IMMUNOLOGIC RESPONSE OF NEONATAL AND OLDER RABBITS TO ANTIGENS OF RABBIT LEUCOCYTES*

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(Received for publication, June 1, 1962)

It was reported earlier that the production of antibody by rabbit lymph node cells which had been incubated *in vitro* with *Shigella* antigen and transferred to recipient rabbits could be markedly reduced by the prior injection into the recipient rabbits of leucocytes of the prospective donor rabbits (1). A number of observations made in the system suggested that the suppression of the transferred lymph node cells thus produced was a result of the active induction of an immunologic response in the tissues of the recipient animal to the transplantation antigens of the donors' cells (2). The suppressive effect could be transferred adoptively: if leucocytes of prospective donor rabbits were injected into other rabbits (not the prospective recipients) and at suitable intervals cells were obtained from the draining lymph nodes, these cells could confer on the recipient rabbits the suppression of antibody formation by antigen-incubated lymph node cells obtained from the donors (3). It was further found that if rabbits were injected with pooled rabbit leucocytes and bled at appropriate intervals, the sera thus obtained could cause the suppression of antibody formation by transferred lymph node cells, if such antisera were injected into the recipient animals or were incubated with the lymph node cells before transfer. A number of the properties of the suppressive agent in such sera indicated that it was probably antibody to the transplantation antigens of the donors' cells (3).

The effects of isoantibodies in suppressing the activity of immunologically competent cells have since been described in other experimental situations. Siskind and Thomas (5) were able to protect newborn mice from the runting syndrome which follows the injection of adult spleen cells (4), by the injection of sera of mice immunized with the donor strain of spleen cells. Gorer and Boyse (6) and Garver and Cole (7) were able to prevent the protective effect of bone marrow cells in lethally irradiated mice by antisera prepared against cells of the strain of mice used as donors of the bone marrow cells. The effect of an antibody in suppressing immunologically competent cells was also suggested in the recent study of Capalbo, Urso, and Makinodan (8) who transferred mouse spleen cells in Millipore chambers to mice of isologous and homologous strains and found that antibody production was somewhat

* This study was supported by Research Grant HE-04598 of the National Heart Institute, National Institutes of Health, United States Public Health Service.

diminished after the 10th day in the isologous recipients, but more so in the homologous mice.

In other studies, in which antigen-incubated lymph node cells were transferred to neonatal recipient rabbits, it was found that the prior injection of leucocytes of prospective donors induced a suppression of antibody formation by the lymph node cells subsequently transferred, as had been found to be the case in older rabbits (9). Since a role of antibody in the suppression of the transferred cells had been clearly demonstrated in the case of the older rabbits, and since it has been shown in a number of systems that neonatal animals produce far less antibody than do adult animals, the production of suppressive antibodies by neonatal rabbits was studied. A marked difference was, in fact, found between the response of neonatal and adult rabbits in the production of antisera effective in the suppression of lymph node cells. Data will be presented below on the immunologic response of adult and neonatal rabbits as observed in the active induction of suppression of lymph node cells, in the adoptive transfer of such suppression, and in the relative effectiveness of antisera obtained in the two groups of rabbits in suppressing antibody formation by transferred lymph node cells.

Materials and Methods

1. Preparation of Pools of Rabbit Anti-Rabbit-Leucocyte Serum.—Heparinized blood was obtained from a group of adult rabbits. The blood was centrifuged for 5 minutes at 2000 RPM at 5°C, after which the upper layer of the sediment plus a small volume of plasma was collected. This suspension was well mixed and centrifuged again at the same time and speed. The layer of white blood cells was collected with some of the plasma layer and transferred to a tube containing a small amount of heparin. If the leucocytes were to be injected into larger rabbits they were then counted and the appropriate amount injected intradermally. For injection into neonatal rabbits, the centrifugation was repeated in order to concentrate the cells.

2. Suspending Medium for the Cells.—A modified Tyrode's solution was used throughout. To make 1 liter of the Tyrode's solution, the following salts were dissolved in distilled water, which had been passed through a mixed resin ion exchange bed, to a volume of 900 ml of solution: NaCl, 8.0 g; KCl, 0.2 gm; dextrose, 1.0 gm; $MgCl_2$, 0.1 gm: $CaCl_2$, 0.2 gm; Na_2HPO_4, 0.142 gm. To this was added 100 ml of phosphite buffer solution, prepared by adding to 50 ml of 0.2 M H_3PO_3 enough 1 M NaOH solution to bring the pH to 7.5, and then adding water to 100 ml. The completed Tyrode-phosphite solution, of which the pH was also 7.5, was filtered, with the addition of phenol red, through a washed Seitz filter pad. This suspending medium was used as prepared by this procedure, or with the addition of gelatin to 0.13 per cent (Tyrode-phosphite-gelatin), or with rabbit serum albumin at 0.5 per cent (Tyrode-phosphite-albumin).

