STUDIES ON RABBIT LYMPHOCYTES IN VITRO

IX. The Suppression of Antiallotype-Induced Blast Transformation in Lymphocyte Cultures from Allotypically Suppressed Donors*

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The immunoglobulins of the rabbit belong to three major groups: IgG, IgA, and IgM (1–3). These immunoglobulins are generally believed to be produced in plasma cells and to be secreted or released into the humoral fluids (4–8). Immunofluorescence studies of plasma cells indicate that a given plasma cell contains only one immunoglobulin group at a time (9–11). In contrast, studies on the induction of lymphocyte transformation in vitro with sheep antisera specific for IgG, IgA, and IgM indicate that each lymphocyte contains the determinants of IgG and IgM at the same time; with approximately one-third of the lymphocytes containing all three major immunoglobulin groups (IgG, IgA, and IgM) at a time (12).

In addition to the three major immunoglobulin group specificities indicated above, rabbit immunoglobulins also have antigenic specificities, that may differ from one rabbit to another, termed allotypes (13). Rabbit immunoglobulin allotypes are genetically controlled by two loci, termed "a" and "b." Allotypes Aa1, Aa2, and Aa3 are controlled by the a locus and are located on the L chain of rabbit immunoglobulins; allotypes Ab4, Ab5, Ab6, and Ab9 are controlled by the b locus and are located on the H chain of rabbit immunoglobulins (14–17). Other allotypic specificities have been claimed, but only the above seven allotypic specificities have been well characterized (18). Breeding experiments indicate that the a locus determinants segregate independently of the b locus determinants (18). Since each locus (a or b) controls one set of allotypes, a given rabbit may be homozygous at both loci, heterozygous at both loci, or homozygous at one locus and heterozygous at the other. For an excellent coverage of the current state of immunoglobulin allotypes, the reader is referred to the recent review of Kelus and Gell (18).

The plasma cells of an allotypically heterozygous rabbit contain only one of the two allotypic specificities supplied genetically (11, 19). This failure of expression of one of the allotypic specificities by plasma cells has been termed "allelic exclusion." The preceding report in this series presented data which demonstrated that approximately twice as many lymphocytes from a homozygous (i.e. b4 b4) donor would trans-

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form when cultured in vitro with the appropriate antiallotype serum (i.e. anti-Ab4) when compared to the lymphocytes of a heterozygous donor (i.e. b4 b5) (20). These data were considered to be consistent with the concept of allelic exclusion as evidenced by plasma cells.

The possibility that the control of expression of allotypes by plasma cells is similar to that of lymphocytes may be tested further by the experimental

TABLE I

Serum Allotype Concentrations and Blast Transformation of Normal and Suppressed Rabbits

Allotype	Allotype serum levels of donors*			
	Normal b4 b5		Ab4-suppressed b4 b5	
	mg/ml		*mg/ml*	
Aa1	>3		>3	
Ab4	1.8		0.16	
Ab5	2.5		2.9	
Serum added	**Allotypic transformation in vitro**			
	Blasts	Thymidine-^{14}C uptake	Blasts	Thymidine-^{14}C uptake
ml	%	*counts/10 min*	%	*counts/10 min*
Autologous 1/2	<1	486	<1	951
Anti-Ab4 1/4	36	1772	<1	985
1/8	21	922	2	1225
1/20	26	1640	4	1417
1/40	18	672	3	1130
Anti-Ab5 1/4	2	850	17	1517
1/8	21	1007	38	1946
1/20	25	1985	26	1991
1/40	23	681	—	—

* Age = 15 wk.

phenomena of allotypic suppression discovered by Dray (21, 22). In this situation the expression of the paternally supplied allotype in heterozygous offspring may be suppressed by immunizing a homozygous doe to the allotype of a homozygous buck to which she is bred. Thus, if a homozygous b5 b5 doe is immunized against allotype Ab4 and mated to a homozygous b4 b4 buck, the resulting offspring will be genetically b4 b5, but the expression of the b4 allotype in these offspring will be suppressed, i.e. only very low serum immunoglobulin levels of Ab4 will be detectable. The purpose of the present paper is to report that a suppression of allotypically induced lymphocyte transformation also occurs in such allotypically suppressed rabbits. Reasons for the apparent difference in expression of immunoglobulin group specificities by plasma cells and by lympho-

cytes in view of the apparent similarity in expression of immunoglobulin allotype specificities are discussed.

Material and Methods

Rabbits: Three homozygous a1 a1 b5 b5 does were immunized with Ab4–*Proteus vulgaris* complexes made from anti-*P. vulgaris* sera obtained from a homozygous a1 a1 b4 b4 rabbit immunized with *P. vulgaris* (23). These three does were mated with an a1 a1 b4 b4 buck. Of the three matings only one was productive of a viable litter, seven offspring of which survived to make up one group of suppressed rabbits. This doe was remated to a different a1 a1 b4 b4 buck and a second litter of six was obtained. These 13 allotypically suppressed rabbits made up the experimental group.

Six a1 a1 b4 b5 rabbits obtained from mating a normal a1 a1 b5 b5 doe with a normal a1 a1 b4 b4 buck made up the control group of normal rabbits.

