THE TRANSFER OF LYMPH NODE CELLS IN THE STUDY OF THE IMMUNE RESPONSE TO FOREIGN PROTEINS*, ‡

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The transfer of cells capable of producing antibody, from donor to homologous recipient animals, is an accepted laboratory model for the study of the immune response. This technique was developed by Landsteiner and Chase in 1942 to investigate the cell types involved in the immune response (1). Most of the work has consisted of transferring cells, usually from lymphoid organs or the buffy coat of peripheral blood of hosts already stimulated by antigen to non-immunized recipients. Skin testing and/or serologic methods then demonstrated the presence of antibody in the recipients, much sooner than would be expected with a primary response in the recipients (2–10). Recently, antigenic stimulation of transferred cells in the recipient has been demonstrated using tetanus toxoid (11), and dysentery bacilli (12) as antigens.

We have used the cell transfer technique in studying the response to soluble foreign serum proteins in order to: (*a*) find out how much antibody a known number of transferred cells can produce; (*b*) determine effects of time of administration and dose of antigen on the ability of the transferred cells to make an immune response; (*c*) observe the effects of *in vitro* x-radiation on these transferred cells; and (*d*) study the morphology of these transferred cells during an immune response.

The present report deals with the first two of the above investigations.

Materials and Methods

Immunization of Donors.—White male rabbits weighing between 2 and 4 kilos were used as cell donors in all experiments.

Bovine gamma globulins (BGG),[1] lot C-904, and crystalline bovine serum albumin (BSA)[2] lots 128–175 and R-370295B, obtained from Armour and Company, were used as antigens. After dilution with 0.85 per cent saline and addition of 1 per cent of 1:1000 merthiolate solution, the usual concentration was about 20 mg./ml. Donors were immunized by subcutaneous

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[1] BGG, bovine gamma globulin.

[2] BSA, bovine serum albumin.

injections of 10, 10, 20, 20 mg. of antigen on consecutive days and on the 5th day of the primary series, 30 mg./kilo intravenously. Three weeks later they received subcutaneous injections of 10 or 20 mg. antigen in each hind foot pad and 50 or 100 mg. intravenously. Ten days after these second injections serum antibody determinations were performed. Any variations from this course of immunization are mentioned under specific experiments.

Approximately 1 week after antibody determinations, 5 weeks after the first antigen injection of the primary series, cell donors were exsanguinated and mesenteric, popliteal, and axillary (when found) lymph nodes were obtained intact with aseptic precautions, and stored in cold Petri dishes. The rate of antibody synthesis in the donors at the time of cell transfer is relatively low.

Preparation of Cells.—In general, our techniques were patterned after those of Harris *et al.* (10) with the following exceptions and additions.

As suspending fluid in early experiments, we used Hanks's solution without bicarbonate both with or without ⅛ per cent simple animal gelatin. However, PVP-macrose with added phosphate buffer[3] was found to be a preferable vehicle and was used in most of the work to be reported here. The cell suspension obtained after tearing the nodes was filtered through a cold stainless steel funnel containing a double layer of 50 mesh tantalum gauze. Approximately one billion cells were recovered from the lymph nodes of each donor. About 80 per cent of these cells did not stain with trypan blue, and were considered viable (10). Most of the cells floated freely in the suspension; roughly 10 per cent were in clumps of not over 10 cells, usually attached to a reticulin fiber. These suspensions were usually injected directly without any washing of the cells.

The cell suspension remaining after injection of the recipients was smeared on clean glass slides, half of which were fixed for 1 hour in ethyl ether–ethyl alcohol mixtures, and half air-dried. Those fixed in ether-alcohol were air-dried and stained according to the Papanicolaou technique; unfixed, air-dried slides were stained with Wright's stain followed by May-Grünwald-Giemsa stain.

Treatment of Recipients.—White male or female rabbits weighing approximately 2 kilos were used as recipients in all experiments. All recipients, unless otherwise noted, received 400 r of whole body x-radiation 48 hours before cells and antigen were injected. It has been demonstrated that 400 r whole body x-radiation given 48 hours prior to primary injection of these antigens will completely inhibit the primary immune response (13).