3. Preparation of Lymph Node Cells.—From donor rabbits, either uninjected or injected 4 days earlier in the fore and hind foot-pads with 1 ml of a 5 per cent solution of human γ-globulin, popliteal and axillary lymph nodes were obtained. The nodes were teased into Tyrode-phosphite-gelatin solution, filtered through an 80-mesh stainless steel filter, and washed in approximately 25 volumes of the suspending medium. After centrifugation, the cell sediment was suspended in six times its volume of Tyrode-phosphite-albumin. The cell

suspension was then incubated for 30 minutes at 37°C, in roller tubes, with an equal volume of 10^4 dilution of *Shigella*-trypsin filtrate (10). After incubation, the cells were washed twice in approximately 20 volumes of Tyrode-phosphite solution, and a third time in 10 volumes. The cell sediment was then suspended in 6 volumes of Tyrode-phosphite-albumin solution. Both the cell suspensions and the suspending medium were kept in an ice bath throughout the manipulations. The cells were then either incubated with anti-leucocyte serum and transferred, or injected directly into recipients.

4. "Adoptive" Transfer of the Cell-Suppressive Effect.—Leucocytes were obtained from prospective donors of lymph node cells and injected subcutaneously into the hind feet of rabbits, each injection being of 10^7 cells. After 4 days the leucocyte-injected rabbits were sacrificed, and cells from the popliteal lymph nodes were washed and transferred to 1 kg irradiated recipients. Within 2 hours the same recipients were given lymph node cells from the donors of the leucocytes, the lymph node cells having been incubated *in vitro* with *Shigella*-trypsin filtrate.

5. Measurements of Agglutinins for Shigella.—The procedure for the agglutination test has been described in detail elsewhere (11). In brief, the agglutination test was carried out by adding 0.2 ml of a 0.05 per cent suspension of alcohol-treated *Shigella* to 0.4 ml volumes of 2-fold dilutions of serum, and, after appropriate incubation, observing the pattern formed by the sedimented organisms on the bottoms of the tubes.

6. Irradiation of Recipient Rabbits.—The animals were exposed, 24 hours before cell transfer, to 425 r of deep Roentgen rays, with the following factors: 200 kv, 20 ma, 67.5 cm distance to the bottom of the container, yielding 18 r per minute in air. Filtration was by 1 mm aluminum + 0.5 mm copper.

RESULTS

1. Active Induction of Suppression of Transferred Lymph Node Cells in Neonatal and Older Recipient Animals.

Blood leucocytes obtained from prospective donor rabbits and prepared as described above, were injected at levels of 10^5, 10^6, or 10^7 cells into neonatal rabbits and rabbits of approximately 1 kg. After 7 to 9 days, lymph node cells obtained from the donors of the leucocytes were incubated with *Shigella*-trypsin filtrate (10^{-4} mg/ml), washed, and transferred to the previously injected rabbits as well as to control recipient rabbits of each age group.

In the sera of the control recipients, agglutinins to *Shigella* appeared at the threshold of measurement on the 4th day, rose to a maximal concentration on the 6th to 8th day, and then declined. The maximal agglutinin titers of the recipients previously injected with leucocytes were found to be lower than those of the control titers at all levels of leucocytes used. The geometric mean maximal titers for recipients of both groups are shown in Fig. 1. It can be seen that at each of the levels of leucocytes injected the degree of reduction of mean maximal agglutinin titer in comparison with the control level is very nearly the same for the neonatal and the older recipients.

2. Active Induction of the Suppressive Effect at Shorter Intervals after Leucocyte Injection.—An attempt was made to detect any difference in the active induction of the suppressive effect in neonatal and older rabbits by testing them at smaller intervals of time between the injection of leucocytes and the transfer

of lymph node cells. The intervals chosen were in the range which had been found to correspond to the earliest detection of the suppressive effect in older recipient rabbits injected with this number of leucocytes,–2, 3, and 4 days.

In these experiments one part of a litter of rabbits was injected intradermally with 10^7 donor leucocytes, and part remained uninjected. In each experiment 1 kg rabbits were included, both leucocyte-injected and non-injected. After 2, 3, or 4 days the rabbits (which were irradiated 24 hours earlier) were injected intravenously with *Shigella*-antigen–incubated lymph node cells obtained from the leucocyte donors.

