ANTIBODY-PRODUCING CAPACITY OF ADULT CHICKEN SPLEEN CELLS IN NEWLY HATCHED CHICKS

A Study of Sources of Variation in a Homologous Cell Transfer System*

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The formulation of natural selection theories of immunity (1–3) has stimulated intensive investigation of the cellular dynamics of antibody production in a number of laboratories (4–14). Although determinations of the activity of isolated, single cells offer a direct approach (15–17), such analyses have thus far been limited to cells from secondarily stimulated animals. There have as yet been no reports of antibody production by isolated, single cells primarily stimulated *in vitro*, and most investigators have studied the activity of populations of immunologically competent cells toward antigenic stimuli using the cell transfer models as reviewed by Cochrane and Dixon (18) and used first with *in vitro* antigenic stimulation by Harris, Harris, and Farber (19).

Following the demonstration by Sterzl that the newborn can act as a suitable recipient for transferred adult homologous spleen cells (20), other investigators (21–23) showed that antibody to *Brucella* antigen appears in the serum of newly hatched chicks following transfer of adult chicken spleen cells stimulated primarily *in vitro*. In our own preliminary studies with this model (23), the level of antibody titer in recipients was highly variable and not directly related to the number of spleen cells transferred. When several chicks received spleen cells from a single donor, variability within the subgroups seemed to be reduced.

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These initial studies confirmed the usefulness of the experimental system for characterization of the behavior of immunologically competent cells toward an antigenic stimulus and suggested that analysis of the sources of variability in antibody titer, using large enough groups to permit statistical analysis, was warranted.

The present report presents experimental data bearing on the following points: (*a*) the variability of antibody titer attributable to genetic antibody-producing capacity of the donor spleen cells; (*b*) the variability attributable to homograft response, particularly in serial passage of spleen cells through newly hatched recipients; (*c*) the effect on variability of response of alterations in the lymphoreticular system of the host by splenectomy, thorotrast treatment, and treatment of the developing embryo with 19-nortestosterone.

This series of experiments indicated that variability in antibody titer of recipients was primarily a function of the genetic capacity of the donor cell population to respond to *Brucella* antigen. However, the homograft reaction, manifested by absence of measurable antibody, was present in the newly hatched chick and secondarily masked expression of the ability of the donor cell population to respond in a high percentage of recipients. This was controlled to some degree by prior splenectomy and thorotrast treatment of recipients, but most effectively by treatment of the embryo with 19-nortestosterone.

Materials and Methods

Chickens.—A non-inbred strain of white Leghorn chickens (Ghostley strain)[1] was used in all experiments. Chicks were housed in specially constructed brooders with ambient temperature control provided by electric lights, with the exception of 19-nortestosterone–treated chicks which were housed in custom-made, lighted Horsfall units. All were fed Purina startena chicken feed and given free access to water.

Eggs were incubated in a forced air incubator at 38°C and relative humidity of 68 per cent to the 18th day, and at the same temperature, but with 90 per cent humidity, from day 18 to hatching.

Antigen.—A standardized suspension of killed *Brucella abortus*, described previously (23), was used in all experiments. It was added to spleen cells at the time of transfer and was also used in the measurement of titers.

Cell Transfers.—Adult chicken donors were given intravenous injections of sodium pentobarbital; the spleens were removed, dispersed into a single cell suspension, mixed with antigen (one splenic white cell to two bacterial cells), and injected intraperitoneally into recipients (23). The procedure is illustrated in Text-fig. 1.