According to the number of cells needed, each recipient rabbit received a 15 to 35 ml. aliquot of cell suspension, injected subcutaneously and intramuscularly into the abdominal wall over as large areas as possible. Anterior abdominal wall injection, rather than intravenous injection, was chosen so that we might recover the injection sites and correlate the morphologic appearance of the transferred cells with the immunologic activity. In about ¼ of the injections, some of the cells were inadvertently deposited in the peritoneal cavity. Injections were performed immediately after total and trypan blue counts of the cell suspensions.

With the exception of specific experiments, varying amounts of BGG and BSA trace-labelled with I^{131} (I*BGG and I*BSA) as previously described (14) were injected in the ear veins of recipients within the hour after injection of cells in the anterior abdominal walls. Three

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[3] PVP-macrose, a product of Schenley Laboratories, Inc., consisted of the following: 3.5 per cent polyvinylpyrrolidone (K-29-30) in sterile, pyrogen-free salt solution containing sodium chloride 0.65 per cent, potassium chloride 0.04 per cent, calcium chloride 0.025 per cent, magnesium chloride: $6H_2O$ 0.0005 per cent, and sodium bicarbonate 0.024 per cent. Immediately before use, 5 ml. of phosphate buffer, 2 molar with respect to Na_2HPO_4 was added to 500 ml. of PVP-macrose. This final solution had a pH of 6.8. Additional ionic strength did not exceed 0.08.

days after antigen injection, and subsequent days as necessary, determinations of circulating antigen were made according to techniques reported previously (14). Recipients were exsanguinated on the 3rd day after antigen disappearance, when the serum antibody concentration is maximal (15). Quantitative antibody determinations and initial combining ratios were performed, according to the techniques originally described by Heidelberger and coworkers (16). Initial combining ratios (2R) were used to differentiate between antibody to BSA produced by previously sensitized cells, and virgin cells. With antibody produced after secondary stimulation of immunized rabbits, more antibody is precipitated in regions of antibody excess per unit of antigen than with antibody produced after primary stimulation. Initial combining ratios observed after primary stimulation are from 5–6, secondary ratios are from 8–12.

Unless noted, all experiments were controlled by the injection of antigen into rabbits which were x-radiated but did not receive cells, and/or the injection of cells into x-radiated rabbits which did not receive antigen.

The usual period of time from autopsy of the first donor to injection of cells in the last recipient was approximately 4 hours.

EXPERIMENTAL PROCEDURES

In the experiments described in detail below, the primary and secondary immune responses of transferred cells to I*BGG and I*BSA injected into the recipients, as well as the response of cells after *in vitro* incubation with I*BGG and I*BSA, were studied.

Calculations.—In each experiment the number of viable cells transferred to each recipient rabbit, and the number of antigen molecules per viable cell were calculated. The number of viable cells per recipient was calculated by the use of the trypan blue test, according to the techniques of Harris *et al.* (10). The number of antigen molecules per viable cell transferred was calculated by multiplying Avogadro's number (6.0247×10^{23}) by the weight in grams of antigen injected, and dividing this value by the molecular weight of the antigen (BSA = 65,000; BGG = 165,000) and by the number of viable cells transferred.

Primary Response

Experiment 128.—Lymph node cells were obtained from eight non-immune healthy rabbits. Each of three recipients received 3.6 billion viable cells and 5 million antigen molecules per viable cell (2.25 mg. I*BSA). One control rabbit received 2.25 mg. of I*BSA only.

Experiment 109.—Lymph node cells from nine non-immune healthy rabbits were transferred to two recipient groups of three rabbits each. Each recipient in group I received 970 million viable cells that had been incubated for ½ hour at 37°C. with 2.5 million antigen molecules per viable cell (1.5 mg. I*BGG), and did not receive antigen subsequently. These recipients were studied for antibody production 3, 5, 7, and 11 days after the transfer of cells. In group II, 970 million viable cells, incubated for ½ hour at 37° C. without antigen, were injected into each recipient. These rabbits were then stimulated with 2.5 million antigen molecules per viable cell (1.5 mg. I*BGG). Group III, a control group of two rabbits, received 1.5 mg. I*BGG per rabbit only.