Serum immunoglobulin allotypes: Serum immunoglobulin allotype levels were determined by specific hemagglutination inhibition (24). Sheep erythrocytes in Alsever's solution were washed, tanned (25, 26), and coated with Aa1 Aa2 Ab4 Ab5 rabbit IgG obtained by diethylaminoethyl cellulose (DEAE) chromatography (27). After determination of the specific agglutination titers of anti-Aa1, anti-Ab4, and anti-Ab5 sera, a dilution of each of these antisera that gave good agglutination was chosen for the inhibition test. The unknown serum samples and known immunochemically pure IgG samples homozygous for Aa1, Ab4, and Ab5 were then separately diluted in 1:100 normal rabbit serum of allotypic specificity Aa2 Aa3 Ab6 Ab9 using the Takatsy microtiter system (28, 29). The degree of inhibition of hemagglutination by the unknown sera was compared to that of the known IgG samples and the allotypic concentrations of the unknown sera were calculated as described previously for IgG (24). Since this technique is extremely sensitive it is especially useful for determining the allotypic concentrations when they are very low. At high concentrations the method is not accurate, so that when samples gave a reading of 3 mg/ml or greater they were recorded as $>$ 3 mg/ml. Triplicate determinations were carried out for each serum sample.

Lymphocyte cultures: Lymphocyte suspensions were obtained from the peripheral blood of the rabbits used and were cultured as described previously (20). The amount of blast transformation was estimated both morphologically and by the amount of thymidine-^{14}C incorporation after 48 hr in vitro (20).

Experimental protocol: Lymphocyte cultures were obtained from control and allotypically suppressed rabbits from 3 to 40 wk of age at approximately 4-wk intervals. These cultures were treated with at least three, and usually five, dilutions each of anti-Ab4 and anti-Ab5 sera. In some individual experiments the number of antisera dilutions tested was limited by the number of lymphocytes obtained. To minimize dilution effect, the maximum per cent blast transformation observed with a range of dilutions of each antiallotype sera was recorded. To facilitate evaluation of the thymidine-^{14}C uptake data, the counts/10 min of the control (unstimulated) cultures were subtracted from the maximum thymidine-^{14}C uptake of the stimulated cultures. In a given culture experiment made from lymphocytes of one donor obtained at one time, the maximum stimulation (counts/10 min) with anti-Ab4 was then subtracted from the maximum stimulation with anti-Ab5. A positive value indicates that for a given animal at the time of culture the stimulation with anti-Ab5 was greater than that with anti-Ab4. A negative value indicates greater stimulation with anti-Ab4 than with anti-Ab5.

RESULTS

The results of a typical experiment comparing the serum immunoglobulin allotype levels, the per cent blast transformation induced in lymphocyte cul-

