STUDIES ON THE TRANSFER OF LYMPH NODE CELLS

XI. Effect on the Anti-Shigella Agglutinin Titers of Recipient Rabbits of the Prior Injection of Leucocytes from the Donor Animals*

By T. N. HARRIS, M.D., SUSANNA HARRIS, Ph.D., and MIRIAM B. FARBER

(From the Children's Hospital of Philadelphia (Department of Pediatrics, University of Pennsylvania), and the Division of Immunology, Department of Public Health and Preventive Medicine, Graduate School of Arts and Sciences, University of Pennsylvania, Philadelphia)

(Received for publication, February 17, 1958)

The present series of studies has involved the transfer to a homologous recipient of lymph node cells from rabbits injected with *Shigella paradysenteriae* or of lymph node cells incubated *in vitro* with soluble antigenic material derived from this organism. Following the transfer of such cells, agglutinins to *Shigella* have been found in the sera of the recipient rabbits (1, 2), and there has been evidence that the transferred cells have some physiological role in the production of this antibody. There are thus aspects of the lymph node cell transfer system which are parallel to other areas of tissue transplantation, a field in which there has recently been a considerable body of experimental work, especially in the transplantation of skin (3). It seemed likely that the application of methods in the studies of tissue transplantation to the transfer of lymph node cells might shed some light on the mechanisms involved in lymph node cell transfer or on factors involved in transplantation immunity. In early experiments of this kind it was observed that if the prospective recipients of lymph node cells were injected a week before the cell transfer with blood leucocytes of the prospective donors, agglutinins did not appear in the sera of the recipients in the usual manner, a finding which suggested an effect on the transferred cells of a specific alteration in their new, host-tissue environment as a result of the pre-injection of the recipient (4). Further studies in this area are reported below.

Materials and Methods

Pre-Injection of Donor Leucocytes.—Blood from prospective donors was obtained by cardiac puncture and collected with heparin (3 mg. per 50 ml. of blood). To this volume of blood was

* This study was supported by Research Grant H-869 of the National Heart Institute, National Institutes of Health, United States Public Health Service.

added an equal volume of a 3 per cent solution of gelatin in 0.75 per cent NaCl.[1] The tubes were maintained in the vertical position in an ice bath. After 1 hour the sedimentation of erythrocytes was such that the overlying columns of fluid could be collected by suction. The leucocytes were sedimented from the pooled supernates by centrifugation at 2000 R.P.M. for 10 minutes, and were then washed with Tyrode's solution. Various amounts of the suspensions of leucocytes were injected intradermally in a number of sites on the backs of the prospective recipients.

Cell Transfer.—Lymph node cells were obtained from the popliteal and axillary lymph nodes of donor rabbits, in some cases from those donors whose blood leucocytes had been used for pre-injection and in other cases from unrelated donors, in accordance with the protocol of the experiment. The cells were washed in Tyrode's solution containing 12.5 per cent gelatin. Approximately 225×10^6 cells were incubated with 1 ml. of a 10^{-4} dilution of filtrate of trypsin-treated *Shigella paradysenteriae* (ST) at 37°C. for 30 minutes. The cells were then washed 3 times in Tyrode gelatin solution and finally suspended in Tyrode's solution containing 0.5 per cent of bovine plasma albumin (Armour & Co., Chicago)

Serologic Tests.—Serial 2-fold dilutions of rabbit serum were made in volumes of 0.4 ml. To these were added 0.2 ml. of a 0.06 per cent suspension of alcohol-treated *Shigella paradysenteriae.* After shaking and 1 hour of incubation at 37°C., the tubes were stored at 4°C. for 48 hours, and then examined for evidence of agglutination, as described in detail previously (5).

Irradiation of Recipient Rabbits.—The animals were exposed, on the day before cell transfer, to 425 roentgens 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.

EXPERIMENTAL

1. Variations in Interval between the Pre-Injection of Donor Leucocytes and Lymph Node Cell Transfer.—Leucocytes were obtained from the blood of a group of prospective donors and injected intradermally on the backs of prospective recipients at various intervals prior to lymph node cell transfer. On the appropriate day the same donors were sacrificed, and the popliteal and axillary lymph nodes excised. Cells obtained from the nodes were incubated *in vitro* with ST (*Shigella*-trypsin) filtrate, washed, and then transferred to the pre-injected recipients, which had been irradiated 24 hours earlier. Sera of the recipients obtained at regular intervals after transfer were tested for the presence of agglutinins to *Shigella paradysenteriae.* In the sera of control (non-pre-injected) recipients, agglutinins appeared on the 4th day after transfer and generally reached a maximal titer by the 6th to 8th day. In the sera of recipients pre-injected 1 day prior to transfer, agglutinins appeared at the same time and followed the same pattern as in the controls. Among the recipients pre-injected 2 days before transfer there were many which developed agglutinins to *Shigella* in lower titer than the controls. The geometric mean peak titer of this group of recipients was markedly lower than the mean of the controls or the group of 1 day pre-injected animals. With increasing time between the pre-injection of donor leucocytes and lymph node cell trans-