Secondary Response

Experiment 99.—Cells from six donor rabbits, having an average anti-BGG level of 210 γ N per ml. of serum 7 days before sacrifice, were transferred to two recipient groups of three

rabbits each. Each recipient in group I received 970 million viable cells and 23 million I*BGG molecules per viable cell (6 mg. I*BGG); each recipient in group II received 970 million viable cells and 46 million I*BGG molecules per viable cell (12 mg. I*BGG). Group III, a control group of three rabbits, received 6 mg. I*BGG per rabbit only.

Experiment 137.—Cells from eight donor rabbits, with an average anti-BSA level of 950 γ N per ml. of serum 12 days before sacrifice, were transferred to two recipient groups of five rabbits each. Each recipient in group I received 840 million viable cells from mesenteric lymph nodes, and 37 million I*BSA molecules per viable cell (3.4 mg. I*BSA). Each recipient in group II received 580 million viable cells from extremity lymph nodes, and 37 million I*BSA molecules per viable cell (2.3 mg. I*BSA). Group III, a control group of two rabbits, received 2.3 mg. I*BSA per rabbit only. The foot pads of the donors had been injected with 0.1 ml. Lilly typhoid vaccine 3 days before sacrifice, to induce lymphoid hyperplasia in the regional nodes (10).

Experiment 136.—Cells from seven donor rabbits, with an average anti-BSA level of 1300 γ N per ml. of serum 10 days before sacrifice, were transferred to three recipient groups of four rabbits each. Each recipient of group I received 510 million viable cells and 37 million I*BSA molecules per viable cell (2 mg. I*BSA). Each recipient of group II received 510 million viable cells and 148 million I*BSA molecules per viable cell (8 mg. I*BSA). Each recipient of group III received 510 million viable cells only and was studied for antibody production 3, 5, 7, and 11 days after the transfer of cells.

Experiments 100, 131, and 147 were performed to study the effects of incubation and/or long periods of refrigeration of the cells on their ability to participate in a subsequent secondary response in the recipient.

Experiment 100.—Cells from five donor rabbits, with an average anti-BGG level of 450 γ N per ml. of serum 8 days before sacrifice, were transferred to four recipient groups of two rabbits each. Each recipient of group I received 360 million viable cells that had not been incubated or refrigerated, and 35 million I*BGG molecules per viable cell (3.4 mg. I*BGG). Each recipient of group II received 400 million viable cells that had been incubated for ½ hour at 37°C., and 31 million I*BGG molecules per viable cell (3.4 mg. I*BGG). Each recipient of group III received 230 million viable cells that had been incubated for 1½ hours at 37°C., and 52 million I*BGG molecules per viable cell (3.4 mg. I*BGG). Each recipient of group IV received 395 million viable cells that had been refrigerated at 2–4°C. for 12 hours, and 31 million I*BGG molecules per viable cell (3.4 mg. I*BGG).

Experiment 131.—In this and the following experiment (147), ⅓ of the donor cells (those transferred to recipients in group I) were incubated for ½ hour at 37°C., as part of a study concerning the effects of *in vitro* x-radiation on the cells. Cells from seven donor rabbits, with an average anti-BSA level of 780 γ N per ml. of serum 8 days before sacrifice, were transferred to three recipient groups of four rabbits each. Each recipient of group I received 610 million viable cells and 37 million I*BSA molecules per viable cell (2.4 mg. I*BSA).

Experiment 147.—Cells from eight donor rabbits, with an average anti-BSA level of 990 γ N per ml., were transferred to three recipient groups of four rabbits each. Each recipient of group I received 590 million viable cells and 28 million I*BSA molecules per viable cell (1.75 mg. I*BSA).

Incubation of Cells with Antigen

In these experiments, an attempt was made to stimulate an antibody response by an *in vitro* contact of cells and antigen. The original cell suspension

was centrifuged at 2–4°C. for 5 minutes at 1400 R.P.M., the supernate removed and tested for antibody, and the cell mass resuspended in fresh suspending fluid. Radioiodinated antigen was added and the mixture gently shaken. This mixture was counted in a well-type Geiger-Muller counter before incubation. After incubation at 37°C. for periods of ½ or 1 hour, the cell mixture was again centrifuged; the supernate withdrawn and counted. The cells were resuspended in fresh fluid, washed by gentle shaking, recentrifuged, and the supernate and cell mass counted separately. The first supernate following incubation was analyzed for protein and non-protein–bound radioactivity. The cells were resuspended uniformly, counted, and injected into recipient rabbits. Those recipients which did not receive antigen subsequent to the injection of cells were studied for antibody production 3, 5, 7, and 11 days later.