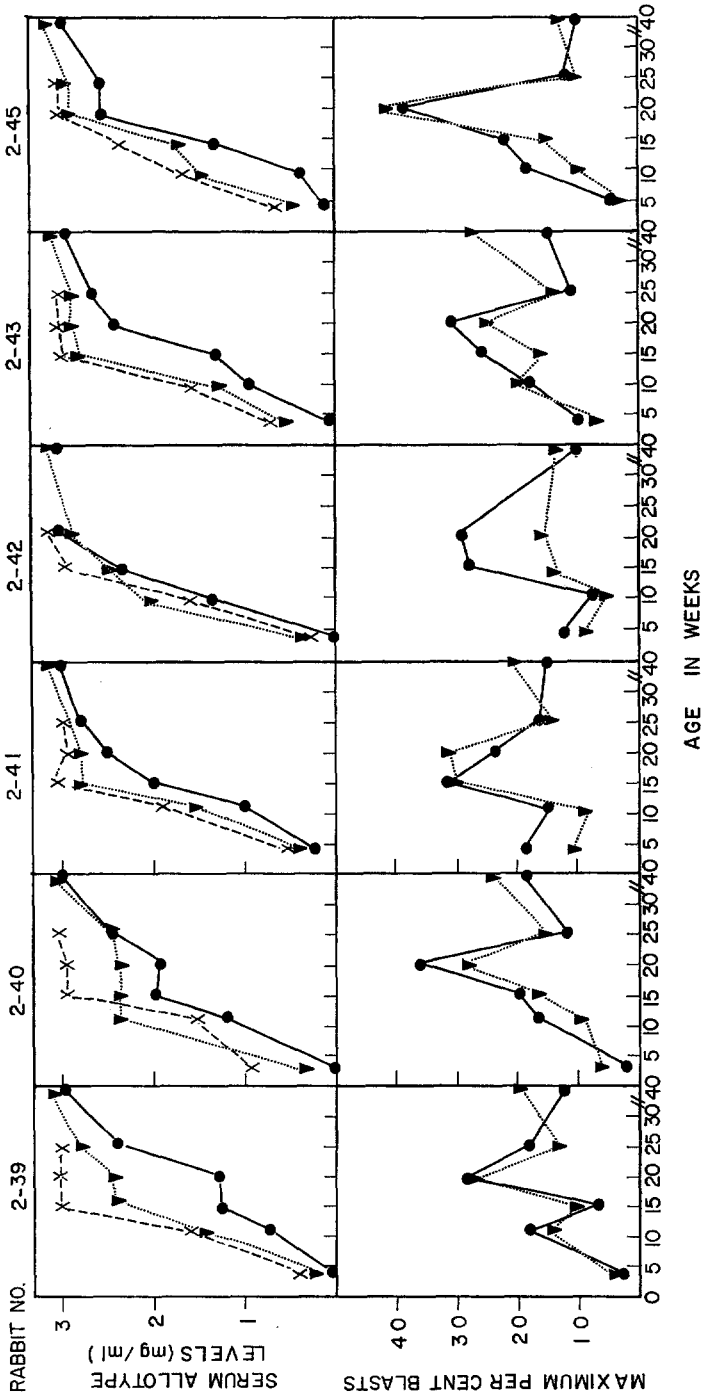

FIG. 1. Serum immunoglobulin allotype levels and per cent blast transformation induced by antiallotype sera in lymphocyte cultures obtained from normal a1 b4 b5 lymphocyte donors. X---X Aa1, ●—● Ab4, ▼---▼ Ab5. At 5 wk of age all these allotypes are found in low concentrations in the serum. Normal adult concentrations are found between 25 and 40 wk of age. There is a lag in the serum concentration of the paternally supplied allotype Ab4. There is no difference in the percentage of blast transformation induced in lymphocyte cultures by anti-Ab4 and anti-Ab5 sera.

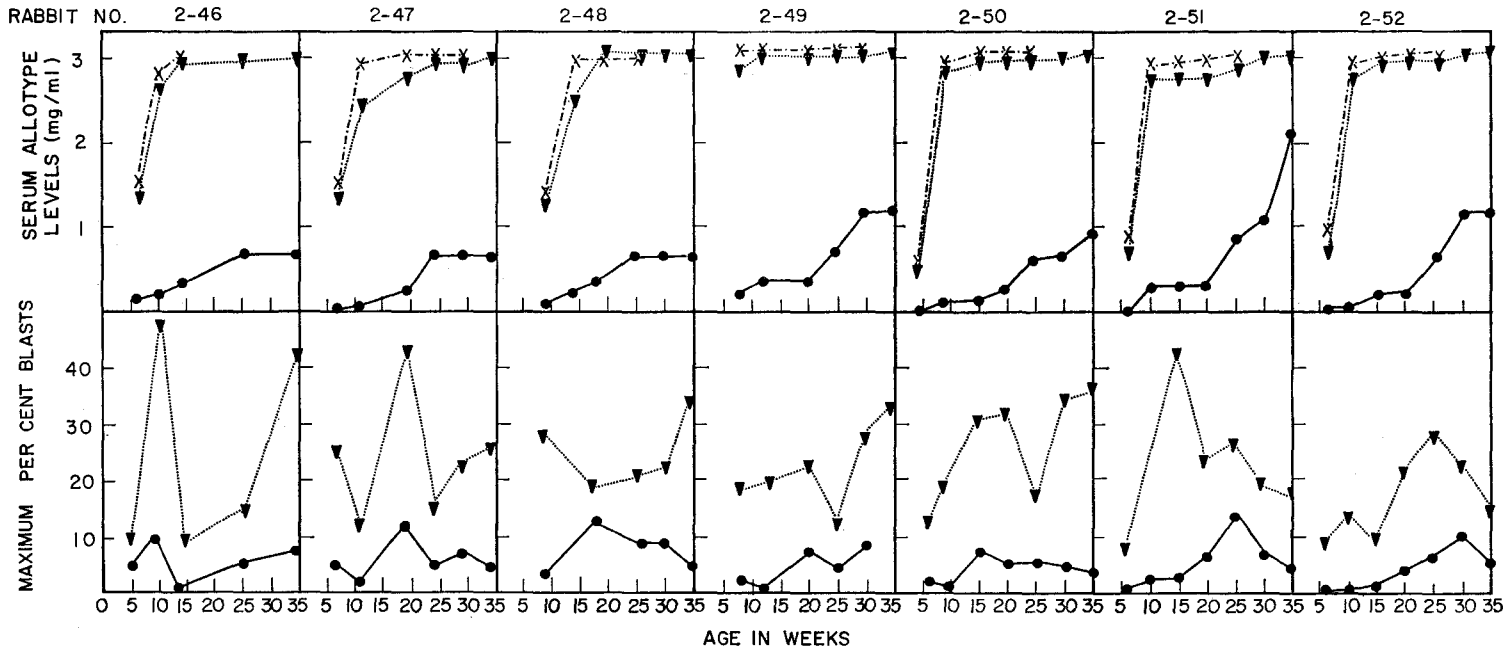

FIG. 2. Serum immunoglobulin allotype levels and per cent blast transformation induced by antiallotype sera in lymphocyte cultures obtained from the first litter of Ab4-suppressed a1 a1 b4 b5 donors. X----X Aa1, ●—● Ab4, ▼---▼ Ab5. The serum levels of Aa1 and Ab5 reach adult levels at 20–25 wk of age. The suppressed allotype Ab4 remains at very low (just detectable) levels until 20–25 wk of age. Then there is a gradual increase, but the levels remain at about 20% of normal during the 35 wk followed. The per cent of transformed lymphocytes stimulated by anti-Ab5 sera is significantly greater than that induced by anti-Ab4 sera during the 35 wk period.

tures with anti-Ab4 and anti-Ab5 sera, and the amount of thymidine-^{14}C uptake induced by the antisera is given in Table I. One lymphocyte and serum donor

