Immunology, 1962, 5, 603.

The Quality of Antibody Produced by Transferred Cells*

HOWARD M. GREY

Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California

(*Received 22nd May*, 1962)

Summary. Comparisons of the rate of dissociation of antibody produced by recipients and donors in cell-transfer experiments were made in a BSA-anti-BSA system using the ammonium sulphate salting-out technique. It was found that: (1) antibody formed in most normal and irradiated adult recipients had dissociation rates very similar to those of the donor animals; (2) antibody formed in most newborn recipients was similar to the antibody formed in the donors with respect to the kinetics of antigen-antibody dissociation. These data were considered as evidence for the hypothesis that the recipient animal plays no direct role in the formation of antibody in cell transfer, and that the recipient environment to which the transferred cells are exposed does not appreciably affect the quality of the antibody produced.

INTRODUCTION

When lymphoid cells from a previously immunized rabbit are transferred to recipient animals an immune response ensues. The role of the transferred cells in this immune response is not entirely clear although previous data obtained are consistent with the hypothesis that the transferred cells are responsible for the production of the antibody and that the recipient's role is solely a supportive one (Chase, 1953; Harris, Harris and Farber, 1954, 1958; Roberts and Dixon, 1955). The present studies on the quality of antibody produced in cell transfer were performed for two reasons: (1) to give additional information regarding the importance of the pre-immunized transferred cells in the production of the antibody; (2) to determine the effect of different host environments on the quality of antibody produced after transfer of pre-sensitized cells.

Previous studies on the quality of antibody produced in rabbits during the course of immunization have resulted in the following pertinent information: (*a*) antibody produced shortly after a primary immunization invariably dissociates from antigen more rapidly than antibody formed by the same animal after either subsequent antigenic stimulation or after a long time interval has elapsed since the primary stimulation, and (*b*) there is a wide variation in the dissociability of antibody from antigen when antibodies made by different animals at the same stage of immunization are compared (Farr, 1958; Grey, 1961 and unpublished; Jerne, 1951). Because of these facts, it is possible to distinguish between an early primary and a secondary response in any given animal and to distinguish, in extreme examples at least, between the secondary immune responses made by two or more animals.

* Publication Number 5 of the Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California.

This work was supported in part by a U.S. P.H.S. grant and an Atomic Energy Commission research contract.

The findings of the present study indicate that the quality of the antibody produced in donor animals is closely approximated by the quality of antibody made in homologous recipients of their lymphoid cells. This is taken as evidence in support of the theory that the antibody produced upon transfer of sensitized cells is made by the transferred cells. Also, data are presented which indicate that the quality of antibody produced by transferred cells is constant regardless of changes in the host environments to which they are exposed.

MATERIALS AND METHODS

Albino donor rabbits weighing 2–3 kg. were used as sources of immunized cells. Donors were immunized with two similar courses of bovine serum albumin (BSA), Armour fraction V. Each donor received a total of 200–250 mg. of protein, the primary course preceding the second course by 3 weeks (Roberts and Dixon, 1955). Animals were bled 10 days after the last injection to determine antibody concentrations and the relatively good antibody producers were used as cell donors 18–30 days after the last injection of BSA.

Cell transfers were performed as previously described by Roberts and Dixon (1955). Transfers to newborn recipients were made with cells obtained from the spleen, mesenteric and popliteal nodes of individual donors. Lymph-node cells only were used for adult transfer experiments.

Newborn recipients (3–5 days old) received 0.5 mg. BSA intramuscularly 2–4 hours before transfer to insure exposure of the transferred cells to antigen at the time of transfer. Subcutaneous and intraperitoneal injections of 2×10^8 cells from an individual donor animal were given to each newborn recipient, the number of recipients being determined by the quantity of cells obtained from a single donor. Animals were bled for antibody 10 days after cell transfer. Adult recipients received $5–7 \times 10^8$ cells from individual donors. The cells were injected intramuscularly into the ventral abdominal wall and this injection was immediately followed by an intravenous injection of 2 mg. BSA. These animals were also bled for antibody studies 10 days after transfer. Normal neonates and adults which were either untreated normals or X-irradiated (400 r. whole-body X-irradiation delivered 1–2 days prior to cell transfer) were used as recipients.