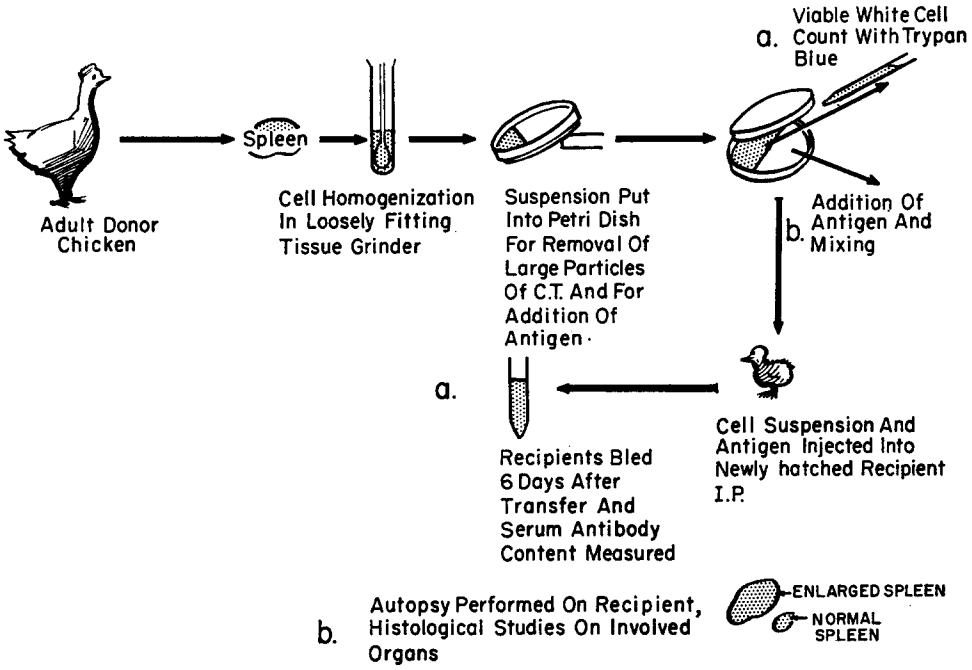
Recipients were newly hatched chicks of the same strain and were between 12 and 48 hours old at the time of transfer. Controls for the antibody-producing capacity of adult spleen cells were as described earlier (23), and consisted of normal spleen cells transferred without antigen, transfer of heated cells with antigen, and injections of antigen only into recipients.

Alteration of Reticuloendothelial System in Recipients

1. Thorotrast Blockade.—Chicks were given 2.5 ml per kg of thorotrast, *via* the jugular vein, 24 hours before cell transfer (24, 25).

[1] We are indebted to Dr. George Ghostley, Mr. Fred Ghostley, and the Ghostley Chicken Hatchery of Anoka, Minnesota, for supplying newly hatched chicks and fertile eggs.

2. Splenectomy.—Chicks were anesthetized with ether, and a transverse incision made below the sternum. An eyelid retractor was inserted, and skin, muscle, and peritoneum retracted. The gizzard was retracted laterally, exposing the spleen below the third lobe of the liver in the midline. It was lifted up by its pedicle and hemostasis achieved with a small, curved forceps. Since the spleen is only 2 mm in diameter in the newly hatched chick, it was removed intact with a pituitary rongeur. Hemostasis was maintained either by continued pressure on the pedicle with the forceps or by insertion of a small piece of sterile gelfoam soaked in thrombin. The incision was closed by a single 5–0 silk suture through all layers of the abdominal wall. Spleen cells and antigen were given 24 hours after surgery.



TEXT-FIG. 1. Diagram of cell transfer system. Tissue homogenizer consisted of a 16 × 150 mm test tube and the pestle of a Potter homogenizer. C. T. = connective tissue. I. P. = intraperitoneally.

In sham-operated controls the spleen was left in place.

3. 19-Nortestosterone Treatment.—On the 5th day of incubation, eggs were injected with 0.1 cc containing 0.63 mg of 19-nortestosterone (Elite Chemical Co., Newark, New Jersey), in corn oil into the albumin end, according to the method of Meyer *et al.* (26), and the shells were sealed with a mixture of 20 per cent beeswax and 80 per cent paraffin. Controls received 0.1 cc of corn oil. Chicks hatching on the 21st day were observed for 24 hours, and cell transfers were made on the next day or within 12 hours after the end of the observation period.