Primary Response.—Rabbits in Experiment 109 group I. described above, received 970 million viable cells previously incubated ½ hour with 2.5 million antigen molecules per viable cell.

Secondary Response.—Experiments 108, 115, and 113: Since death of cells during incubation, or loss of immunologic activity without death of cells, might lead to false negative results, several experiments were performed to rule out these possibilities.

Experiment 108.—Cells from four donor rabbits immunized by five monthly intravenous injections of 500 mg. BSA, with an average anti-BSA level of 490 γ N per ml. of serum 6 days before sacrifice, were transferred to three recipient groups of three rabbits each. Each recipient in group I received 360 million viable cells that had been incubated for ½ hour with 28 million I*BSA molecules per viable cell (2 mg. I*BSA), and did not receive antigen subsequently. These cells had not been washed after incubation, but after centrifugation the cell mass contained only 2.4 per cent of the antigen. Each recipient in group II received 370 million viable cells that had been incubated for ½ hour without antigen followed by 49 million I*BSA molecules per viable cell (2 mg. I*BSA). The three recipients in group III had not been x-radiated prior to transfer of cells, and each recipient received the same number of cells that had been incubated with the same amount of antigen as those in group I.

Experiment 115.—Cells from six donor rabbits, having an average anti-BGG level of 140 γ N per ml. of serum, were transferred to three recipient groups. Each of three recipients in group I received 660 million viable cells that had been incubated for 1 hour with 24 million I*BGG molecules per viable cell (2.5 mg. I*BGG), and did not receive antigen subsequently. These cells were washed once after incubation and the cell mass contained 1.8 per cent of the antigen. Each of three recipients in group II received 660 million viable cells that had been incubated for 1 hour with 24 million I*BGG molecules per viable cell (2.5 mg. I*BGG) followed by 21 million I*BGG molecules per viable cell (3.8 mg. I*BGG) in addition. These cells were washed once after incubation and the cell mass contained 1.8 per cent of the antigen. Each of the two recipients in group III received 790 million viable cells that had been refrigerated at 2–4°C. about 2 hours (the time involved in procedures for groups I and II), and 24 million I*BGG molecules per viable cell (5 mg. I*BGG).

Experiment 113.—Cells from six donor rabbits immunized to both BSA and BGG were transferred to two recipient groups of three rabbits each. The donors in this experiment had an average anti-BSA level of 370 γ N per ml. of serum, and an average anti-BGG level of 530 γ N per ml. of serum. These cells were all incubated for ½ hour with 39 million I*BSA

molecules per viable cell (12 mg. I*BSA). After one washing the cell mass contained 0.3 per cent of the antigen. Each recipient in group I received 450 million viable cells and no further antigen. Each recipient in group II received 570 million viable cells, and 20 million I*BGG molecules per viable cell. Determinations for both anti-BSA and anti-BGG were performed subsequent to the elimination of I*BGG. Group III, a control group of three rabbits received 5 mg. I*BGG per rabbit only.

TABLE I

*Differential Cell Counts**

Experiment	Lymphocytes			Plasma cells	Reticulo-endothelial cells	Myeloid cells	Erythrocytes
	Small	Large	Total				
Primary response							
128	57	24	82	0.5	14	0.5	3
Secondary response							
99	84	7	91	3	6	0	1
127	80	7	87	2	7	1	2
136	76	8	84	5	9	0.5	1
137 I	77	9	86	3	9	0.5	2
137 II	82	5	87	4	7	1	1
Incubation without antigen							
131 I	70	17	87	2	9	0.5	2
147 I	72	12	84	3	11	1	2
Incubation with antigen							
108	67	17	84	2	13	0.5	1
113 I	68	16	84	4	9	0	3
113 II	65	18	83	4	10	0.5	2
115 I	63	21	84	3	11	0.5	1
115 II	62	22	84	3	11	0.5	2
115 III	67	19	86	2	10	1	1

* Listed as per cent of 500 cells counted.