^{131}I-labelled BSA (I*BSA) was used as test antigen in these studies and was iodinated according to the method described by Talmage, Dixon, Bukantz and Dammin (1951). Antibody determinations were made by the ammonium sulphate antigen binding test described by Farr (1958). The ammonium sulphate salting-out technique was also used for the kinetic studies reported below. The procedure for obtaining dissociation rates was similar to that described by Talmage (1960), i.e. a dilution of antiserum was selected so that when 1 ml. was added to 0.1 μg. I*BSA N, 30–50 per cent of the I*BSA would be precipitated upon addition of an equal volume of saturated ammonium sulphate (SAS).† Five ml. of an appropriate dilution of antiserum was added to 5 ml. of a solution of I*BSA containing 0.1 μg. I*BSA N/ml. To determine the amount of non-specific precipitation of antigen present, a control tube containing 5 ml. of 10 per cent normal rabbit serum in borate buffer (1 : 10 NRS) and 5 ml. of I*BSA was used. These solutions were kept for 18 hours at 24°. After equilibrium was established, 1 ml. of a solution containing 650 μg. N

†This degree of antigen excess was chosen because it had been shown by Talmage (1960) that for a given antiserum the rate of dissociation remained stable if the test was performed with an antiserum dilution capable of precipitating 50 per cent or less of the I*BSA present. If higher concentrations of antiserum were used, decreased rates of dissociation were observed.

of unlabelled BSA was added to the reaction mixture. At varying time intervals after the addition of unlabelled BSA, duplicate 1 ml. aliquots were taken from the reaction mixture and added to tubes containing 1 ml. of SAS. The resultant precipitates were kept at 4° for 1 hour and then centrifuged, washed with 3 ml. of 50 per cent SAS, recentrifuged and assayed for radioactivity in a γ well-type scintillation counter. After a correction had been made for the non-specific precipitation of I*BSA,† the per cent I*BSA precipitated as a result of specific antibody was calculated. The dissociation of I*BSA-anti-BSA complexes was calculated for the various time intervals after the addition of unlabelled BSA and is represented by the fraction P_x/P_0, where P_x is the per cent I*BSA specifically precipitated by antibody at time x and P_0 is the per cent I*BSA precipitated by antibody at time 0 after addition of unlabelled BSA.

RESULTS

A. KINETIC STUDIES ON ADULT RECIPIENTS

Cells from individual donors were prepared for transfer and injected into either normal adults or adults that had received 400 r. whole body X-irradiation 24–48 hours before transfer. The results obtained with normal and irradiated adult recipients were similar and will be presented together. The per cent I*BSA fixed to antibody at time x after the addition of unlabelled BSA divided by the per cent I*BSA fixed at time 0 was plotted on a logarithmic scale against time on a linear plot. The logarithmic vertical axis would therefore represent the per cent of I*BSA-anti-BSA complexes that remained undissociated. Representative curves obtained with two donors and their respective normal recipients are shown in Fig. 1. The dissociation pattern of the recipients closely followed that of