[1] We are indebted to Kind and Knox Gelatine Co., Camden, New Jersey, and to Dr. D. Tourtellotte, for generous supplies of the gelatin solution.

fer, lower geometric mean peak titers of the recipients' sera were found, and with an interval of 6 to 9 days the recipients' titers were at the limit of detectable agglutinin level in the present system of measurement or below it. The relation found between the mean peak agglutinin titers and the pre-injection interval is shown in Fig. 1 by the line connecting the solid circles. The data obtained in such experiments are summarized in Table I, which includes also data obtained at pre-injection intervals up to 107 days. The maximum serum titer for each recipient was tabulated, and the titers were grouped

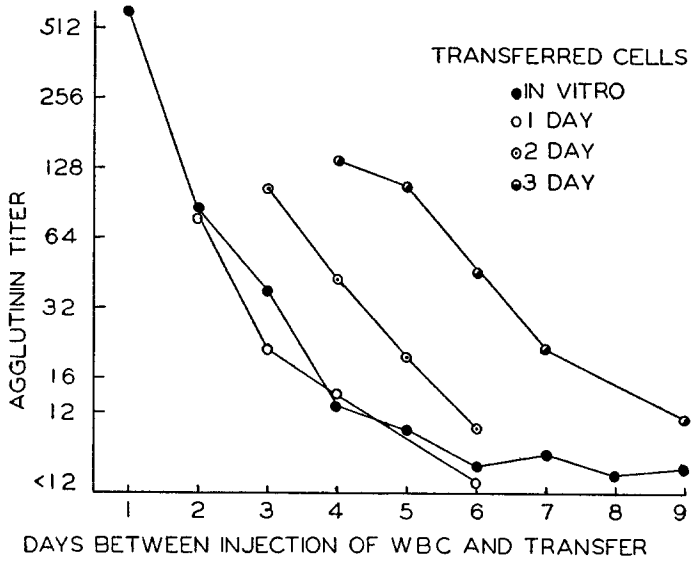

FIG. 1. Geometric mean peak agglutinin titers of recipient rabbits pre-injected with leucocytes of other (donor) rabbits at various intervals prior to lymph node cell transfer. The lymph node cells had been incubated *in vitro* with *Shigella*-trypsin filtrate or had been obtained from donor rabbits injected with whole *Shigella*, 1, 2, or 3 days before the cell transfer.

at 3 levels: 128 or higher, *i.e.* titers which approached or were similar to the controls, 24 to 96, titers which indicated partial reduction in comparison with the control titers, and <12 to 16, which reflected almost complete suppression of agglutinin formation. The percentage frequency of titers within each of these three ranges, as well as the geometric mean, are shown for the recipients pre-injected at each of the intervals employed. It can be seen that at a 6 day pre-injection interval all the pre-injected recipients failed to develop serum agglutinins. The distribution was quite similar for greater intervals, up to 20 days. With larger intervals the percentage of titers showing complete suppression decreased, and the percentage showing partial suppression increased, as did the geometric mean titers. The longest interval tested was 107 days,

at which time there was still evidence of partial reduction of titers, with a mean titer of 3 powers of 2 below that of the control group.

These data were obtained with the pre-injection of approximately 10×10^6 donor leucocytes. It was found that the relation between the pre-injection interval and the effectiveness of the pre-injection shown in Fig. 1 was altered when larger numbers of leucocytes were injected. Thus, in some experiments 250 to 500×10^6 leucocytes were pre-injected and a marked reduction in serum titer was observed when the interval between pre-injection and transfer was only 1 day.