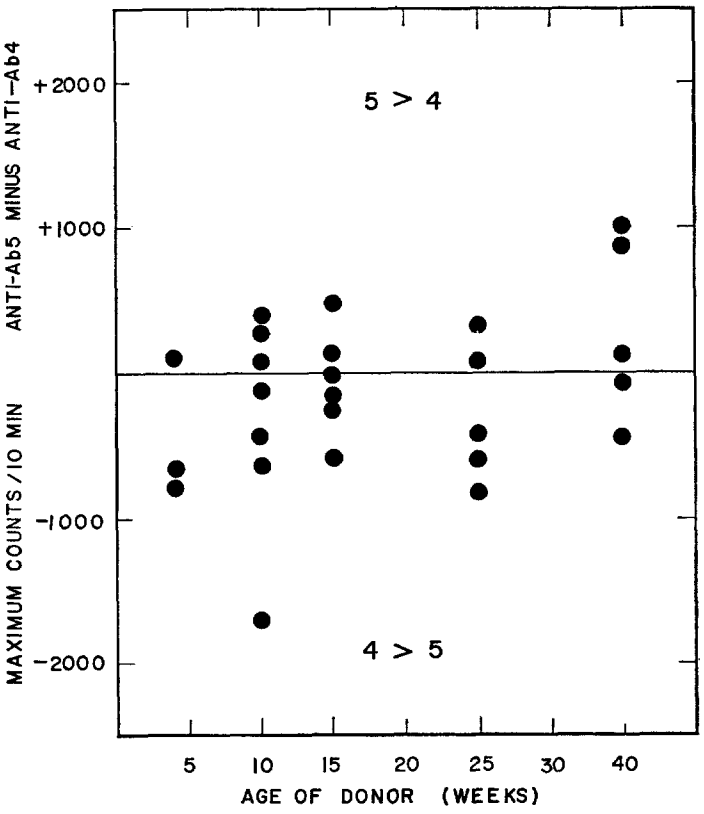

FIG. 3. Uptake of thymidine-^{14}C by allotypically stimulated normal a1 a1 b4 b5 lymphocytes. The counts/10 min for the unstimulated control was subtracted separately from the maximum counts/10 min induced by anti-Ab5 and by anti-Ab4 sera at each test date. The value for the Ab4 stimulation was then subtracted from the value for the Ab5 stimulation. A positive value indicates that stimulation by anti-Ab5 is greater. The values shown indicate no difference between stimulation by anti-Ab5 and by anti-Ab4.

is a normal a1 a1 b4 b5 rabbit, while the other is an Ab4-suppressed a1 a1 b4 b5 rabbit.

The serum levels of each immunoglobulin allotype tested (Aa1, Ab4, and Ab5) and the maximum per cent blast transformation induced in lymphocyte cultures by anti-Ab4 and anti-Ab5 dilutions for the six normal rabbits and for the seven allotypically suppressed rabbits from the first litter are given in graphic form in Figs. 1 and 2. The amounts of thymidine-^{14}C incorporation

expressed as maximum counts/10 min minus control induced by anti-Ab4 subtracted from maximum counts/10 min minus control induced by anti-Ab5 are given for the same cultures in Figs. 3 and 4. The serum allotype levels and maximum per cent blast transformation in cultures obtained from the second

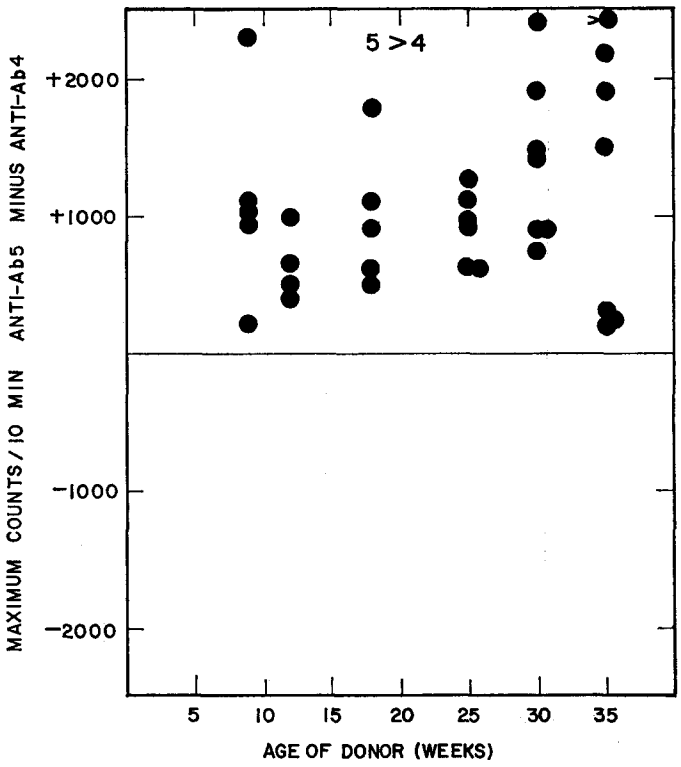

FIG. 4. Uptake of thymidine-^{14}C by allotypically stimulated Ab4-suppressed a1 a1 b4 b5 lymphocytes obtained from the first litter. A positive value indicates that stimulation by anti-Ab5 is greater than that by anti-Ab4. The values shown clearly indicate that stimulation by anti-Ab5 of Ab4-suppressed b4 b5 lymphocytes is always greater than stimulation by anti-Ab4.

litter of Ab4-suppressed rabbits at 4 months of age are given in Fig. 5, and at 6 months of age in Fig. 6. The thymidine-^{14}C uptake of the cultures from the second litter is given in Fig. 7.