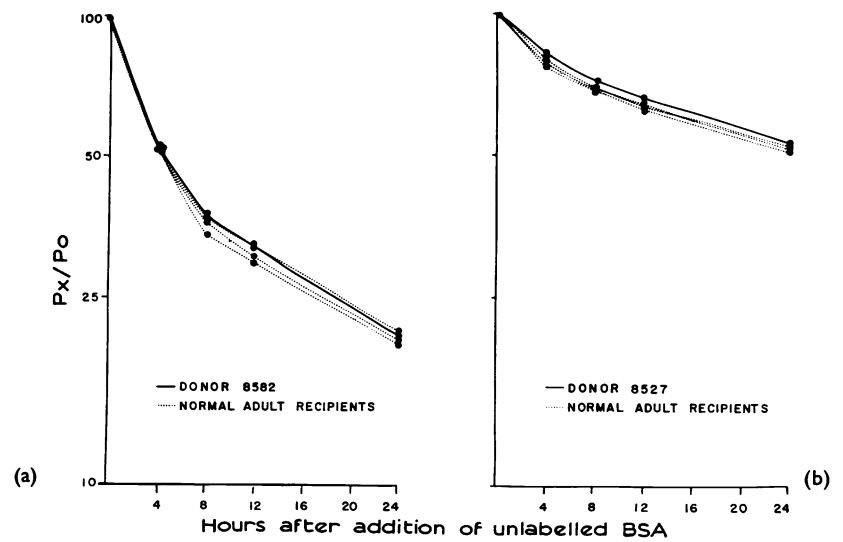

FIG. 1. Dissociation rates of anti-BSA antibody produced in cell transfer. (a) Donor 8527 into three normal adults. (b) Donor 8582 into three normal adults. Vertical axis gives per cent antibody that is still bound to I*BSA after addition of unlabelled BSA.

†The correction factor used is based on the per cent of I*BSA precipitated by ammonium sulphate in the tubes that contained only normal rabbit serum and antigen.

the respective donors, despite the fact that the antibody produced by the two donors differed markedly with respect to the rates of dissociation as evidenced by a one-half dissociation time of 4.5 hours for donor 8582 as compared to 27 hours for donor 8527. Table 1 represents the dissociation data obtained for all donors and adult recipients. Columns 2 and 5 represent the per cent dissociation of I*BSA-anti-BSA complexes 24

TABLE 1

DISSOCIATION OF ANTIGEN-ANTIBODY COMPLEXES IN ADULT RECIPIENTS

Donor	*Per cent dissociation at 24 hours*	*Recipient*	*Pretreatment of recipient (r.)*	*Per cent dissociation at 24 hours*
8436	52	8896	—	57
		8897	—	61
8588	74	9028	—	68
		9029	—	71
		9030	—	71
8582	79	9031	—	79
		9032	—	80
		9033	—	79
8527	47	9025	—	47
		9026	—	48
		9027	—	49
8522	26	8998	400	32
		8999	400	36
8523	32	9000	400	35
		9001	400	35
8525	56	8986	400	53
1046	41	9145	400	37
		9146	400	43

hours after the addition of unlabelled BSA. This was the last point taken for the dissociation rate measurements and was used as the time of comparison in order to bring out more readily any differences that might have existed in the dissociation rates. In six out of eight cases there was good agreement between the per cent dissociation of antibodies from donors and that of the antibodies from the respective recipients. The recipients of donors 8436 and 8522 were somewhat different in that although there was a good agreement among the antibodies of recipients, there was as much as a 10 per cent difference between the recipients and the respective donor antibodies. This difference in dissociability between donor and recipient will be discussed.

B. KINETIC STUDIES ON NEWBORN RECIPIENTS

Cells from individual donors were transferred to normal 3–5-day-old newborn rabbits and also to previously irradiated adult recipients. In addition, in each transfer experiment one newborn recipient received 2×10^8 cells that were killed by heating at 56° for half an hour. The recipients that received killed cells never had detectable antibody in their sera at the time of bleeding 10 days after cell transfer. The results of four separate transfers are given in Table 2. The dissociation that occurred after 24 hours in the sera of the newborn

TABLE 2

DISSOCIATION OF ANTIGEN-ANTIBODY COMPLEXES IN NEWBORN RECIPIENTS

Donor No.	*Percent dissociation after 24 hours*		
	Donor	*400 r. Adult recipient*	*Normal newborn recipient*
164	77	78	80
		76	80
			79
			78
			77
70	78	—	78
			79
168	—*	49	49
		45	49
72	60	51	46
			45
			43

* Donor serum lost.

animals closely paralleled that of the X-rayed adults and the donors, except in the case of donor 72 where there was 15 per cent more dissociation in the serum of the donor than in those of newborn recipients.