TABLE I

Effect of Pre-Injection of Donor Leucocytes on Agglutinin Titers of Recipients of Lymph Node Cells Incubated in Vitro with ST Filtrate

Interval between pre-injection of donor leucocytes and cell transfer	No. of recipients	Agglutinin titers of recipients			Geometric mean titer of group
		Percentage occurrence in the range of			
		128 or higher	24 to 96	<12 to 16	
days					*log₂*
(No pre-injection)	69	81	19		8.6
1	6	100			9.3
2	14	57	7	36	6.4
3	6	17	50	33	5.3
4	8		50	50	3.6
5	12		25	75	3.3
6	6			100	2.8
7	26		11	89	2.9
8	15			100	2.6
9	12		8	92	2.7
20	3			100	2.5
35	5		25	75	3.7
65	5		40	60	4.5
75	4		75	25	4.7
90	4		75	25	4.8
107	8		100		5.1

The foregoing experiments all involved the transfer of lymph node cells incubated *in vitro* with ST filtrate. It was of interest to determine whether pre-injection of donor leucocytes would affect subsequent serum titers of recipients of popliteal lymph node cells from antigen-injected donors. Accordingly, recipient rabbits were pre-injected intradermally with donor leucocytes at various intervals prior to cell transfer. On the day of transfer cells were obtained from lymph nodes regional to injections of *Shigella*, which had been made 1, 2, or 3 days earlier. These cells were transferred to the pre-injected recipient rabbits. The peak agglutinin titers of these recipients were tabulated according to the donor interval (the interval between injection of antigen into the donor and collection of its lymph node cells) and the pre-

injection interval (the interval between pre-injection of donor leucocytes and cell transfer), and the geometric mean peak titer of each group was calculated. For each interval the mean titers were plotted against the pre-injection intervals, and it can be seen in Fig. 1 that in each case the pre-injection of donor leucocytes at appropriate times affected the peak titers of recipients. In the case of lymph node cells of a 1 day donor interval the curve of mean agglutinin titer *versus* pre-injection interval is similar to that observed in the case of lymph node cells incubated *in vitro* with the antigen. In the case of cells obtained at a 2 day donor interval the curve is similar in shape to the others, but with a difference of 1 day in pre-injection interval corresponding to a

TABLE II

Injection of Donor Leucocytes into Recipient Rabbits Prior to Lymph Node Cell Transfer: Effects of Varying the Number of Leucocytes and the Route of Injection

No. of donor leucocytes pre-injected	Agglutinin titers of recipients			
	No. of rabbits	Geometric mean titer		
		log_2		
10^8	6	3.0		
10^7	26	2.8		
10^6	26	4.1		
10^5	10	6.3		
No pre-injection	40	8.8		
Route of pre-injection with donor leucocytes	**Mean titers (log_2) of groups of rabbits injected with various numbers of donor leucocytes**			
	10^8	10^7	10^6	10^5
Intradermal	2.5	2.6	3.8	6.5
Intravenous	3.5	2.6	4.4	5.8

given mean level of agglutinin. In the case of cells obtained at a 3 day donor interval the curve is again of similar shape, with a difference of 1 to 2 days from the curve obtained with cells at the 2 day donor interval.

2. Pre-Injection of Varying Numbers of Donor Leucocytes by Two Routes.—An interval between pre-injection of donor leucocytes and transfer of *in vitro*-incubated lymph node cells was chosen which could be expected to cause essentially complete suppression of agglutinin production in the recipient, *i.e.* 6 or 7 days, and the effect of varying the number and route of injection of donor leucocytes was studied. It was found that the agglutinin titers of the recipients were a function of the number of donor leucocytes pre-injected. In the upper part of Table II are given the mean agglutinin titers of groups of recipients pre-injected with various numbers of donor leucocytes. It can be seen that with 10^8 and 10^7 leucocytes the recipient mean titers are similar to

each other and similar to the levels shown in Fig. 1. The mean titer was higher when 10^6 leucocytes were injected, and still higher following the pre-injection of 10^5 leucocytes. Even at the last level there was still a difference of 2.5 powers of 2 between the mean titers of pre-injected recipients and those of the control recipients, which were not pre-injected.

In comparing the effectiveness of the intradermal and intravenous routes of injecting donor leucocytes, no consistent difference could be found over the range of 10^5 to 10^8 leucocytes, as can be seen in the lower half of Table II. After pre-injection by the intradermal route the mean titers were somewhat lower than after intravenous injection at doses of 10^8 and 10^6 leucocytes, equal at 10^7, and somewhat higher at 10^5.

3. Pre-Injection of Cells of Various Sources.—In a number of experiments leucocytes obtained from sources other than the blood of rabbits were pre-injected into recipients 6 to 7 days prior to transfer. Following the transfer of *in vitro* incubated lymph node cells the geometric mean peak titer was calculated for recipients pre-injected with lymph node cells, leucocytes of peritoneal exudates, thymus cells, or blood leucocytes of rabbits. It was found that after pre-injection with cells from any of these sources the titers were reduced to approximately the same degree, as is shown in the upper part of Table III.