RESULTS

Differential Counts of Cells before Injection.—Differential counts on 500 cells from each experiment are listed on Table I. Under reticulo-endothelial cells are included monocytes, large phagocytes, "blast" or "undifferentiated" forms and fibrocytes. The cell suspensions consisted of 82 to 91 per cent small and large lymphocytes, 2 to 5 per cent plasma cells, 6 to 14 per cent reticulo-endothelial cells, 0.5 to 1 per cent myeloid cells, and 1 to 3 per cent erythrocytes. Following incubation there appeared to be a decrease in number of small lymphocytes and an increase in numbers of large lymphocytes and reticulo-endothelial cells.

Immunologic Observations.—Table II lists the results obtained in all the experiments except those in which the cells were incubated with antigen; the

TABLE II
Immunologic Results in Recipient Rabbits

Experiment	I* Antigen	Recipient groups	Antigen elimination day			Antibody		
			5	6	7	γN/ml. serum	molecules per cell‡	Initial combining ratio
Primary response§								
128	BSA	I	0/3	0/3	0/3	0	0	—
		II	0/1	0/1	0/1	0	0	—
109	BGG	I	0/3	0/3	0/3	0	0	—
		II	0/3	0/3	0/3	0	0	—
Secondary response								
99	BGG	I	3/3	—	—	8.4–49.8	0.3–1.9	—
		II	3/3	—	—	13.4–43.7	0.6–1.6	—
		III	0/3	0/3	0/3	0	0	—
137	BSA	I	5/5	—	—	52.8–82.0	2.6–5.0	12.8, 13.2
		II	0/5	4/5	4/5	5.5–38.5	0.5–3.2	—
		III	0/2	0/2	0/2	0	0	—
136	BSA	I	4/4	—	—	4.0–72.1	0.3–5.1	10.9
		II	0/4	4/4	—	10.1–44.0	0.8–3.3	7.9
		III‖	—	—	—	0	0	—
100	BGG	I	2/2	—	—	23.0–43.0	2.8–4.7	—
		II	0/2	2/2	—	7.4–19.6	0.8–2.1	—
		III	0/2	0/2	0/2	0	0	—
		IV	2/2	—	—	9.0–19.6	0.8–1.8	—
131	BSA	I	2/4	2/4	3/4	0.0–29.2	0.0–1.6	—
147	BSA	I	2/4	4/4	—	4.4–20.9	0.3–1.2	—
127	BSA	I	2/2	—	—	74.8–158.	2.0–4.1	—
		II	2/2¶	—	—	53.9–60.7	1.4–1.5	—
		III	0/3	0/3	0/3	0	0	—

‡ Number of antibody molecules in recipient in hundred millions per viable cell.
§ No immune elimination of antigen in primary response experiments by day 15.
‖ No antibody detected 3, 5, 7, and 11 days after the transfer of cells.
¶ The recipients in which antigen was delayed, completed elimination by day 4.

latter are listed on Table III. Calculations for Tables II and III were made as follows: The values for micrograms of antibody N per milliliter of serum, listed on Tables II and III as "γN/ml. serum" were obtained either directly by quantitative antibody precipitations, or by multiplying the amount of

antigen N precipitated by 1 ml. of serum at 80 per cent antigen precipitation by the average ratio obtained at this point for anibody N to antigen N (BSA = 5.5; BGG = 3.5) (17, 18). The total amount of antibody N per recipient was then calculated by multiplying the amount per milliliter of serum by the estimated globulin pool (20). The total globulin pool has been found to be at least twice the intravascular globulin, and for these calculations

TABLE III

Immunologic Results in Recipient Rabbits

Cells Incubated with Antigen Prior to Transfer.

Experiment	I* Antigen	Recipient groups	Antigen elimination day			Antibody	
			5	6	7	γN/ml. serum	Molecules per cell‡
Primary response§							
109	BGG	I	—	—	—	0	0
		II	0/3	0/3	0/3	0	0
		III	0/2	0/2	0/2	0	0
Secondary response							
108	BSA	I‖	—	—	—	0	0
		II	0/3	1/3	2/3	4.4–13.2	0.4–1.2
		III‖	—	—	—	0	0
115	BGG	I‖	—	—	—	0	0
		II	0/3	1/3	2/3	0	0
		III	0/2	2/2	—	2.8–5.3	0.1–0.2
113	BSA and BGG	I‖	—	—	—	0	0
		II I*BSA I*BGG	0/3	3/3	—	10.2–28.0	0.9–2.2
		III	0/3	0/3	0/3¶	0	0

‡ Number of antibody molecules in recipient in hundred millions, per viable cell.