DISCUSSION

Much evidence has been accumulated in the past to indicate that the antibody response in recipients receiving a transfer of lymphoid cells from an immunized donor is made by the transferred cells and that the recipient's role is a supportive one. First, radiation of the recipient in doses sufficient to markedly depress a primary response by the recipient may actually improve the antibody response made subsequent to the transfer of sensitized cells (Harris, Harris, Beale and Smith, 1954). Second, antibody produced by cell transfer of previously sensitized cells is of the 'secondary type' as measured by initial combining ratio (Heidelberger and Kendall, 1935; Roberts and Dixon, 1955) or by the neutralization of diphtheria toxin by antitoxin (Chase, 1953 a, b). Third, the time at which antibody is first detectable in the serum of the recipient is dependent upon when the donor's cells are exposed to the antigen rather than when the recipient is exposed (Harris and Harris, 1954). Fourth, killing the cells by any one of a number of methods before transfer abrogates the immune response in the recipient (Harris, Harris and Farber, 1954).

When antiserum obtained from animals within the first 3 weeks after a single injection of BSA was studied under the conditions described above, invariably over 90 per cent of the antigen-antibody complexes had dissociated within 24 hours. In contrast to this, when the antiserum obtained from the thirty-five recipients of previously sensitized cells was studied, 80 per cent or less of the antigen-antibody complexes formed dissociated after 24 hours (Tables 1 and 2). Also, the rate of dissociation of the antibody obtained from the animals receiving previously sensitized cells bore a striking resemblance, in most cases, to the rate of dissociation observed in the antibodies formed in the respective donor animal. These observations are considered as strong corroborative evidence in favour of the theory that it

is the transferred cells that produce the antibody formed subsequent to their injection into recipient animals.

Occasionally a donor serum differed significantly from the sera obtained from the recipient animals (a difference of 17 per cent in the case of donor 72). This much change in the dissociation rate is also encountered occasionally in the sera of an actively immunized animal when sera obtained after two, three, four or more exposures of antigen are compared. Since the transferred cells received one further antigenic stimulus which the intact donors did not receive, it is quite possible that whatever mechanisms cause the variations observed in the kinetics of the sera obtained from actively immunized animals were also operative in the cell-transfer situation.

The second problem the present study deals with is the effect of the host environment on the quality of antibody produced by a given population of cells. Previous data suggest that the quality of antibody produced in an animal may, to some extent, be genetically controlled (Farr, 1958; Grey, unpublished). Cell transfer of previously sensitized cells offers a means of keeping the antibody-forming cells genetically constant thereby making possible an evaluation of the effect of manipulation of the host environment on antibody quality.

In the above experiments the transferred cells were exposed to three quite different environments during their restimulation by antigen and their antibody response: to normal adult animals, to X-irradiated adult animals, and to newborn animals. These various environments are known to have considerable effect on the amounts of antibody formed by transferred cells. In non-irradiated recipients the amount of antibody formed is less than in irradiated recipients presumably because the homograft response of the former against the transferred cells is greater and function of the transferred cells is curtailed (Harris, Harris, Beale and Smith, 1954; Mark, 1962). Cells transferred to newborn rabbits have in some situations produced much less antibody than similar cells transferred to adults while in other situations the neonate provides almost as conducive an environment as does the adult (Dixon and Weigle, 1957, 1959; Harris, Harris and Farber, 1962). However, regardless of the environment, the quality of the antibody was not significantly influenced. From this observation it appears that the factors determining amount of antibody formation are separate from those determining at least some of the qualities of antibody. This would be expected if the qualities of the γ globulin synthesized by a cell were predetermined by the make up of the cell but the rate at which γ-globulin synthesis proceeded was subject to environmental factors involving cellular processes in general.