Leucocytes were obtained from the blood of chicken, cow, horse, and man. Approximately 10^7 such leucocytes were injected intradermally into recipients 6 to 7 days before transfer of *in vitro*-incubated lymph node cells. The geometric mean peak titer was calculated for each group and compared with that of the control group. As can be seen in the lower part of Table III, mean titers of recipients pre-injected with leucocytes of chicken, cow, and horse were fairly similar to that of the controls. In the case of recipients pre-injected with human leucocytes the mean titer was lower than that of the control group, but higher than the groups pre-injected with rabbit leucocytes shown in the upper half of the table.

In some of these experiments erythrocytes were obtained from rabbit blood with as little contamination as possible by leucocytes (1:1000) and used in equal numbers for pre-injection of rabbits. On subsequent cell transfer it was found that the mean peak titer of recipients pre-injected with rabbit erythrocytes was quite similar to that of the non-pre-injected controls, as shown in the lower part of Table III.

4. Pre-Injection of Leucocytes from Individual Rabbits, Followed by Transfer of Lymph Node Cells from the Same or Other Rabbits.—In the experiments described thus far, pooled leucocytes of 6 to 8 donor rabbits were used for pre-injection, and pooled lymph node cells obtained from the same group of donors were used for *in vitro* incubation with antigen and transfer. Subsequently, it was found that pre-injection of pooled leucocytes of one group of donors fol-

lowed by transfer of incubated lymph node cells pooled from another set of donors yielded the same results, *i.e.* failure of agglutinins to appear after transfer. This result was obtained in all such experiments performed. It was of interest to obtain some data on the frequency with which the pre-injection of leucocytes of any single rabbit would affect the agglutinin titer of a recipient of antigen-incubated lymph node cells of another single rabbit. In such experiments each pair of recipients was injected with leucocytes from 1 rabbit and

TABLE III

Geometric Mean Peak Titers of Groups of Recipient Rabbits Pre-Injected with Cells of Various Tissues of Rabbits, and Leucocytes of Other Species

Experiments involving pre-injection of cells obtained from rabbits.

	No. of experiments	No. of recipients	Geometric mean titer
			$\log_2$
Blood leucocytes	6	15	3.2
Lymph node cells	4	10	3.0
Peritoneal exudate cells	2	10	3.3
Thymus cells	2	7	3.6
No pre-injection (controls in above experiments)	6	12	8.3

Experiments involving pre-injection of cells obtained from other species, and rabbit erythrocytes.

Leucocytes of	No. of experiments	No. of recipients	Geometric mean titer
			$\log_2$
Chicken	2	5	7.2
Cow	2	5	7.3
Horse	2	7	7.5
Man	3	10	5.3
Erythrocytes of rabbit	5	16	7.4
No pre-injection (controls in above experiments)	5	13	7.6

antigen-incubated lymph node cells from another. Two pairs of recipients received both leucocytes and lymph node cells pooled from all the donors of the experiment, and in two other instances 1 of the donors of leucocytes for pre-injection also served as a source of lymph node cells. The individual peak recipient titers obtained in a number of experiments are shown in Table IV. As can be seen in this table, the 10 pairs of recipients of blood leucocytes and lymph node cells from different, single, rabbits included seven instances in which the agglutinin titers of both recipients were below the threshold of measurement or were low; in two cases the titer of 1 recipient was low but that of the 2nd recipient was similar to or higher than the controls; and in 1

pair, both recipients developed titers in the middle range, below that of the controls.

5. Pre-Injection of Recipients with Their Own Leucocytes or with Leucocytes of Other Rabbits.—The effect of pre-injection of a recipient's own leucocytes was studied in three experimental situations. In one of these, the recipient was bled and a leucocyte suspension was prepared and injected intradermally into the same recipient; in another, the same procedure was followed except that the leucocytes were injected intravenously, and in the third, whole blood was collected from the recipient and re-injected intravenously. In each case

TABLE IV

Maximum Agglutinin Titers of Recipients Pre-Injected with Leucocytes of Individual Donors and Given Incubated Lymph Node Cells from Other Donor Rabbits

Donors of		Peak agglutinin titers of individual recipients		
Leucocytes for pre-injection	Lymph node cells for transfer	Pre-injected		Not pre-injected
A	E	<12	<12	
B	F	<12	<12	192
C	G	32	1024	192
D	H	24	24	
Pooled A–D	Pooled E–H	<12	<12	
I	M	<12	32	
J	N	16	24	1536
K	O	64	192	1536
L	P	<12	12	
Pooled I–L	Pooled M–P	<12	<12	
Q	Q	<12		384
Q	R	<12	384	768
S	S	16		1024
S	T	<12	<12	