It is clear that there is marked suppression of both the Ab4 immunoglobulin concentration in serum and the amount of lymphocyte blast transformation induced by anti-Ab4 in cultures obtained from the a1 a1 b4 b5 rabbits whose mothers had been immunized with Ab4. The suppression of both serum immunoglobulin allotype Ab4 and blast transformation induced in vitro by anti-Ab4 appears to become less with age.

The statistical analysis and comparisons of the four allotypic groups tested are presented in Table II. The conclusions are (*a*) that lymphocyte transformation induced by anti-Ab4 sera of normal b4 b5 cells is significantly greater than that induced by anti-Ab4 of Ab4-suppressed b4 b5 cells; (*b*) that the transformation induced by anti-Ab4 sera is not significantly different than that in-

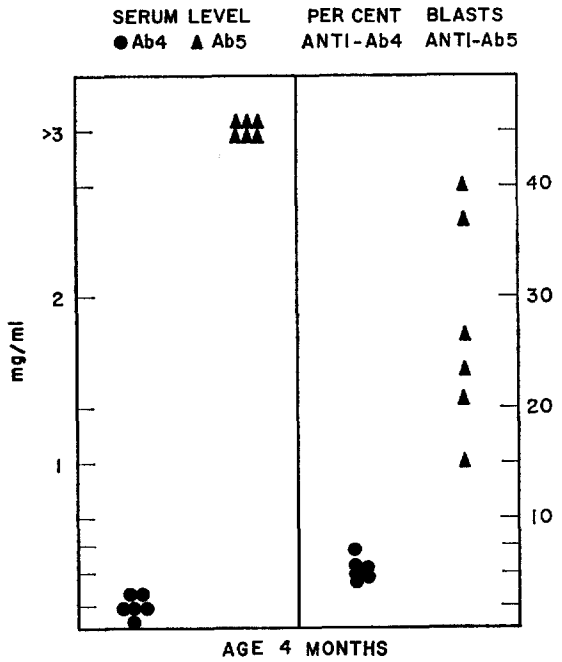

FIG. 5. Serum immunoglobulin levels and maximum per cent transformation induced by antiallotype sera in lymphocyte cultures obtained from the second litter of Ab4-suppressed a1 a1 b4 b5 donors at 4 months of age. Marked suppression of both the Ab4 sera levels and transformation induced by anti-Ab4 is observed.

duced by anti-Ab5 sera when tested on normal b4 b5 cells, but that the transformation induced by anti-Ab4 sera on normal b4 b5 cells is significantly less than that induced by anti-Ab5 on Ab4-suppressed b4 b5 cells; and (*c*) that the amount of lymphocyte transformation induced by anti-Ab5 sera on normal b4 b5 cells is significantly less than that induced by anti-Ab5 sera on Ab4-suppressed b4 b5 cells. Therefore, there is a compensatory increase in the ability of Ab4-suppressed b4 b5 cells to respond to stimulation in vitro by anti-Ab5 sera. In one culture obtained from an Ab4-suppressed donor the per cent of transformed cells is greater than 60%. The highest per cent transformation by a given antiallotype serum in a lymphocyte culture obtained from normal (unsuppressed) heterozygotes yet observed is 54% (31).

DISCUSSION

The data presented in this paper may be summarized simply by stating that the amount of transformation stimulated by antiallotype sera in lymphocyte cultures obtained from allotypically suppressed rabbits is suppressed to a similar extent as the given serum immunoglobulin allotype level (21, 22) and the number

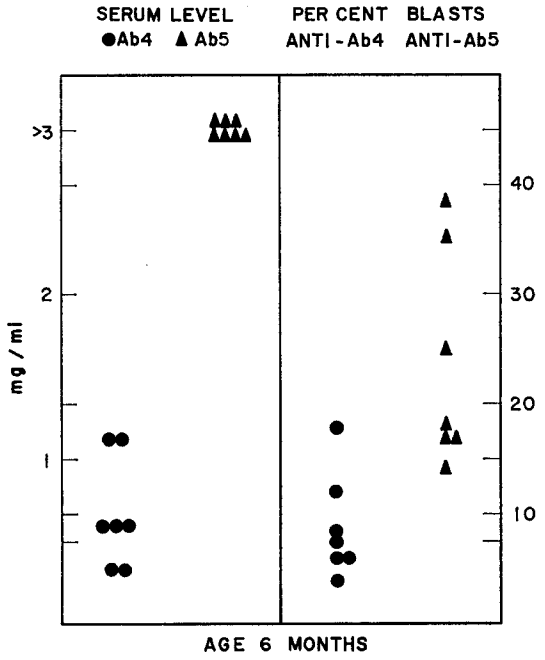

FIG. 6. Serum immunoglobulin levels and maximum per cent transformation induced by antiallotype sera in lymphocyte cultures obtained from the second litter of Ab4-suppressed a1 a1 b4 b5 donors at 6 months of age. The suppression of the Ab4 sera levels and transformation induced by anti-Ab4 is less than that observed at 4 months of age.

of plasma cells that contain the immunoglobulin allotype that is suppressed (30). This finding indicates that in allotypically suppressed rabbits the control of immunoglobulin allotype expression in lymphocytes is similar to that in plasma cells.