§ No antibody observed 3, 5, 7, and 11 days after the transfer of cells which had been incubated. No immune elimination of antigen by day 15 in recipients of cells and antigen.

‖ No antibody observed 3, 5, 7, and 11 days after the transfer of cells which had been incubated with antigen.

¶ No immune elimination of antigen in controls which received only antigen.

was assumed to be the equivalent of 80 ml. of serum globulin per kilo of recipient (19, 20). The number of molecules of antibody detected in the recipient per viable cell transferred, listed on Table II and III as "Antibody molecules per cell," was calculated by multiplying Avogadro's number (6.0247×10^{23}) by the amount of antibody N per recipient and by 6.25, the ratio of protein weight to protein N. This value was divided by the molecular weight of the antibody protein (165,000) and the number of viable cells transferred.[4]

[4] Detailed histologic study of the donor cells from pre-injection smears, and of recipient injection sites obtained 1 hour, 1 day, 2 days, 3 days, 4 days, 5 days, and 6 days after transfer,

Primary Response.—No primary response was detectable in the experiments described herein nor in several preliminary experiments.

Secondary Response.—Antigen elimination: The complete elimination of antigen was observed by day 5 or day 6 after the injection of cells and antigen in all experiments in which cells were transferred as soon as possible to the recipients. When cells were incubated before injection into recipients, the complete elimination of antigen was not usually observed until day 6 or day 7. If one recipient rabbit was found to be immunologically unresponsive, when the other rabbits in the group reacted as expected, autopsy of this rabbit always revealed that most of the cells were in the peritoneal cavity. Cells inadvertently injected in the peritoneal cavity appeared to die much more rapidly than cells injected subcutaneously or intramuscularly.

In Experiment 127, the two recipient rabbits in group II which received a reduced amount of antigen 48 hours after the injection of cells, completely eliminated the injected antigen by day 4.

Antibody Production.—The index of antibody production was considered as the number of antibody molecules present in the recipient on the 3rd day after the complete elimination of antigen, divided by the number of viable cells injected in this recipient at the outset of the experiment. The 10 recipients which eliminated I*BGG by the 5th day after the injection of cells and antigen, had an average of 150 million molecules of specific antibody per viable cell transferred. The 11 recipients which eliminated I*BSA by the 5th day had an average of 350 million molecules of anti-BSA per viable cell transferred. When the complete elimination of antigen was not observed until the 6th day after the injection of cells and antigen, antibody levels were lower. The 8 recipients which did not eliminate I*BSA until the 6th day after the transfer of cells and injection of antigen had an average of 190 million molecules of anti-BSA per viable cell transferred.

The amount of antibody observed on the 3rd day after the complete elimination of antigen was usually decreased when cells were incubated, with or without antigen, prior to transfer. Although the 5 recipients which eliminated I*BGG by the 6th day after the transfer of incubated cells and injection of antigen had an average of 150 million molecules of anti-BGG per viable cell transferred, no recipient had more than 220 million molecules of anti-BGG per viable cell transferred. The 8 recipients which eliminated I*BSA by the 5th or 6th day after the transfer of incubated cells and injection of antigen, had an average of 80 million molecules of anti-BSA per viable cell; the highest value was 160 million molecules of anti-BSA per viable cell.

It should be noted that cells transferred from previously immunized donors

showed less than one definite mitotic spindle per 1000 lymphocytes and reticulo-endothelial cells. This mitotic activity was insufficient to replace cells dying at the normal rate. Therefore the possibility of the donor cell population increasing in the recipient was slight.

to x-radiated recipients, but not stimulated with antigen, did not produce detectable amounts of antibody (Experiment 136 group III).

Initial Combining Ratios.—Because of the amount of serum involved in the tests, initial combining ratios (2R) could not be performed on all sera. However, when performed, all were within the range expected for antibody formed during a secondary response.