recipients other than the one bled were also injected with the leucocytes. One week later all of the recipients were given antigen-incubated lymph node cells pooled from a group of donor rabbits. The peak agglutinin titer for each recipient was determined, and for each experimental group the percentage frequency of these titers was calculated for the higher, middle, and lower range. These results are given in Table V. Within both the intradermally and intravenously injected groups the differences in findings between the recipients injected with their own leucocytes and those injected with leucocytes of other rabbits were marked. The percentage frequency of titers above 128 was high in the group of recipients injected with their own cells and low in the groups injected with other cells. Conversely, the percentage frequency of

titers of <12 to 16 was very low in the groups injected with their own cells and high in the groups injected with other rabbits' cells. In a comparison of rabbits pre-injected with their own cells and those not pre-injected at all, the percentage–frequency distribution of titers was found to be fairly similar, but with the following differences: first, the occurrence of titers of 128 or higher was somewhat lower in the groups pre-injected with their own leucocytes than in the controls, and second, of the rabbits pre-injected with their own leucocytes a small percentage failed to develop measurable agglutinins or did so in very low titer, whereas in the control group this did not occur.

TABLE V

Pre-Injection of Each Recipient with Its Own Leucocytes or Whole Blood, or with Cells of Another Rabbit

Groups of experiments	No. of recipients	Agglutinin titers of recipients		
		Percentage occurrence in the range of		
		128 or higher	24 to 96	< 12 to 16
Intradermal pre-injection of blood leucocytes				
(*a*) None	11	82	18	0
(*b*) Own cells	18	72	22	6
(*c*) Cells of other rabbits	17	24	35	41
Intravenous pre-injection of blood leucocytes				
(*a*) None	12	83	17	0
(*b*) Own cells	26	77	19	4
(*c*) Cells of other rabbits	15	20	27	53
Intravenous pre-injection of whole blood				
(*a*) None	11	82	18	0
(*b*) Own blood	21	81	19	0
(*c*) Blood of other rabbits	13	23	46	31

In the case of recipients injected intravenously with whole blood, the results obtained in the groups of recipients injected with their own blood were indistinguishable from those in control, non–pre-injected recipients. The findings in the group of recipients injected with blood of other recipients were fairly similar to those found in the case of leucocytes of other rabbits except for a shift between the occurrence of very low and moderately low titers, indicating somewhat less suppression of agglutinin formation in these recipients than in those pre-injected with suspensions of leucocytes, either intradermally or intravenously.

6. Effect of Various Treatments of Leucocytes Prior to Pre-Injection.—Leucocytes obtained from donor rabbits' blood were treated in a number of ways and then injected intradermally into recipients. The treatments included heating for 60 minutes at 60°C., alternate freezing in a dry ice alcohol bath at −70°C. and thawing, suspension of the cells in distilled water, addition

of sodium iodoacetate to 10^{-2} M, lyophilization, x-irradiation at 400 r, and oscillation in a magnetostriction oscillator (Raytheon Manufacturing Co., Boston) for 1 hour. One week after pre-injection of the recipients with treated leucocytes, antigen-incubated lymph node cells pooled from the same donors were transferred to these recipients. Within each group the geometric mean peak agglutinin titer was calculated, as well as the percentage frequency of titers within the ranges of 128 or higher, 24 to 96 and <12 to 16. The results are shown in Table VI. It was found that all treatments except oscillation and x-irradiation resulted in a loss of the capacity of the leucocytes to bring about the pre-injection effect. Within this group, the loss was more nearly complete

TABLE VI

Effect on the Agglutinin Titers of Recipient Rabbits of the Pre-Injection of Donor Leucocytes Treated in Various Ways

Treatment of donor leucocytes	No. of recipients	Agglutinin titers of recipients			Geometric mean titer of group
		Percentage occurrence in the range of:			
		128 or higher	24 to 96	< 12 to 16	
					$\log_2$
No pre-injection (controls)	22	77	23	0	7.2
Heating	10	80	20	0	7.3
Iodoacetate	10	70	30	0	7.0
Freezing	6	67	33	0	6.6
Distilled water	15	67	33	0	6.0
Lyophilization	10	70	30	0	6.5
Sonic oscillation					
Whole suspension	16	0	13	87	2.9
Sediment	5	0	20	80	3.3
Supernate	5	100	0	0	7.1
X-irradiation	6	0	17	83	2.8
Untreated	17	0	6	94	3.0

and consistent in the case of some treatments, heating or treatment with iodoacetic acid, than in the others, suspension in distilled water, lyophilization, and freezing and thawing. X-irradiation and oscillation of the leucocytes appeared to have no effect on the capacity of these cells to bring about the pre-injection effect. When oscillated suspensions were centrifuged and supernate and sediment injected separately the active material appeared to be in the sediment.