The lymphocytes (20) and the plasma cells (11, 19) of the normal allotypically heterozygous rabbit appear to contain only one of the two possible allotypic specificities supplied genetically. This conclusion was first made in regard to plasma cells following the results of specific fluorescent antibody studies which demonstrated that the plasma cells of a heterozygous rabbit contained either one or the other allotype specificity supplied by the two alleles at a given locus but not both (11, 19). This phenomenon was termed "alleleic exclusion." The

observation that approximately twice as many lymphocytes from a homozygous rabbit will transform when stimulated with the appropriate antiallotype serum as compared to lymphocytes from a heterozygous rabbit is consistent with the concept of allelic exclusion by the lymphocyte (20). In addition, stimulation

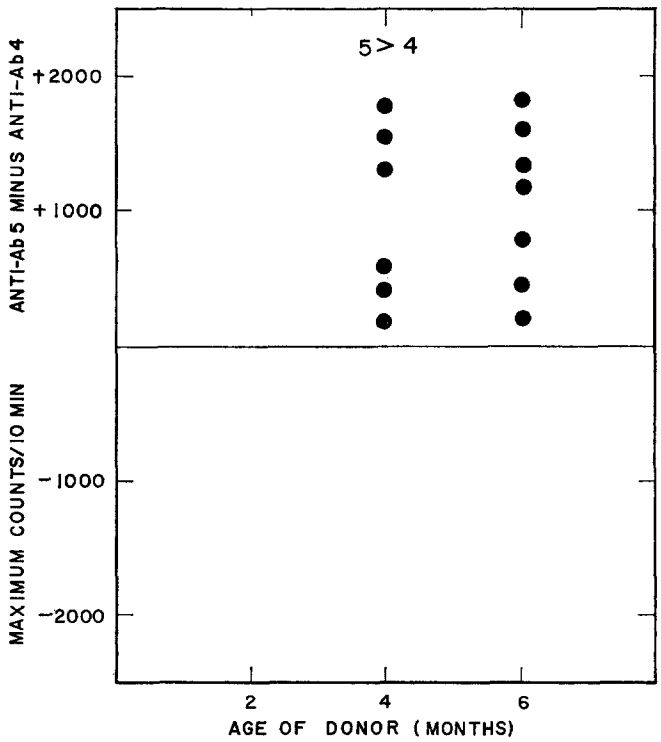

FIG. 7. Uptake of thymidine-^{14}C by allotypically stimulated Ab4-suppressed a1 a1 b4 b5 lymphocytes obtained from the second litter of suppressed rabbits at 4 and 6 months of age. A positive values indicates that stimulation by Ab5 is greater than that by anti-Ab4.

of heterozygous lymphocytes with mixtures of antiallotype sera directed towards both of the allotypic specificities present results in approximately twice as many transformed cells as the addition of only one of the appropriate antiallotype sera (31). This is also consistent with the concept that a given lymphocyte of a heterozygote may carry only one of the two allotypic specificities supplied genetically. The observation that mixtures of antiallotype sera directed toward the two specificities present in double homozygous donors (for example a1 a1 b4 b4 lymphocyte cultures to which are added mixtures of anti-Ab1 and anti-Ab4 sera) also cause a doubling of the amount of blast transformation must have a different explanation (31, 32).

However, whereas the control of expression of allotypic specificity may be similar in lymphocytes and in plasma cells, the expression of immunoglobulin class specificity (IgG, IgA, IgM) appears to be different. A given lymphocyte may contain as many as three different immunoglobulin classes (at least by

TABLE II

Statistical Analysis of Lymphocyte Transformation of Four Culture Groups

Culture group	Cell donor	Stimulating antisera	Number of expt	Maximum % blasts	
				Mean	±SEM
1.	Normal b4 b5	anti-Ab4	35	18.05	1.65
2.	Normal b4 b5	anti-Ab5	35	15.48	1.48
3.	Ab4-suppressed b4 b5	anti-Ab4	56	5.53	0.44
4.	Ab4-suppressed b4 b5	anti-Ab5	56	23.50	1.44

Comparison of statistics

Groups compared	SEDM	*P* by *t* test
1 vs. 2	1.16*	0.005§
1 vs. 3	7.31‡	<0.001‡
1 vs. 4	2.41‡	<0.001
2 vs. 4	3.80‡	<0.001
2 vs. 3	6.42‡	<0.001
3 vs. 4	11.89‡	<0.001

SEM, standard error of the mean; SEDM, standard error of the difference of the mean.
* Not significant.
‡ Highly significant.
§ Probably not significant.

lymphocyte transformation data, 12, 33) whereas a given plasma cell contains only one immunoglobulin class of molecules at a time (9–11).