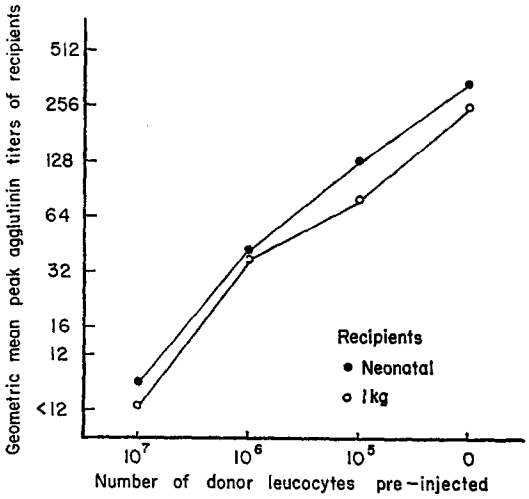

FIG. 1. Geometric mean maximal agglutinin titers of neonatal and 1 kg recipients injected with 10^5, 10^6, and 10^7 donor leucocytes 1 week prior to the transfer of antigen-incubated lymph node cells.

The maximal titers of agglutinin found in recipients which had been injected with leucocytes 2 days before cell transfer were lower than the control levels, and at the 3 and 4 day intervals the levels were still lower. In the case of each interval the geometric mean maximal titer of agglutinin in the neonatal recipients differed only slightly from that of the 1 kg recipients, or was lower in the case of the neonatal rabbits. The geometric mean titer for each group of recipients, for each of these 3 intervals, is shown in Table I.

3. Effect of Anti-Rabbit-Leucocyte Sera Obtained in Neonatal and Older Rabbits.—

Serum pools were obtained from neonatal and older rabbits after intradermal injection of pooled adult rabbit leucocytes. The young rabbits ranged from 1 to 11 days of age at the time of injection. The number of leucocytes injected was 50×10^6, in the preparation of six of the serum pools, 250×10^6, in the case of four serum pools, and 500×10^6 in one case. In each case, leucocytes of the same collection were also injected intradermally into each of a

number of 1 kg rabbits, usually in the same number as was injected into the neonatal rabbits. All of the rabbits were bled 3 times between the 8th and the 11th day. The sera from neonatal and 1 kg rabbits, respectively, were pooled and frozen. Each serum pool was subsequently tested in the following manner: Lymph node cells were pooled from a group of donors, incubated with *Shigella*-trypsin filtrate, washed, and suspended. An aliquot of the cell suspension was mixed with a given volume of anti-leucocyte serum (in the range of 10 to 0.5 ml), incubated at 37°C for 30 minutes, and transferred to a 1 kg irradiated recipient. In each experiment some recipients were injected with lymph node cells not incubated with anti-leucocyte serum.

The geometric mean maximal agglutinin titers of recipients of serum-incubated lymph node cells are shown in the graphs of Fig. 2, and those of the

TABLE I

Geometric Mean Peak Agglutinin Titers of Neonatal and 1 kg Recipients Injected with Donor Leucocytes 2, 3, or 4 Days before Cell Transfer

Interval between pre-injection and cell transfer	Recipients							
	Neonates				1 kg			
	Pre-injected		Control		Pre-injected		Control	
	No.	Titer	No.	Titer	No.	Titer	No.	Titer
days		log_2		log_2		log_2		log_2
2	15	5.4	9	8.3	15	7.6	9	8.7
3	16	5.7	14	9.0	5	5.6	4	9.5
4	14	4.4	7	9.0	7	4.6	6	7.8

control recipients in these experiments are given as isolated symbols in the right upper corner of the figure. Each point in the graphs represents the geometric mean of the peak titers of 3 to 9 recipients of lymph node cells incubated with the indicated volume of anti-leucocyte serum. Because of the limited quantities of serum which could be obtained from the neonatal rabbits it was not always possible to test them over the full range of interest.

Anti-leucocyte sera of 1 kg rabbits injected with 50×10^6 leucocytes caused suppression of transferred lymph node cells, the degree of suppression in relation to the amount of serum being similar to that previously found for first injection sera (3). Sera of 1 kg rabbits injected with 250×10^6 leucocytes were no more effective. Among the sera obtained in neonatal rabbits there was, again, no difference among pools from rabbits injected with 50×10^6, or 250×10^6 leucocytes, or even, in one case, 500×10^6 cells. On comparing the group of antisera obtained in the neonatal rabbits with those of the 1 kg animals it can be seen that the sera of the neonatal rabbits were far less effective. None of the serum pools obtained in the neonatal rabbits caused reduction of the geometric mean maximal titers of the recipients to a level as low as 64,

even when used in volumes of 10 ml, whereas in the case of anti-leucocyte serum pools from older rabbits, almost all of these caused reduction of the mean anti-*Shigella* titer to this level or below at a 1 ml volume.