DISCUSSION

A. The Effect of Pre-Injection of Donor Leucocytes on Lymph Node Cell Transfer.—It is clear, from the data presented above, that the results of lymph node cell transfer can be substantially affected by the prior injection

of blood leucocytes from the donors of the lymph node cells, if the leucocytes are injected at a sufficient interval before the cell transfer and in sufficient numbers relative to this interval. Such an effect, as indicated by lower concentrations of agglutinins to *Shigella* in the sera of recipient animals, was demonstrated under various experimental conditions: in terms of the transferred lymph node cells, whether these had been incubated *in vitro* with *Shigella*-trypsin filtrate or whether they had been obtained from donors injected previously with *Shigella*; in terms of the pre-injected leucocytes, whether these were pooled from groups of rabbits which were to be used as donors of the transferred lymph node cells or pooled from groups of rabbits other than the lymph node cell donors, or, in some cases, even from individual rabbits,—either the prospective donors of the transferred lymph node cells or others.

That this "pre-injection effect" is immunologic in nature is suggested by several observations. First, the intervals between the pre-injection of leucocytes and the lymph node cell transfer which is required for the partial and the complete effect, respectively, are consistent with the time after injection of cellular antigens at which one could expect the appearance of significant and maximal titers of antibody. (To the 2 day and 6 day intervals indicated in Table I and Fig. 1 for the partial and complete effects, respectively, on *in vitro* incubated cells, should be added 3 days, the time after transfer of such cells that anti-*Shigella* antibody appears. It should also be noted that these intervals are decreased when larger numbers of donor leucocytes are used for the pre-injection.) Further evidence of an immunologic mechanism based on rabbit leucocyte antigens is offered by the species specificity of the reaction—that the pre-injection of leucocytes of several mammalian species other than the rabbit failed to affect the results of subsequent transfer of rabbit lymph node cells. Finally, an immunologic mechanism is suggested by the failure of pre-injection of the recipient's own leucocytes to affect substantially its subsequent agglutinin titer: in this case no new cell antigens of rabbit leucocytes would be introduced into the recipient's tissues.

The first experiments on this point indicated a clear difference between the effects of the recipient's own leucocytes pre-injected intradermally or intravenously and those of other rabbits (upper two-thirds of Table V). However, the data in the first two rows of these sections of the table indicated a slight difference between rabbits pre-injected with their own leucocytes and the non-pre-injected controls, in that the former group contained somewhat fewer titers in the highest range and 1 rabbit in the lowest range of titers. The question arose as to whether the manipulation of the blood in concentrating the leucocytes could have caused enough denaturation of leucocyte proteins to have rendered some of these slightly antigenic to the rabbit from which the blood was drawn. Accordingly, the experiments were repeated with leucocytes as they occur in whole bood, drawn and injected into the same or other rabbits without further manipu-

lation, in amounts containing the same number of leucocytes as hitherto. In these experiments (lowest part of Table V) the results were no different following pre-injection of the recipient's own blood or no pre-injection at all; however, the pre-injection of blood of other rabbits did cause a substantial difference in the distribution of the subsequent agglutinin titers of the recipient rabbits. This difference was fairly similar to that found in recipients pre-injected with prepared suspensions of leucocytes of other rabbits but with somewhat less reduction in titers. These data again support the conscluion that the effects of pre-injection of leucocytes reported here are due to differences in antigenic structure in leucocytes among members of the species, but indicate that a small part of the effect of leucocyte pre-injection on the lymph node cell transfer observed throughout this study could be due to changes in leucocyte protein *per se*.

Thus the effect of pre-injection of leucocytes on subsequent lymph node cell transfer would seem to rest on individual tissue specificity within members of the rabbit species. This interpretation would also be consistent with the finding that substantial pre-injection effects were obtained invariably in the case of leucocytes pooled from several rabbits, and sporadically in the case of leucocytes from individual rabbits.