There are at least three possible explanations for this difference in content of immunoglobulin classes between lymphocytes and plasma cells in view of the similar content of allotypic specificities. (*a*) The sensitivity of lymphocyte transformation by anti-immunoglobulin sera may be much greater than that of the fluorescent antibody technique used for immunoglobulin identification in plasma cells. Therefore, the difference between lymphocytes and plasma cells in regard to immunoglobulin class content may only be quantitative. (*b*) The control of allotypic expression may be the same for lymphocytes and for plasma cells, but the control of immunoglobulin class expression may be different. Thus, each lymphocyte of a heterozygote may be able to produce only one allotypic specificity, but may be able to produce all three immunoglobulin class specificities; while each plasma cell may be able to produce only one allotypic specificity

and only one immunoglobulin class specificity. The plasma cell would then be more differentiated or at least more limited in the variety of its immunoglobulin production. (*c*) The control of production of allotypic specificities and immunoglobulin class specificities by lymphocytes and by plasma cells may be the same, but the control of release or secretion may be different. Thus, both the lymphocyte and the plasma cell may be able to manufacture all of the major immunoglobulin classes, but only one class at a time. If the plasma cell rapidly secretes its immunoglobulins, it may be impossible to detect more than one given immunoglobulin class in a plasma cell at a time. All of the first class synthesized may be released before significant amounts of the second class are present. On the other hand, if the lymphocyte does not release or secrete a major portion of its immunoglobulins, detectable ammounts of one class may remain attached to or within the cell when conversion to synthesis of another class occurs. Thus, the plasma cell produces immunoglobulins for secretion; the lymphocyte produces immunobloulins for individual local use. This concept was elaborated further in the preceding paper of this series (20). The prolonged attachment of synthesized immunoglobulin to the lymphocyte provides a convenient explanation for the long-lived immunological specificity of these cells (34, 35).

The mechanism of the suppression of allotypic expression in lymphocytes and in plasma cells is unknown. Immunization of the mother to an allotypic specificity contributed by the father (21, 22) or passive injections of the appropriate antiallotype serum to the paternally contributed allotype into a newborn heterozygous rabbit (36–38) result in a prolonged suppression of the appearance of the given allotypic specificity. Such a suppression might occur either on a cellular or subcellular level. At least four possible mechanisms may play a role in allotypic suppression: (*a*) death of the cells destined to produce the suppressed specificity (39), (*b*) a failure of proliferation of cells producing the suppressed allotype (21), (*c*) a repression of the production of immunoglobulin molecules containing the suppressed allotype with compensatory production of another class of immunoglobulin molecules by the affected cells (38, 40), or (*d*) a repression of production of the part of the immunoglobulin molecule bearing the allotypic antigenic specificity with retention of production of other immunoglobulin antigenic specificities. At the present time, it is impossible to select one mechanism from these alternatives.

SUMMARY

The number of lymphocytes transformed in vitro by antiallotype sera in cultures obtained from allotypically suppressed rabbits is significantly less than that induced in cultures from normal rabbits. There is a compensatory increase in the amount of transformation induced by antiallotype sera directed toward the unsuppressed allotype. Thus the control of allotypic expression is suppressed rabbits appears to be the same for lymphocytes and for plasma cells.

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BIBLIOGRAPHY

1. Small, P. A., Jr., and M. E. Lamm. 1966. Polypeptide chain structure of rabbit immunoglobulins. I. γG-immunoglobulin. *Biochemistry*. **5**:259.
2. Lamm, M. E., and P. A. Small, Jr. 1966. Polypeptide chain structure of rabbit immunoglobulins. II. γM-immunoglobulins. *Biochemistry*. **5**:267.
3. Cebra, J. J., and P. A. Small, Jr. 1967. Polypeptide chain structure of rabbit immunoglobulins. III. Secretory γA-immunoglobulin from colostrum. *Biochemistry*. **6**:503.
4. White, R. G. 1954. Observations on the formation of Russell bodies. *J. Exptl. Pathol.* **35**:365.
5. Coons, A. H., E. H. Leduc, and J. M. Connolly. 1955. Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. *J. Exptl. Med.* **102**:49.
6. Leduc, E. H., A. H. Coons, and J. M. Connolly. 1955. Studies on antibody production. II. The primary and secondary responses in the popliteal lymph nodes of the rabbit. *J. Exptl. Med.* **102**:61.
7. Nossal, G. J. V. 1962. Cellular genetics of immune responses. *Advan. Immunol.* **2**:163.
8. Van Furth, R., H. R. E. Schuit, and W. Hijmans. 1966. The formation of immunoglobulins by human tissues in vitro. III. Spleen, lymph nodes, bone marrow and thymus. *Immunology*. **11**:19.
9. Chiappino, G., and B. Pernis. 1964. Demonstration with immunofluorescence of 19S macroglobulins and 7S gamma globulins in different cells of the human spleen. *Pathol. Microbiol.* **27**:8.
10. Bernier, G., and J. J. Cebra. 1965. Frequency distribution of α, γ,κ and λ polypeptide chains in human lymphoid tissues. *J. Immunol.* **95**:246.
11. Cebra, J. J., J. E. Colberg, and S. Dray. 1966. Rabbit lymphoid cells differentiated with respect to α-, γ-, and μ-heavy polypeptide chains and to allotypic markers Aa1 and Aa2. *J. Exptl. Med.* **123**:547.
12. Sell, S. 1967. Studies on rabbit lymphocytes in vitro. VI. The induction of blast transformation with sheep antisera to rabbit IgA and IgM. *J. Exptl. Med.* **125**:393.
13. Dray, S., S. Dubiski, A. Kelus, E. S. Lennox, and J. Oudin. 1962. A notation for allotypy. *Nature*. **195**:785.
14. Feinstein, A., P. G. H. Gell, and A. S. Kelus. 1963. Immunochemical analysis of rabbit γ-globulin allotypes. *Nature*. **200**:653.
15. Stemke, G. W. 1964. Allotypic specificities of A and B Chains of rabbit γ-globulin. *Science*. **145**:403.
16. Dubiski, S., and P. J. Muller. 1967. A "new" allotypic specificity (A9) of rabbit immunoglobulin. *Nature*. **214**:696.

