03
	3			0.28	0.61	0.20	0.14	0.03	0.06	0.04
	4			0.10	0.31	0.12	0.06	0.03	0.03	<0.01
	5			0.96	2.88	3.84	2.22	1.96	0.85	0.37
	6			0.36	0.66	0.72	0.43	0.49	0.24	0.24
		1		0.56	0.31	0.36	0.20	0.06	0.07	0.01
		2		0.39	5.28	1.52	0.61	0.38	0.36	0.12
		3		0.48	2.88	0.88	0.61	0.38	0.24	0.03
			1	0.09	0.33	0.66	0.21	0.22	0.12	0.06

Results presented here refer to experiments E and F in Table II, in which all of the recipients were of allotype a^1a^3, whereas the donors were a^2a^3.

individuals, typically at ages of 8–12 wk. A gradual decline in a2 levels occurred in most of the rabbits in this group, but even at 1 yr of age, chimerism was still demonstrable in all except one. On the other hand, Ig with the b5 allotype, which had been deeply suppressed in the donor, was never detectable except in trace amounts (2–15 μg/ml) in occasional bleedings, nor were appreciable numbers of

lymphocytes with membrane-bound Ig of the b5 allotype found in the blood of these animals when tested at ages of 2–12 mo (0–2%).

Discussion

Our earlier successes in producing stable lymphoid cell chimeras in a small percentage of randomly selected outbred rabbits (8, 9) led to undertaking the present study. The strong possibility existed that the earlier chimeras that maintained stable proportions of donor and recipient products had resulted from accidental RLA matching. This idea was reinforced by later findings of limited polymorphism in the RLA complex, as reported by Tissot et al. (13). Indeed, we now show that in a colony of RLA-matched rabbits (all of RLA haplotype 3), in which deliberate efforts have been made to avoid inbreeding, transplantation of lymphoid cells from adults to untreated newborn recipients uniformly leads to long-lasting chimerism. On the other hand, attempts to transfer up to 6×10^8 spleen or LN cells into older recipients (2 mo of age) have so far failed. In these latter experiments (data not shown), which involved a total of four donors and eight recipients, donor products in serum of the recipients never exceeded 40 μg/ml and were no longer detectable 4 wk after cell transfer. Whether it is the relative immunoincompetence of the newborn or other as yet undefined factors, including that of "biological space" (19), which contribute to the age restriction for engraftment has not yet been explored. Possibly immunosuppression of more mature rabbits would allow engraftment of compatible lymphoid tissue, thereby greatly extending the convenience and flexibility of our model.

The chimerism observed in this study has been of long duration (up to 20 mo to date), but has not been equally stable in all of the individual animals studied. In the litter depicted in Fig. 1–4, the amounts of donor type Ig remained remarkably stable over the entire period of observation (1–20 mo; only the first 6 mo are shown). However, the proportion of donor allotype diminished as the animals attained normal adult Ig levels at around 4 mo of age. In other litters, some individuals showed a gradual loss of donor allotype but even at 20 mo of age were still synthesizing it at a low level. This is illustrated by data presented in Fig. 5 and Table V. Incidentally, it is apparent from these data that the stability of chimerism is not related to whether the source of the transplanted cells was spleen, LN, or BM. Such fluctuation in chimeric composition, termed "chimeric drift" by Warner et al. (20), is frequently seen in allophenic mice and also has been documented in two allophenic rabbits constructed by Bordenave and Babinet (21). The cause of the drift has not been determined, but in our rabbits it may involve differences in undefined minor histocompatibility antigens and/or shifts in immunoregulatory mechanisms that may control chimeric composition.

Control of Ig synthesis in our chimeric rabbits, whether the result of immune regulation or nonimmune homeostatic mechanisms, was manifested by the attainment of a normal Ig level within the first few weeks of life and its maintenance thereafter (Fig. 3 and Table IV). Synthesis of Ig by donor cells is seen to be associated with a slight lowering of Ig with allotypes attributable to the recipient's own cells. Similarly, suppression of host allotype in chickens made chimeric with normal histocompatible lymphoid cells was noted by Ivanyi and Makings (22), who attributed this phenomenon to a primitive surveillance mechanism. Further evidence for the operation of normal control mechanisms in the chimeric rabbits was apparent in their maintenance

of the normal "pecking order" of Ig allotypes seen in rabbits which are heterozygous with regard to the b group allotypes, namely b4 > b6 > b5 > b9 (17). Thus, there is a preponderance of the b4 allotype in b^4b^6 rabbits chimeric with a $(b^4)b^5$ donor (Fig. 2) and in b^4b^5 rabbits chimeric with a $(b^4)b^6$ donor shown in Table IV.

Our main purpose in establishing this cell transfer model was to extend our base for investigating the mechanism of allotype suppression in the rabbit. In the first series of experiments reported here, we have attempted to learn whether allotype suppression is transmissible by means of lymphoid cells. Results obtained thus far show that although cells from suppressed rabbits established themselves in newborn recipients, the engrafted cells did not interfere specifically with synthesis of the target allotype. As noted above, slight suppression of both b group allotypes made by the recipients was noted and is probably attributable to mechanisms of an as yet undefined nature that control Ig synthesis in rabbits. Another manifestation of this kind of control mechanism is seen in allotype-suppressed rabbits that maintain normal Ig levels by synthesizing abnormally high concentrations of Ig bearing the nonsuppressed allotype (23).