Incubation of Cells with Antigen.—The original suspending fluid of the cells contained less than 1 γ of antibody N per ml. After incubation of cells with antigen, centrifugation alone removed all but 3 per cent of the radioiodinated antigen. One washing, which we found optimal for cell survival, was sufficient to remove all but 0.3 to 1.8 per cent of the radioiodinated antigen. The significance of this small amount of residual antigen was difficult to determine since the amount of radioactive label approached the limits of detection by our methods. No change could be detected in the protein- to non-protein-bound radioactivity ratio of the supernates obtained after incubation. Detectable serum antibody levels were not observed in recipients of any experimental group which received only washed cells that had been incubated with antigen.

However, when antigen was injected into recipients subsequent to the injection of these washed, incubated cells, the complete elimination of antigen and antibody production paralleled those experiments in which cells were incubated without antigen. Antigen elimination was usually not complete until day 6 or day 7 after injection, and detectable antibody on day 3 after antigen elimination was never more than 220 million antibody molecules per viable cell transferred.

DISCUSSION

By observing the secondary immune response made by lymph node cells obtained from previously immunized rabbits and transferred to x-radiated recipient rabbits prior to antigenic stimulation, it has been possible to estimate the amount of antibody synthesized by an individual cell making a secondary response.

As a basis for calculation, let us take the average anti-BSA value of 350 million molecules per viable cell transferred for those recipients eliminating I*BSA within 5 days. Three hundred-fifty million molecules of rabbit antibody amount to 0.1 $\gamma\gamma$ of protein, or more than $\frac{1}{3}$ of the weight of an average transferred lymphoid cell.[5] This average figure is no doubt much less than the maximum amount of antibody which a single lymph node cell is capable of synthesizing for two reasons: first, since it is probable that all the trans-

[5] Assumed values: Volume of lymphocyte: 250 μ^3 (22). Specific gravity of lymphocytes: 1.2 gm./cm.^{3} Thus, $\frac{250\ \mu^3 \times 1.2\ \text{gm.}}{1\ \text{cm.}^3} = 0.26\ \mu\mu\text{g}$. Or, 4 billion lymphocytes weigh 1 gm. (22). Thus, 1 lymphocyte weighs 0.25 $\mu\mu$g.

ferred cells do not participate equally in this anamnestic response, the amount of antibody made by the most active cells would probably be much greater than this average figure; second, the amount of antibody present on the 3rd day after antigen elimination, which we used as the basis of our calculation, obviously does not represent the total antibody production by the transferred cells, and is probably low by a factor of at least 2. It does not include the antibody which combined with antigen *in vivo* and was rapidly catabolized (21); nor does it account for the non-immune catabolism of the antibody protein which has a half-life of 5 days (21). Thus, it would appear that the cells most actively producing antibody probably produce more than their own weight of antibody protein during the first 8 days of the secondary response. This figure is within the same range as that calculated for protein formation by the salivary glands and pancreas.

Is the output of antibody per given number of cells in the artificial situation of a cell transfer experiment comparable to that in the secondary immune response in an intact immunized rabbit? In these cell transfer experiments, 500 million to 1 billion lymph node cells were involved in the immune responses observed. The transferred cells were stimulated by amounts of antigen ranging from 23 million to 148 million molecules of I*BGG or I*BSA per viable cell. By comparison, if we assume that in the intact animal the lymphoid tissue is the principal site of antibody production, then approximately 20 to 40 billion cells per kilo of body weight would be available for participation in an immune response (22). Amounts of antigen for an intact 2 kilo rabbit comparable, on the basis of lymphoid cells available, to the amounts used in the cell transfer experiments, would range from 120 to 1100 mg. of I*BGG or I*BSA. If such doses of antigen are given to intact, previously immunized rabbits, the time necessary for the elimination of antigen is comparable to that seen in these cell transfer experiments (23). In addition, antibody production, measured by the peak amount of antibody detected after the elimination of antigen in these cell transfer experiments and in intact rabbits, is of the same order of magnitude when expressed as molecules of antibody per available lymphoid cell. If the highest serum anti-BSA levels observed in this laboratory are converted into total amounts of antibody present in the intact rabbit, and this figure divided by the estimated number of lymphoid cells in the intact rabbit, the number of anti-BSA molecules per lymphoid cell would be about 150 million, as compared to the average of 350 million molecules of anti-BSA per viable cell, observed in the cell transfer experiments. Thus, it would appear that the transferred cells are responding to antigenic stimuli in much the same way as do similar cells in the intact rabbit.