Measurement of Serum Antibody

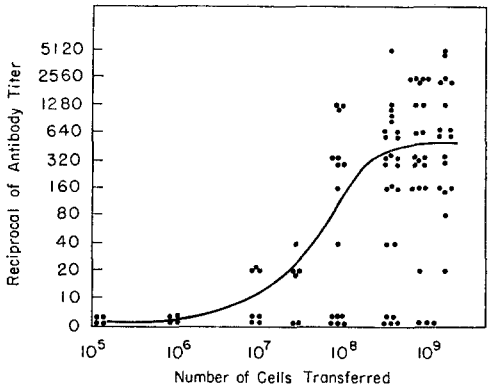
Recipients were bled by cardiac puncture 6 days after transfer. *Brucella* agglutinins were measured by serial, twofold dilutions of the antiserum, beginning with a 1:10 dilution (23), considered to be the limit of reliability for this agglutination method.

For statistical analysis of some experiments, agglutinin titers were transformed to a logarithmic scale by conversion to $1 + \log_2 \frac{\text{titer}}{10}$. Thus, the values 10, 20, 40 . . . , 5120 were transformed to a $\log_2$ scale with values 1, 2, 3 . . . , 10, facilitating calculation of mean, variance, and other statistics.

Graft versus Host Assay

Simonsen's measure of the relative activity of adult, immunologically competent cells in the neonatal host (27, 28) was used as another measure of function of the transferred cells. Spleen weight was divided by body weight:

$$\frac{\text{Spleen weight (milligrams wet weight)}}{\text{Body weight (grams live weight)}}$$



TEXT-FIG. 2. Scattergram showing antibody titer in chicks receiving increasing numbers of spleen cells stimulated *in vitro* with *B. abortus* antigen. Each dot represents a single recipient.

to produce a value, the *GVHR index* (graft *versus* host reaction index), a semiquantitative appraisal of the presence and activity of proliferating, immunologically competent cells in the chick host.

Morphologic Studies

Autopsies were performed on all recipients at intervals of 4 to 12 days after cell transfer, and the tissues were examined in the gross. Specimens of lung, spleen, bone marrow, peripheral blood, liver, kidney, heart, gut, mesentery, peritoneum, bursa of Fabricius, skin, and subcutaneous tissue were taken for histologic examination. Marrow and peripheral blood smears were stained with Wright-Giemsa stain. Other tissues were fixed in 10 per cent formalin and/or absolute alcohol, and stained with hematoxylin and eosin or methyl green–pyronin (29).

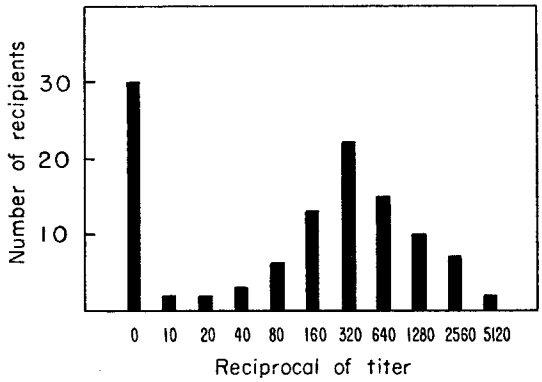
RESULTS

Variability of Antibody Response in Recipients of Cells from Random Donors

As shown in Text-fig. 2, newly hatched chick recipients show good antibody responses when 10^8 and 10^9 cells are transferred. On this basis, a large number

of transfers were carried out using 5×10^8 spleen leukocytes mixed with 10^9 killed *Brucella abortus* cells. The antibody titers, 6 days after transfer, were plotted in the form of a histogram (Text-fig. 3). The sera that did not agglutinate at the minimal dilution of 1:10 (the limit of reliability for the method) were assumed to form distribution about a point at the origin of the horizontal scale. If these are deleted, the balance of the titers, ranging from 10 to 5,120, appear to be normally distributed. Titers were transformed to the logarithmic scale, and a normal curve fitted by the conventional chi-square test for goodness-of-fit (30) to a good approximation ($p = 0.70$).