Sera were obtained from young rabbits injected with leucocytes at 4 or 6

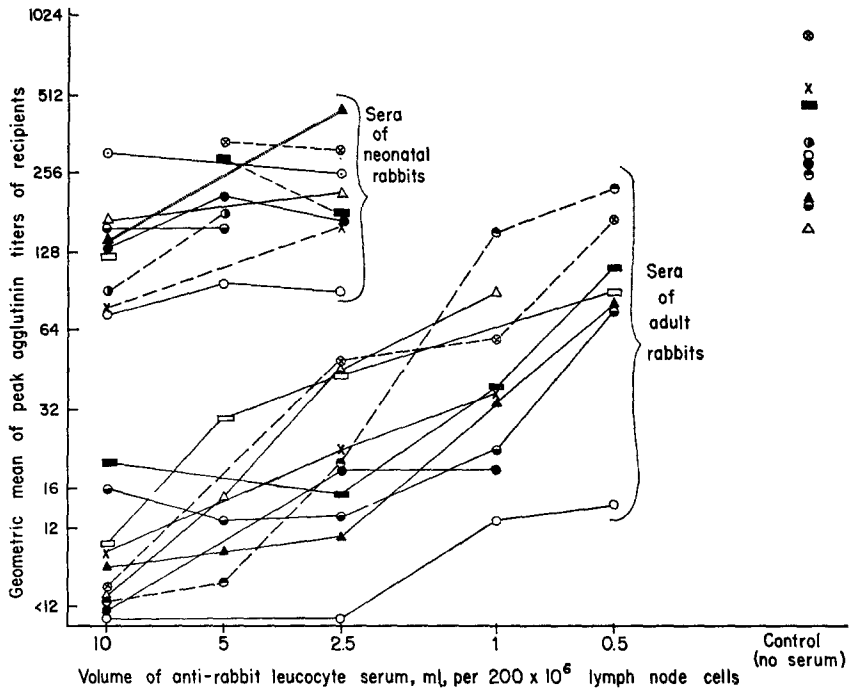

FIG. 2. Suppressive effect on transferred lymph node cells of sera obtained from neonatal and adult rabbits injected with pooled rabbit leucocytes. Solid lines, sera obtained following injection of 50×10^6 leucocytes; dashed lines, 250×10^6; or broken line, 500×10^6. Symbols represent different serum pools, each symbol being used for the serum pool of the neonatal and the adult rabbits injected with the same suspension of pooled leucocytes. Isolated symbols in the upper right corner represent the geometric mean maximal titers of control recipients, those given lymph node cells not incubated with either anti-leucocyte serum.

weeks of age, again in comparison with 1 kg or adult rabbits. These sera were tested in the manner described above, and the results are shown in Fig. 3. It can be seen that in the case of anti-leucocyte serum pools obtained in 4-week-old rabbits, one of two such pools showed suppressive activity, in the range found in older rabbits, and in the case of 6-week-old rabbits both of the anti-leucocyte pools did so.

The possibility was considered that the ineffectiveness of neonatal sera in suppressing the transferred cells was due not to decreased production of

anti-leucocyte antibody in the neonatal rabbit, but to a difference in the catabolism or utilization of this antibody. For this reason, in a number of experiments antigen-incubated lymph node cells were transferred in the usual number to both neonatal and 1 kg irradiated recipients and rabbit anti-leucocyte serum produced in adult rabbits was then injected intraperitoneally in