The data obtained following the pre-injection of leucocytes from single rabbits is of interest from the point of view of the random distribution of the individual antigens involved in tissue specificity. The experiments shown in Table IV involved the pre-injection of leucocytes from 1 rabbit to 2 recipients, and the transfer to the latter of lymph node cells from another individual rabbit. A number of immunologic relationships could exist among these animals, which would determine the outcome of such experiments. If the donor of the pre-injected leucocytes and that of the lymph node cells have at least one common antigen which the recipient does not have, and to which it is therefore able to react immunologically, one could expect a substantial degree of "suppression" of the transferred cells (reduction in subsequent agglutinin titer of the recipient). On the other hand, if the 2 donor rabbits have no common antigen, or if they have common antigens but the recipient also has any antigen common to both donors, and is thus unable to react immunologically to it, one would expect no "suppression" of the cells, *i.e.*, no reduction in titers. Intermediate recipient agglutinin titers, indicating partial "suppression" of the transferred cells, would reflect degrees of effective antigenicity of the tissue factors, perhaps on the basis of smaller differences in configuration between antigenic groupings of the tissue factors involved. In a random population, such as the rabbits used here, it would therefore be possible to encounter any combination of results in experiments such as those shown in Table IV. The fact that of 20 recipients shown in that table as receiving the pre-injected and the transferred cells from 2 different rabbits 16 had markedly reduced titers would suggest that the total number of individual tissue antigens in leucocytes of rabbits is considerable, and that each animal carries several of these, so that the probability of a common antigen in the leucocytes of 2 rabbits chosen at random would be high, but that each animal has substantially less than half the total number, so that the probability that a randomly chosen recipient would not have the antigens common to the donors would again be high.

B. Implication of These Data as to the Function of the Transferred Cells.—The data obtained in this study, and the discussion above, suggest that in this experimental situation the pre-injection of homologous leucocytes has caused an alteration in the host tissue environment into which the lymph node cells are transferred, this alteration being specific with respect to the transferred cells. The fact that this alteration results in the failure of appearance of antibody, such as would be expected after the transfer of antigen-incubated lymph node cells, supports the thesis that a function of the transferred lymph node cells is involved in the production of the antibody normally found under these circumstances.

This thesis is further supported by the data obtained with the transfer of cells from antigen-injected donor rabbits.

In the early studies of this series lymph node cells were transferred from rabbits which had been injected with *Shigella*. It was found that if the lymph node cells were obtained 3 days after the injection of the donor animal with the antigen, antibody appeared in the recipient 1 day after cell transfer. If less time was allowed between injection and sacrifice of the donor, 2 days or 1 day, detectable levels of antibody appeared in the serum of the recipient at longer intervals after cell transfer, 2 and 3 days respectively. Thus it appeared that the transferred cells were involved in a process which required a given time—about 3 days—between contact of lymph node cell with antigen and appearance of detectable concentrations of antibody in the recipient's serum. Returning to the data of the present study, it can be seen in Fig. 1 that the transfer of 3 day cells (*i.e.* cells obtained from draining lymph nodes 3 days after the injection of *Shigella* into the donors) is not followed by the appearance of agglutinins in the recipients if the latter have been pre-injected 1 week earlier with leucocytes from the donor rabbits. Since the transfer of lymph node cells into a specifically adverse environment even within 1 day of the time antibody would appear in the recipient results in the failure of appearance of that antibody, it seems likely that the function of the transferred cells is not merely to contribute some factor which the tissues of the recipient can use to undertake the synthesis of the antibody, but rather to carry out at least a major part of the synthesis of the antibody themselves. It is also significant, in this regard, that the curves relating the effectiveness of pre-injected leucocytes to the interval between pre-injection and cell transfer, shown in Fig. 1, are quite uniform in the case of all four kinds of transferred lymph node cells—whether these are incubated *in vitro* with the antigen, or obtained 1, 2, or 3 days after injection of antigen into the donors—and that these curves are separated by time intervals approximately the same as the differences in the time of appearance of antibody after cell transfer, in the case of these four preparations of cells.

C. The Relationship of These Observations to Those Reported in Studies of Skin Grafting.—The difference in observed events subsequent to the transfer of lymph node cells according to whether the recipients are encountering cells from the donor for the first time or the second time (after pre-injection) is analogous to observations made in the area of skin homografting.

Medawar reported that a first skin graft of a given donor rabbit to a given recipient rabbit was rejected in approximately 10 days, but when a second skin graft from the same donor was placed on the same recipient the graft was rejected in 4 to 6 days (the "second set" phenomenon) (6, 7). Thus the difference in response to a second graft involved a difference of time, in comparison with the time of rejection of a first graft, whereas in the present system antibody fails to appear altogether. This difference between the two situations is in all probability due merely to the fact that there are differences in the respective experimental conditions. Thus, in the case of skin grafting the transplanted tissue must await vascularization for full contact with the blood of the recipients, whereas in lymph node cell transfer the transplanted cells are introduced directly into the blood by intravenous injection.