The bimodal distribution suggested that factors other than sampling error alone were affecting survival and function of donor cells. To evaluate the



TEXT-FIG. 3. Distribution of titers in random donor host combinations. All titers were measured in chicks bled 6 days after receiving 5×10^8 adult homologous spleen cells mixed with 10^9 killed *B. abortus* organisms.

contribution to this variation of donor cell capacity to respond to the antigen, responses were measured among recipients of cells from single donors.

Variability of Antibody Response in Recipients of Cells from Single Donors

In these studies spleen cells from a single adult donor were administered to two or more newly hatched recipients; otherwise, the procedures were those described in the preceding section. The individual titers are given in Table I. Responses within single donor subgroups were consistent enough to suggest that the ability of a discrete donor spleen cell population to respond to *Brucella* antigen is transferable to recipients. A statistical analysis of variance confirmed that the titers were more consistent within donor subgroups than among samples taken at random from the entire experimental group ($p < 0.001$).

Occasional large differences were noted in certain subgroups (donors 23, 25, and 27 in Table I), suggesting that the host destroyed donor cells in some instances.

TABLE I

*Antibody Titers in Recipients of Spleen Cells from Single Donors**

Donor	Recipient titers $\left(1 + \log_2 \frac{\text{titer}}{10}\right)$‡						Donor	Recipient titers $\left(1 + \log_2 \frac{\text{titer}}{10}\right)$‡					
1	10	7	6				21	6	5	5	5	2	
2	8	8	7				22	5	3				
3	9	6					23	8	5	—			
4	8	7					24	6	4				
5	8	7					25	6	4	4	—		
6	9	7	6				26	7	6	6			
7	8	8	7	5			27	6	4	4	—	—	—
8	7	6					28	6	5	3	—	—	
9	7	6					29	5	—				
10	7	6	6				30	6	—	—			
11	5	5					31	6	3	—	—		
12	7	6	6	6			32	3	1				
13	7	6	4				33	3	1	—			
14	7	7	3				34	3	—	—			
15	7	4					35	5	—	—	—		
16	6	6	6	4			36	—	—	—			
17	6	5					37	—	—				
18	7	6	2				38	—	—				
19	5	5	5				39	—	—				
20	6	6	5	5	2								

* 5×10^8 spleen cells were stimulated primarily *in vitro* with 10^9 *B. abortus* and administered intraperitoneally.

‡ Each titer represents a single recipient. The blank value (—) refers to serum showing no agglutination at the 1:10 dilution.

Preliminary analysis of the above data indicated that the variability of antigen titer within single donor subgroups was lower than that of random donor-host pairs. Statistical analysis showed that the variance within single donor groups was 2.054; the variance of the entire sample was 3.135. An analysis of variance was done to determine the statistical significance of this difference, using Fisher's variance ratio "*F* test" (26):

$$F = \frac{\text{Mean square among donors}}{\text{Mean square among chicks within donors}}$$

The basis for this calculation is given in the following analysis of variance table:

Source	Degrees of freedom	Sum of squares	Mean square
Among donors	34	163.93	4.821
Among chicks within donors	55	112.97	2.054

Thus, $F = \frac{4.821}{2.054} = 2.347$ which, with 34 and 55 degrees of freedom, is highly significant ($p < 0.001$). The authors are indebted to Dr. Richard McHugh of the Department of Biostatistics, University of Minnesota, for the analysis of variance and for the fitting of the normal curve to Text-fig. 3.

Serial Transfer of Spleen Cells

In the next group of experiments, the recipients of adult spleen cells were bled after transfer and their spleens removed for transfer of cells to another group of newly hatched chicks. Responses were evaulated both by antibody titer and by the graft *versus* host assay.