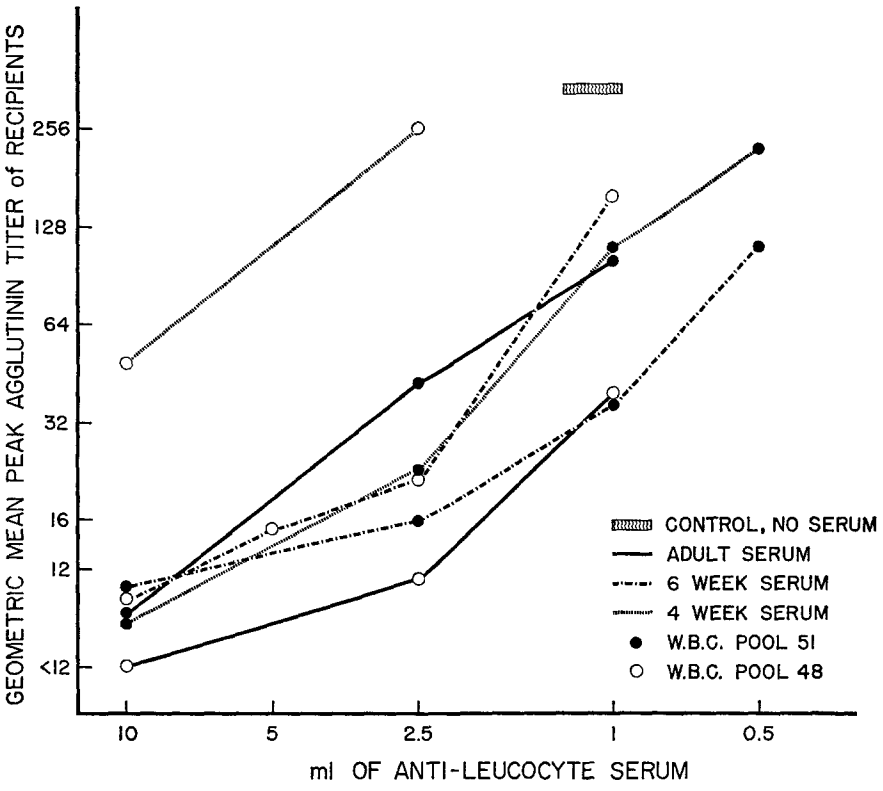

FIG. 3. Suppressive effect on transferred lymph node cells of sera obtained from rabbits injected with leucocytes at 4 and 6 weeks of age and adult rabbits so injected.

various volumes to both groups of recipients. It was found that over a given range of anti-leucocyte serum per kilogram of weight of recipient animal, the geometric mean peak titers of the two groups of recipient rabbits were not markedly different, as can be seen in Fig. 4.

4. Adoptive Transfer of the Lymph Node Cell Suppressive Effect.—

Leucocytes were obtained from the blood of prospective donors of lymph node cells and injected into neonatal and 1 kg rabbits. Each rabbit received 10×10^6 leucocytes in each hind foot-pad and 10×10^6 leucocytes intravenously. After 4 days both groups of leucocyte-injected rabbits were sacrificed. Cell suspensions were prepared from the respective sets of

popliteal lymph nodes and spleens, washed, and transferred to irradiated 1 kg recipient rabbits, the number of cells transferred being between 100 and 200 $\times$ 10^6. Within 2 hours the recipients were also injected with 200 $\times$ 10^6 antigen-incubated lymph node cells obtained from the donors of the leucocytes. In each experiment some recipients were injected only with antigen-incubated lymph node cells. The recipients were bled at regular intervals and the agglutinin titer to *Shigella* was determined in the sera.

The control rabbits, which received only antigen-incubated lymph node cells, developed agglutinins in the usual pattern. The recipients which had also received cells from popliteal lymph nodes or spleens of the leucocyte-injected

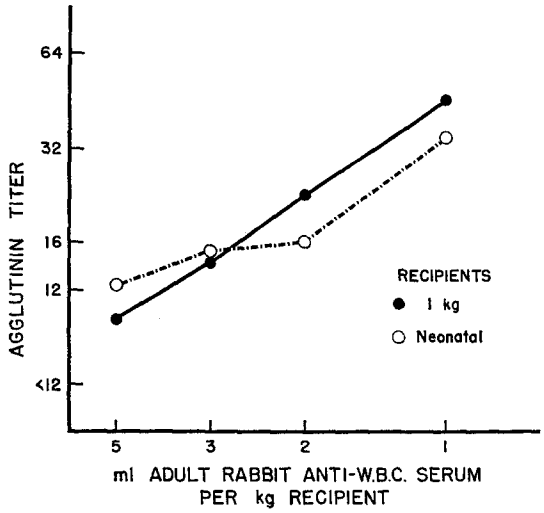

FIG. 4. Passive transfer of suppressive effect on lymph node cells by anti-rabbit-leucocyte serum produced in adult rabbits and injected into 1 kg and neonatal recipients.

rabbits developed agglutinins over a wide range of titer, from below the threshold of measurement to moderately high titers. The peak agglutinin titer of each recipient rabbit is shown in Fig. 5. Among recipients of popliteal lymph node cells those given cells of neonatal rabbits showed a scatter of maximal titers from below the threshold of measurement to 128, with a geometric mean for the group of $\log_2$ 5.1, and those given cells from 1 kg rabbits also showed a scatter of titers, but in a somewhat higher range, the geometric mean of the peak titers being $\log_2$ 5.9. The recipients of the splenic cells showed very little reduction from that of the control levels, in the case of neonatal or older rabbits. Because of the lack of effect of the splenic cells, a number of neonatal rabbits were injected intravenously with a larger number of leucocytes, 50 $\times$ 10^6, and their splenic cells were used for adoptive transfer of the suppressive effect. The maximal titers of this group of recipients, shown in the fifth column of Fig. 5,

were, in fact, in a lower range than the recipients of splenic cells from rabbits given 10×10^6 leucocytes intravenously.