17. Sell, S. 1968. Further characterization of rabbit immunoglobulin allotype Ab9. *Immunochemistry*. In press.
18. Kelus, A. S., and P. G. H. Gell. 1967. Immunoglobulin allotypes of experimental animals. *Progr. Allergy*. **11**:141.
19. Pernis, B., G. Chiappino, A. S. Kelus, and P. G. H. Gell. 1965. Cellular localization of immunoglobulins with different allotypic specifications in rabbit lymphoid tissues. *J. Exptl. Med.* **122**:853.
20. Sell, S. 1968: Studies on rabbit lymphocytes in vitro. VIII. The relationship between heterozygosity and homozygosity of lymphocyte donor and per cent blast transformation induced by antiallotype sera. *J. Exptl. Med.* **127**:1139.
21. Dray, S. 1962. Effect of maternal isoantibodies on the quantitative expression of two allelic genes controlling γ-globulin allotypic specificities. *Nature* **195**:677.
22. Mage, R., G. O. Young, and S. Dray. 1967. An effect upon the regulation of gene expression: allotypic suppression at the a locus in heterozygous offspring of immunized rabbits. *J. Immunol.* **98**:502.
23. Dubiski, S., Z. Dudziak, D. Skalba, and A. Dubiska. 1959. Serum groups in rabbits. *Immunology*. **2**:84.
24. Sell, S. 1963. Measurement of gamma globulin in germfree guinea pigs by hemagglutination inhibition. *Proc. Soc. Exptl. Biol. Med.* **114**:362.
25. Stavitsky, A. B. 1954. Micromethods for the study of proteins and antibodies. I. procedure and general applications of hemagglutination and hemagglutination-inhibition reactions with tannic acid and protein-treated red blood cells. *J. Immunol.* **72**:360.
26. Stavitsky, A. B., and E. R. Arquilla. 1958. Studies of proteins and antibodies by specific hemagglutination and hemolysis of protein coated erythrocytes. *Intern. Arch. Allergy Appl. Immunol.* **13**:1.
27. Levy, H. B., and H. A. Sober. 1960. A simple chromatographic method for preparation of gamma globulin. *Proc. Soc. Exptl. Biol. Med.* **103**:250.
28. Takatsy, G. 1955. The use of spiral loops in serological and virological micromethods. *Acta Microbiol. Acad. Sci. Hung.* **3**:191.
29. Sever, J. L. 1962. Application of a microtechnique to viral serological investigations. *J. Immunol.* **88**:320.
30. Lummus, Z., J. J. Cebra, and R. Mage. 1967. Correspondence of the relative cellular distribution and serum concentration of allelic allotypic markers in normal and "allotype-suppressed" heterozygous rabbits. *J. Immunol.* **99**:737.
31. Gell, P. G. H., and S. Sell. 1965. Studies on rabbit lymphocytes in vitro. II. Induction of blast transformation with antisera to six IgG allotypes and summation with mixtures of antisera to different allotypes. *J. Exptl. Med.* **122**:813.
32. Sell, S. 1967. Studies on rabbit lymphocytes in vitro. V. The induction of blast transformation with sheep antisera to rabbit IgG subunits. *J. Exptl. Med.* **125**:289.
33. Sell, S. 1968. Speculations on anti-immunoglobulin and anti-allotype transformation of rabbit lymphocytes. Proceedings of the Fourth Developmental Immunology Workshop at Sanibel Island. In press.
34. Gowans, J. L., and D. D. McGregor. 1965. The immunological activities of lymphocytes. *Progr. Allergy*. **9**:11.

35. Sell, S., and R. Asofsky. 1968. Lymphocytes and immunoglobulins. *Progr. Allergy.* **12**:68.
36. Mage, R. G., and S. Dray. 1966. Persistance of altered expression of allelic γG-immunoglobulin allotypes in an "allotype suppressed" rabbit after immunization. *Nature.* **212**:699.
37. Dubiski, S., and K. Fradette. 1966. The feed-back mechanism in immunoglobulin synthesis. *Proc. Soc. Exptl. Biol. Med.* **122**:126.
38. Chou, C.-T., B. Cinader, and S. Dubiski. 1967. Allotypic specificity and hemolytic capacity of antibodies produced by single cells. *Intern. Arch. Allergy Appl. Immunol.* **32**:583.
39. Dubiski, S. 1967. Suppression of synthesis of allotypically defined immunoglobulins and compensation by another sub-class of immunoglobulin. *Nature.* **214**: 1365.
40. Herzenberg, L. A., L. A. Herzenberg, R. C. Goodlin, and E. C. Rivera. 1967. Immunoglobulin synthesis in mice: suppression by an anti-allotype antibody. *J. Exptl. Med.* **126**:701.