Other experiments reported here have shown that lymphoid cells from allotype-suppressed donors became established in compatible hosts without a break in suppression. Thus, Ig of the target allotype made in normal heterozygous recipients of lymphoid cells from allotype-suppressed donors was most likely a product of their own cells rather than those of the donor. In fact, our data indicate that the suppressed state of transferred cells seemed to be maintained indefinitely, as no more than occasional traces of b5 Ig were found in the sera of a group of 10 rabbits engrafted with cells from b5-suppressed donors and observed over a 1-yr period (Table V). Moreover, examination of the blood lymphocytes of these chimeras for evidence of cells with membrane-bound b5 Ig also yielded negative results, thus eliminating the possibility that cells capable of synthesizing the suppressed Ig product emerged from suppression but exhibited a block in secreting capacity (2).

In the natural course of events, intact donors such as those used in experiments E and F (Table II) would have been expected to emerge from complete suppression and synthesize gradually increasing quantities of the suppressed Ig allotype by age 6–12 mo (15). Indeed, the presence of small numbers of lymphocytes marked with membrane-bound b5 in two of the donors at the time of killing signifies imminent release from complete suppression (1, 24). Pre-B cells and other even less fully differentiated cells in the marrow are the probable precursors of cells which eventually escape from suppression (25). Thus, one possible explanation of the failure of transplanted cells to recover from suppression would be that such precursors were absent or present in ineffective numbers in the cell populations transferred. Furthermore, we had observed in earlier studies that spleen cells from allotype-suppressed heterozygous rabbits made anti-phage antibodies only of the nonsuppressed allotype when transferred to an in vitro environment, unless cultured in the presence of factors postulated to provide specific interference with an active suppression mechanism (3, 4). Similar intervention might be needed to demonstrate escape from suppression in an in vivo transfer system as well.

Allotype suppression was originally described in heterozygous rabbits that were exposed perinatally to maternal antibodies specific for Ig of the paternal type (23). Chronic suppression of the a and b group allotypes lasting well into adulthood or for

the animal's entire lifetime is readily and reproducibly achieved in the rabbit (26). Complete suppression of paternally derived allotype lasting up to 16 wk has also been induced in heterozygous chickens given anti-allotype antibodies during embryonal life (27). Although chronic suppression in mice has so far been demonstrated for only one allotype, Ig-1b, found on H chains of the γ 2a subclass and with only one mouse strain combination, SJL × BALB/c, suppression in this system has clearly been shown to be associated with the presence of T cells that specifically inhibit production of the Ig-1b allotype when transferred to normal irradiated adults or nonirradiated 2–3-wk-old recipients (28).

Considerable indirect evidence suggested the existence of an allotype-specific suppression mechanism in rabbits (1–4) and called for direct in vivo tests that had previously been impossible to perform. In the experiments described here, we have not thus far obtained any evidence for specific suppression when lymphoid cells of suppressed rabbits were transplanted to potentially susceptible newborn recipients. Among possible reasons for these negative results that should be considered are: (a) suppressor cells could have been present in less than effective numbers when the donors were killed at 3–6 mo of age; and (b) the engraftments observed may be selective and exclude cell types required for active suppression, such as T cells. Interestingly, evidence for selective B cell chimerism has been obtained after transfer of congenic lymphoid cells into nonirradiated immunodeficient mice of the CBA/N strain, whereas irradiation of the recipients allowed both T and B cell populations of the donors to be accepted (29, 30). The authors of these reports concluded that engraftment in unprepared hosts occurs only if the host has a deficiency with regard to a population of lymphoid cells (B cells in the CBA/N mouse). This suggests the possibility that histocompatible B but not T cells could be engrafted in normal neonates because of the low numbers and relative immaturity of B cell functions in young animals (31–33), in contrast to the high numbers and activities of T cells that have been noted (34–36).

The observations recorded here provide unequivocal evidence for the reproducible, successful, and lasting engraftment of donor B cells in recipients selected from an outbred population by matching with respect to major histocompatibility antigens. Thus, a model has become available that should supplement that provided by the inbred mouse and, being outbred, provide information potentially applicable to human situations. To what extent it will be successful depends on the neonatal state of the recipients, and more specifically, whether the relative immunoincompetence of such recipients is the determining factor, remains to be established. Work in progress is designed to answer this question as well as others raised in previous portions of this discussion.

Summary

Noninbred rabbits, matched with regard to the major histocompatibility complex (RLA-A and RLA-D loci) but mismatched for Ig allotypes, served as donors (adult) and recipients (newborn) of lymphoid cells. Lasting chimerism regularly followed the transfer of 1×10^8–3×10^8 spleen, lymph node, or bone marrow cells, as indicated by the continued production of Ig with allotypic determinants of both donor and recipient. Typically, Ig of donor allotype accounted for 25–50% of total allotypic Ig

at 4 wk of age and the amount of donor Ig produced remained stable for up to 20 mo. Total allotypic Ig levels remained normal in the chimeric rabbits. "Chimeric drift" or a gradual diminution of donor products over a period of several months, occurred in some individuals.

Transfer of lymphoid cells from allotype-suppressed adult donors to newborns of appropriate allotypes did not result in specific suppression of the target allotype in the recipients. Other experiments showed that lymphoid cells from suppressed donors adoptively transferred to histocompatible recipients continued to synthesize Ig of the nonsuppressed type only. The suitability of using an outbred population of histocompatible but allotype-mismatched rabbits for analyzing allotype suppression and other immunoregulatory phenomena is demonstrated by the results presented here.

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