As indicated above, these recipients were given separate injections of the two preparations of lymph node cells, those of leucocyte-injected rabbits and those incubated with *Shigella* antigen. In a number of experiments the two sets

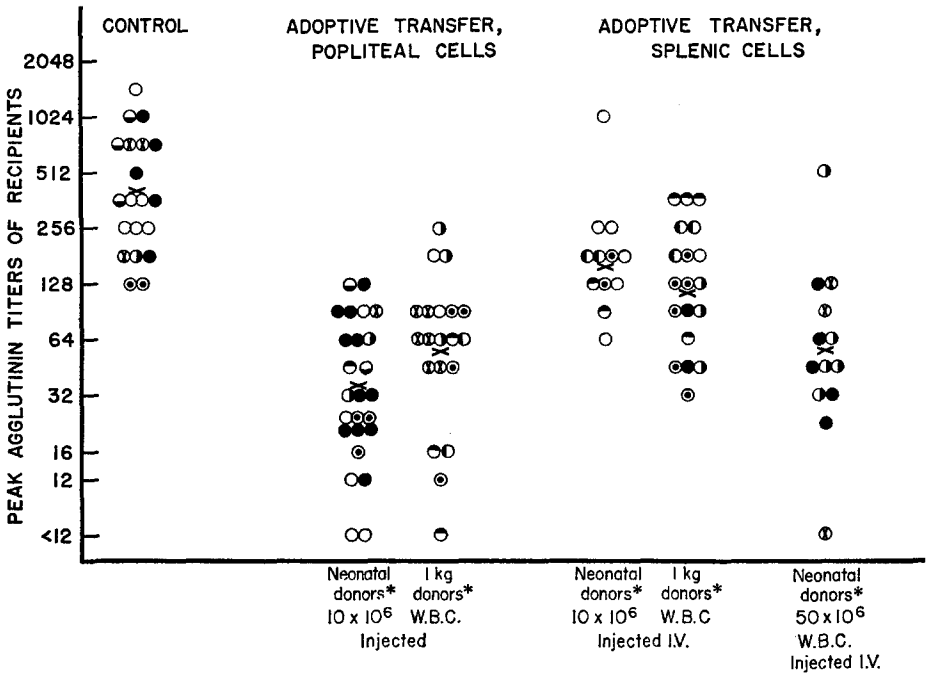

FIG. 5. Peak titers of individual recipients of lymph node cells from draining lymph nodes or spleens of leucocyte-injected neonatal or 1 kg rabbits. The transfer of these anti-leucocyte lymph node cells was followed by transfer of *Shigella*-antigen–incubated lymph node cells. For each group of recipients the geometric mean of the peak titers is indicated by an ⋈. The different symbols indicate different experiments.

* Donors referred to are donors of anti-leucocyte lymph node cells.

of lymph node cells were prepared, incubated together and transferred into the recipient animals, by analogy with experiments recently reported by Winn (12). In studies of the adoptive transfer of anti-tumor immunity by cells of regional lymph nodes in mice, Winn had found much greater anti-tumor activity when lymph node cells were injected together with tumor tissue than when they were injected separately in different sites. In the present experiments, no increase in the suppressive effect of the anti-leucocyte lymph node cells was found to result from incubation with the *Shigella*-antigen–incubated cells prior to transfer.

DISCUSSION

Active Induction of Lymph Node Cell Suppression.—In the 1 kg rabbits the extent of the immunologic reaction to the injection of leucocytes in various doses was approximately the same as was found previously in such animals (2): Essentially complete suppression of transferred lymph node cells following the injection of 10^7 leucocytes, a smaller but still marked degree of suppression induced by the injection of 10^6 cells (reduction of mean maximal agglutinin titer to about 10 to 15 per cent of the controi level), and a smaller degree of suppression following the injection of 10^5 cells (reduction of mean titer to about 40 per cent of the control level). In the direct comparison between the induction of this homograft reaction in 1 kg rabbits and in neonatal rabbits as prospective recipients of lymph node cells, the neonatal animals gave evidence of essentially the same degree of immunologic reaction, at each of the immunizing doses of leucocytes used.

It should be pointed out that within each of the groups of rabbits a relation was shown between number of cells injected and the degree of immunologic response. Since it is likely that following the injection of a given number of cells into neonatal and larger rabbits the concentration of solubilized transplantation antigens would be higher in the smaller volume of tissue fluids of the neonatal animals, it may be that the immunologic response of the older rabbits is actually somewhat greater, in relation to existing concentration of available antigenic material, but that the difference was offset by the higher concentration of solubilized antigen in the tissue fluids of the neonatal rabbit.