That the transferred donor cells were responsible for the anamnestic responses observed in these experiments seems certain for several reasons. First, the time for complete elimination of antigen from the serum was consistent with

an anamnestic response. If the recipients had not been rendered incapable of participation in an immune response by 400 r of whole body x-radiation 48 hours before injection of antigen and had made the response instead of the transferred cells, the elimination of antigen would have been from 2 (BGG) to 5 (BSA) days later than observed. Second, the initial combining ratios of the recipients' antisera were in the secondary range. Third, the serum antibody levels observed were higher than are seen when normal rabbits are exposed for the first time to small doses of these antigens (23).

Since approximately 90 per cent of the transferred cells are lymphocytes, the temptation to attribute much of this immunologic activity to the lymphocyte is great. However, the lymphocyte has great potentialities, and after injection may not remain a lymphocyte throughout the immune response, so that forms derived from it may be of equal importance. The role of the recticuloendothelial cells, 10 per cent of the transferred cells, in this response is difficult to evaluate. It does not seem likely that they alone could produce the observed amounts of antibody. Certainly, the occasional plasma cell present at the time of transfer could not alone account for the entire antibody synthesis.

In the experiment in which mesenteric and extremity cells were compared, there was no significant difference in the amounts of antibody produced, although those recipients receiving mesenteric cells eliminated antigen 1 day earlier than those receiving extremity lymph node cells. Harris *et al.* (10) found that when dysentery bacilli injected in the foot pads were used as antigens, extremity nodes were more dependable sources of antibody-producing cells than mesenteric nodes. This difference is most likely due to the method of donor immunization and the type of antigen involved in the two systems. BSA and BGG are soluble and no doubt come into contact with almost all cells of the body after intravenous or subcutaneous injections. Dysentery antigens are particulate and in large part, phagocytized locally or by regional nodes after injection.

In an attempt to determine how long the transferred cells survive and retain their ability to initiate an immune response in the new host, we delayed antigen injection for periods up to 48 hours after the transfer of cells. When 1/4 the usual antigen dose was given 48 hours after transfer of the cells, there was a prompt immune response, suggesting that at least some of the transferred cells remained immunologically active in the non-stimulated recipient for 48 hours.

It might appear that the transferred cells behave differently, in several respects, when bacillary antigens, rather than soluble protein antigens, are used. Both the initiation of a primary response by transferred cells and the initiation of a response by transferred cells after *in vitro* contact with antigen have been accomplished with a bacillary antigen (12). Our studies with soluble protein antigens failed to demonstrate either of these. The greater sensitivity

of the agglutinin test for antibodies to bacillary antigens, than the antigen elimination or precipitin determinations for antibodies to soluble protein antigens, may explain the success of the former and the failure of the latter in detecting a primary response. *In vitro* contact between transferred cells and bacillary antigens may well result in the carrying along of antigen with cells into the recipient because of either phagocytosis or non-specific adsorption of antigen to cells. Such a carry-over of antigen, which was not observed with the labelled protein antigens, may well allow an antigen-cell contact long enough to produce an immune response. Here again, the greater sensitivity of the agglutinin test may also play a role.

CONCLUSIONS

A secondary immune response to the soluble foreign protein antigens I*BSA and I*BGG has been demonstrated when lymph node cells, largely lymphocytes with a few reticulo-endothelial and plasma cells, from previously immunized rabbits were transferred to x-radiated recipient rabbits, and the recipients then challenged with antigen.

The total specific antibody synthesized by the transferred cells during the first 8 days of the secondary response amounted to approximately ⅔ of the wet weight of the transferred cells.

In an attempt to elicit a primary response, lymph node cells were obtained from normal, non-immunized donors, and transferred to x-radiated recipients. No immune response was observed upon antigenic stimulation.

When normal or previously immunized lymph node cells were incubated with antigen for periods up to 1 hour, washed and injected into recipients, no antibody production was observed.

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