ACKNOWLEDGMENT

The author wishes to express thanks for the encouragement and advice given to him by Dr. Frank J. Dixon in whose laboratory this work was done.

REFERENCES

CHASE, M. W. (1953). 'Avidity of diphtheria antitoxin produced by transfer of cells from immunized rabbits.' *Fed. Proc.*, **12**, 438.

CHASE, M. W. (1953). *Riassunti delle Comunicazioni*, **1**, 453.

DIXON, F. J. and WEIGLE, W. O. (1957). 'The nature of the immunologic inadequacy of neonatal rabbits as revealed by cell transfer studies.' *J. exp. Med.*, **105**, 75-83.

DIXON, F. J. and WEIGLE, W. O. (1959). 'The nature of the immunologic inadequacy of neonatal rabbits. II. Antibody formation by neonatal splenic cells transferred to adult recipients.' *J. exp. Med.*, **110**, 139-46.

FARR, R. S. (1958). 'A quantitative immunochemical measure of the primary interaction between I*BSA and antibody.' *J. infect. Dis.*, **103**, 239-62.

GREY, H. M. (1961). 'Studies on the binding between streptococcal M protein and antibody.' *J. exp. Med.*, **115**, 671-83.

GREY, H. M. Unpublished observations.

HARRIS, S. and HARRIS, T. N. (1954). 'Studies on the transfer of lymph node cells. III. Effects of variation in the interval between the injection of antigen into the donor and collection of the lymph node cells.' *J. exp. Med.*, **100**, 269-87.

HARRIS, S., HARRIS, T. N. and FARBER, M. B. (1954). 'Studies on the transfer of lymph node cells. I. Appearance of antibody in recipients of cells from donor rabbits injected with antigen.' *J. Immunol.*, **72**, 148-60.

HARRIS, S., HARRIS, T. N. and FARBER, M. B. (1958). 'Studies on the transfer of lymph node cells. XII. The effect of anti-rabbit-leukocyte serum on the transfer of antigen incubated lymph node cells.' *J. exp. Med.*, **108**, 411-29.

HARRIS, T. N., HARRIS, S., BEALE, H. and SMITH, J. J. (1954). 'Studies on the transfer of lymph node cells. IV. Effect of X-irradiation of recipient rabbits on the appearance of antibody after cell transfer.' *J. exp. Med.*, **100**, 289-300.

HARRIS, T. N., HARRIS, S. and FARBER, M. B. (1962). 'Transfer of rabbit lymph node cells to neonatal recipient rabbits.' *J. Immunol.*, **88**, 199-205.

HEIDELBERGER, M. and KENDALL, F. E. (1935). 'A quantitative theory of the precipitin reaction. III. The reaction of crystalline egg albumin and its homologous antibody.' *J. exp. Med.*, **62**, 697-720.

JERNE, N. K. (1951). 'Study of avidity based on rabbit skin responses to diphtheria toxin-antitoxin mixtures'. *Acta path. microbiol. scand.*, Suppl. **87**, 3-183.

MARK, R. (1962). 'Anti-bovine serum albumin (BSA) formation in mice by transferred isologous and homologous spleen cells.' *Fed. Proc.*, **21**, 28.

ROBERTS, J. C. and DIXON, F. J. (1955). 'The transfer of lymph node cells in the study of the immune response to foreign proteins.' *J. exp. Med.*, **102**, 379-92.

TALMAGE, D. W., DIXON, F. J., BUKANTZ, S. C. and DAMMIN, G. J. (1951). 'Antigen elimination from blood as early manifestation of immune response.' *J. Immunol.*, **67**, 243-55.

TALMAGE, D. W. (1960). 'The kinetics of the reaction between antibody and bovine serum albumin using the Farr method.' *J. infect. Dis.*, **107**, 115-32.