Adoptive Transfer of Lymph Node Cell Suppression.—The experiments with adoptive transfer gave further evidence that the immunologic reaction to rabbit leucocyte antigens was approximately of the same degree in neonatal as in older rabbits. The degree of adoptive suppression conferred by 250 million popliteal lymph node cells of 1 kg rabbits given foot-pad injections of 10^7 leucocytes, was quite similar to that observed in a previous study (3). In that study it had been found that the injection of 5 times that number of leucocytes yielded no greater a degree of adoptive suppression by 250 million lymph node cells, so that the antigenic stimulus due to the injection of 10^7 leucocytes was a maximal one in these experimental conditions. Therefore, the fact that popliteal lymph node cells of neonatal rabbits injected with 10^7 leucocytes were as effective in conferring suppression of transferred cells as similar cells from 1 kg rabbits could not be due to submaximal antigenic stimulation of the cells of the older rabbits by the transplantation antigens of the donor rabbits, but, rather, indicates that a maximal degree of stimulation with this antigen produced as great a degree of immunologic response in cells of neonates as of older rabbits.

The degree of immunologic response of lymph node cells of neonatal rabbits to rabbit leucocyte antigens is of interest in contrast with recent observations

on neonatal rabbits injected with *Shigella paradysenteriae*. In that study (13), it was found that serum of very young rabbits injected with *Shigella* contained no detectable agglutinin for *Shigella* or very low concentrations of this antibody, and that cells from the regional lymph nodes of such rabbits, when transferred to recipient rabbits, did not lead to the appearance of agglutinins in the sera of the latter. In the present study, sera of leucocyte-injected neonatal rabbits were found to have very little suppressive antibody, but lymph node cells of such rabbits were as effective as those of adult rabbits in the adoptive transfer of this suppressive effect to recipient rabbits. However, no paradox is necessarily implied by the apparent difference in effectiveness of the lymph node cells of neonates in their immunologic response to the two antigens, or, within each system, by the difference in effectiveness of lymph node cells relative to the concentration of antibody detectable in sera of actively immunized neonatal rabbits. First, it is not possible to compare the sensitivity of the measurements of serum antibody in the two systems. (The detectable level of agglutinin to *Shigella*, in the present method of testing, can be approximated, but we have at present no means of determining the amount of transplantation antibody necessary to suppress antibody formation by 2×10^8 lymph node cells). Second, the conditions of estimating the effectiveness of the transferred cells differ in the two systems, since the anti-*Shigella* agglutinins are measured *in vitro*, whereas the degree of suppression of lymph node cells is determined *in vivo*, the target cells being in the blood or tissues of the recipient animal in which the anti-leucocyte antibody is being released by the transferred lymph node cells. Finally, if, as indicated at the end of this Discussion, antibody of higher affinity for the transplantation antigen may be produced in the leucocyte-injected neonatal rabbit than that produced in the adult, the possibility should be considered that among antibodies produced in the neonatal animal the transplantation antibody may be of higher affinity than the bacterial agglutinin, so that the concentration of free transplantation antibody in the serum would not be as great, in relation to the total amount formed, as in the case of the agglutinin.

Suppressive Antibody in Sera of Leucocyte-Injected Rabbits.—The level of suppressive antibody in the serum of adult rabbits injected with 50 million leucocytes was in the same range as that previously found (14). The similarity between the degree of suppression in sera obtained after injection of 50 million leucocytes and that found in the present study after the injection of 250 million leucocytes indicates that maximal antigenic stimulation was produced by the 50 million cell dose. The sera of neonatal rabbits injected with leucocytes in either of these amounts, and in the case of one pool with 500 million leucocytes, were found to contain far less suppressive antibody than the sera of the 1 kg rabbits, since the sera of the neonatal rabbits even in the largest amounts used, 10 ml, did not lead to the production of as low a range of anti-*Shigella* titers by

transferred lymph node cells as did 1 ml of the anti-leucocyte sera of adult rabbits. Because of the possibility that the failure of appearance of suppressive antibodies in the sera of the neonatal animals might be due to the effect of injection of excessive amounts of antigen, a series of experiments were carried out in which the dose of leucocytes was reduced to one-fifth (10^7 cells) for both neonatal and 1 kg rabbits. In the older rabbits it was found that the suppressive effect of the resulting antisera was lower than that obtained by the injection of 50 million leucocytes, (3 or 4 such pools showing only partial suppression even at the largest amount of serum used, 10 ml), and in the neonatal rabbits, no evidence of suppressive antibody was obtained.