A number of the observations made in this study are analogous to the corresponding observations reported in studies of skin homografting. First, a definite minimum interval of time between leucocyte pre-injection and lymph node cell transfer was found necessary in this study for the suppressive effect on the transferred cells, and a certain minimum of time must elapse between first and second skin homografts in order to elicit the second set phenomenon (7). Second, the accelerated rejection of a second skin graft in mice was observed as long as 120 days after the first skin graft was placed (8), and in this study some suppressive effect on transferred lymph node cells was found as long as 107 days after the pre-injection of leucocytes. Third, in this study it was found that, within a given range of numbers of leucocytes pre-injected, the degree of suppression of activity of the transferred cells was a function of the number of leucocytes; in the case of skin homografts Medawar reported that the survival time of skin homografts bears an inverse relationship to the dose of grafted skin (7).

Other parallel observations have also been made between the two systems. Medawar (9) had found that the second set phenomenon could be induced not only by prior skin grafting but also by the injection of leucocytes; the injection of erythrocytes did not, however, have a similar effect. Thus the antigens of rabbit leucocytes—at least those shared with skin—were found not to occur in erythrocytes. In the present study there was no evidence of common antigens between leucocytes and erythrocytes of the rabbit, in that the pre-injection of erythrocyte suspensions did not lead to decreased agglutinin titers.

Finally, there have been similarities in the results of treatment of the cells injected for the primary contact between the animals. Of the treatments of pre-injected leucocytes found in this study to inactivate the effect, several (heating, lyophilization, and freezing) had been applied to rabbit tissue and mouse splenic cells in skin grafting studies, with similar effects, *i.e.* failure of induction of the second set phenomenon (10, 11). Of the two treatments found not to inactivate the pre-injection effect—x-irradiation and sonic oscillation—

the latter had been found not to inactivate splenic cells of mice for induction of the second set phenomenon (11). Also, the active material has been found in both systems to be present in the insoluble sediment of the oscillated cell suspension (11).

One observation made in these studies is at variance with a parallel observation reported in the skin homografting studies. In preparing rabbits for accelerated rejection of skin grafts by injection of leucocytes, Medawar found intradermal injections of the leucocytes to be 18 times as effective as intravenous (9). Later, Billingham and Sparrow reported that rabbits injected intravenously with suspensions of homologous, dissociated, viable epidermal cells (1 to 5 million) failed to show accelerated skin graft rejection, and in some of the experimental animals the survival of the graft was increased by a factor of 2 or 3 (12). In the present study no difference was found between the effectiveness of leucocytes pre-injected intravenously or intradermally in the range between 10^5 and 10^8 cells, as shown in Table II. It may be mentioned in this connection that in the case of pure bred strains of mice, Billingham *et al.* found the intravenous route effective for inducing accelerated skin graft rejection by injection of leucocytes (13).

SUMMARY

The transfer to rabbits of homologous lymph node cells which have been incubated *in vitro* with *Shigella*-trypsin filtrate leads to the appearance of agglutinins to *Shigella* in the sera of the recipients. In the present study it has been found that the prior injection of the prospective recipients with blood leucocytes from the donor animals prevented the appearance of anti-*Shigella* agglutinins. The following observations have been made in this system:

1. The degree of the pre-injection effect was found to be a function of the number of leucocytes injected and of the interval between such pre-injection and the transfer of the antigen-incubated lymph node cells.

2. The pre-injection of leucocytes at appropriate intervals could also cause the failure of antibody to appear in sera of recipients of lymph node cells when these were obtained from donor rabbits injected with *Shigella*, 1, 2, or 3 days prior to cell transfer.

3. Agglutinins failed to appear in cell-transfer experiments after the pre-injection not only of blood leucocytes, but also of lymph node cells, peritoneal exudate cells, or thymus cells of rabbits. This effect was not brought about by pre-injection of erythrocytes of rabbits or leucocytes of chicken, cow, or horse. The pre-injection of leucocytes of human blood had an effect of partial suppression.

4. When the leucocytes for pre-injection were pooled from groups of rabbits, either the prospective donors of the lymph node cells or other rabbits, essentially complete suppression of agglutinin titers occurred regularly. When the

leucocytes for pre-injection were obtained from an individual rabbit and the lymph node cells from another rabbit the suppression of the recipients' titers occurred sporadically.

5. When the recipient's own leucocytes were pre-injected the subsequent agglutinin titers were somewhat lower than those of the non–pre-injected controls. When the recipient's whole blood was re-injected as the source of leucocytes the subsequent agglutinin titers were as high as those of the non–pre-injected controls.

6. The pre-injection effect was not obtained if the leucocytes had been heated, frozen and thawed, suspended in distilled water, lyophilized, or treated with sodium iodoacetate. However, sonic oscillation or x-irradiation of the leucocytes had no effect on their capacity to bring about the pre-injection effect.

We are indebted to the Department of Radiology of the Graduate Hospital of the University of Pennsylvania for its generosity in carrying out the irradiations of recipient rabbits throughout these studies.

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