This failure of production of suppressive antibodies, or production in far lower concentration, was observed in young rabbits as far as the 17th day of life. In young rabbits 4 weeks of age, however, some production of suppressive antibody was indicated. It would have been desirable to obtain serum from young rabbits injected a second time with leucocytes, but this was not feasible. At the time the young rabbits would be ready for the second injection they would have been approaching the age (4 weeks) at which it had been found that a first injection of leucocytes could give rise to the production of anti-leucocyte antibodies.

The Actively Induced Rejection of Transferred Lymph Node Cells in the Neonatal Rabbit, in the Light of the Low Concentration of Suppressive Antibody in Their Sera.—Because of the earlier demonstration of a role of antibody in the suppression of transferred lymph node cells (3), and of the evidence of far lower concentration of such antibody in the sera of neonatals than of older rabbits injected with rabbit leucocytes, a problem of considerable interest was raised by the equal effectiveness of the active induction of lymph node cell suppression in neonatal and older rabbits following the injection of leucocytes, and the equal effectiveness of appropriate lymph node cells of rabbits of these two age groups in adoptively conferring this suppressive effect.

Since the extent of the actively induced suppression had been tested at 7 to 9 days after the injection of the leucocytes, when an approximately maximal suppressive effect had been reached, the question arose of whether even with a lower concentration of suppressive antibody in neonatal rabbits the actively induced suppression could be attributed to such antibody, *i.e.* whether the extent of the suppressive effect in the neonatal animals was due to the uptake, even from a lower concentration of antibody in the serum, of a total amount of antibody sufficient for complete suppression by cells able to combine with the antibody as it was being released. This question was approached by reducing the interval of time between the immunizing injection of leucocytes and the transfer of lymph node cells to that of the earliest detectable effect. However, there was no less suppression of transferred lymph node cells in the neonatal than the 1 kg rabbit during this period, as was seen in Table I, even with the shorter interval and the partial degree of this effect. An alternative approach

for explaining the degree of actively induced suppression in terms of an effect of antibody would be to postulate higher affinity of the anti-leucocyte antibody produced in the neonate than in the older rabbit, so that considerably less free antibody would be found in the serum in relation to the concentration of bound antibody. However, because the transplantation antigen is not as yet available in isolated, or even in soluble form, it is not feasible to approach this question at the present time.

SUMMARY

The immunologic response of neonatal and of older rabbits to the tissue (transplantation) antigens of pooled rabbit leucocytes was studied, the test system being the suppression of formation of agglutinin to *Shigella paradysenteriae* by transferred lymph node cells which had been incubated with *Shigella* antigen.

In the active induction of the suppressive effect on the transferred cells it was found that neonatal rabbits reacted as vigorously as 1 kg rabbits to the prior injection of a given number of rabbit leucocytes pooled from prospective donors of lymph node cells. The suppressive effect was dose-related, and within the range of number of leucocytes used was similar for both age groups of recipients. An attempt to detect a difference in the response during the first few days after leucocyte injection, before the full suppressive effect is reached, failed to show any difference between rabbits of the two age groups.

Since it had been found possible to transfer the suppressive effect passively with sera obtained from older rabbits injected with rabbit leucocytes, attempts were made to do so with sera obtained from neonatal rabbits injected with similar numbers of pooled adult rabbit leucocytes. No consistent suppression of transferred lymph node cells was observed with sera from neonatal rabbits, even with relatively large amounts of such serum. In sera of rabbits which had been injected with rabbit leucocytes at the age of 4 to 6 weeks, suppressive antibody could be detected. When anti-rabbit-leucocyte serum obtained in adult rabbits was injected into neonatal and 1 kg recipients at a given volume per gram of animal weight the suppressive effect of the serum was of similar extent in the two groups of recipients.

In the adoptive transfer of the lymph node cell suppressive effect, by cells of lymph nodes draining the sites of injection of pooled rabbit leucocytes, it was found that the popliteal lymph node cells of neonatal rabbits were as effective as those of 1 kg rabbits. Splenic cells of neonatal rabbits were also effective, when an adequate number of rabbit leucocytes had been injected intravenously.

Thus, in conferring adoptive immunologic response, as in active immunization, the neonatal rabbits were as effective as the older rabbits in their response to homotransplantation antigens, in contrast to the considerable difference in

concentration of the suppressive antibody in sera of neonatal and older rabbits injected with rabbit leucocytes.

We are grateful to the Radiology Departments of the Graduate Hospital of the University of Pennsylvania and the Children's Hospital of Philadelphia for their generosity in carrying out the irradiation of recipient rabbits in this study.

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