TABLE II

Serial Passage Studies with Single Antigenic Stimulation

Passage	Donor	No. of spleen cells		Recipient data at sacrifice*			
		Recovered from donor	Transferred to recipient	Mean body weight	Mean spleen weight	GVHR index‡	Mean log titer§
				gm	*mg*		
Controls			Heated cells, antigen only, and untreated chicks	64.5 (49.3–73.5)	58.8 (49.4–67.8)	0.91 (0.73–1.08)	‖
I	Adult chicken	10^8–10^9	$5 \times 10^8 + 10^9$ *B. abortus* antigen	62.5 (50.1–71.8)	102.5 (47.8–153.6)	1.64 (0.78–3.05)	5.44 (1–10)
II	Recipients from I	10^7–5×10^7	10^8 pooled cells only	54.4 (48.2–73.7)	84.9 (52.5–173.0)	1.56 (1.01–2.22)	2.63 (1–5)
III	Recipients from II	10^6–10^7	1.5×10^8 pooled cells only	60.1 (49.0–72.0)	82.5 (46.3–112.3)	1.37 (0.86–1.72)	‖
IV	Recipients from III	10^5–10^6	10^7 pooled cells only	53.4 (46.3–67.3)	48.6 (42.6–66.3)	0.91 (0.81–0.98)	‖

* Numbers in parentheses indicate the range of values represented by the mean above, on chicks sacrificed at 6 days after transfer and in controls of the same age.

‡ GVHR index $= \frac{\text{Spleen weight (mg)}}{\text{Body weight (gm)}}$, an indication of splenomegaly and the graft *versus* host reaction.

§ Log titer $= 1 + \log_2 \left(\frac{\text{titer}}{10}\right)$

‖ No detectable antibody in recipients.

Results of a typical experiment involving serial transfer of cells through four passages (following a single, primary antigenic stimulation *in vitro* at the time of the first transfer) are shown in Table II. Antibody production was demonstrated through two transfers, but not in the third, although the number of spleen cells to each recipient was increased in the third transfer. The GVHR index, reflecting splenomegaly, was significantly increased only in the first three passages.

In the absence of splenomegaly, the number of cells obtained from recipient spleens was reduced after the second passage. Table III shows the decreasing number of recipients as a result of the poor cell recovery. A number of similar

studies were done, and in each instance the recovery after the second passage was poor, limiting further serial passage studies.

When recipients in serial passage experiments received cells from a single donor, consistency of titer was evident only after the first transfer. About one-third of the secondary recipients demonstrated antibody, but the titers did not correlate with those of the spleen cell donors, the primary recipients.

Similar results were recorded when spleen cells from primary recipients at the extremes of the antibody titer distribution were pooled into "high" and "low" groups and transferred to secondary recipients. There was no perpetuation of the high or low antibody response. Few of the secondary recipients had measurable antibody in their serum, and low titers were the rule among those with significant antibody regardless of the classification of the donor in the high or low antibody-producing group.

TABLE III

*Antibody Production and Splenomegaly of Recipients in Serial Passage Studies with Single Antigenic Stimulation**

Passage	No. of recipients showing antibody	No. of recipients with splenomegaly
I	12/18	15/18
II	6/19	14/19
III	0/4	3/4
IV	0/4	0/4

* Adult donor cells were mixed with *Brucella abortus* antigen *in vitro*.

To evaluate the effect of antigenic stimulation on the proliferation of immunologically competent cells in this system, antigen was added between transfers. In a few instances (Table IV) titers were higher in secondary recipients than they had been in primary recipients. The correlation of responses of primary and secondary recipients was poor, however, and reduced titers were usual on secondary transfer.

The serial passage studies offered further evidence of a factor in the host environment that destroyed proliferating adult donor spleen cells in newly hatched recipients.

Alteration of the Reticuloendothelial System in Chick Hosts

To assess the role of the homograft reaction of the newly hatched chick in the function of spleen cell transplants, transfers were done following alterations of the lymphoreticular system of the host by administration of thorotrast (24, 25), splenectomy, and treatment with 19-nortestosterone (26).

1. Thorotrast Blockade.—Groups of chicks 12 hours old were given 2.5 ml per kg of thorotrast 24 hours before transfer of 5×10^8 spleen cells and antigen.

The antibody titers from bleedings on the 6th day were higher than those in the control group (Text-fig. 4), but the differences were not significant ($p = 0.11$) when compared by the Mann-Whitney rank test (31).

2. Splenectomy.—Since many recipients demonstrated splenomegaly following transfer of adult spleen cells, the effect of splenectomy was also studied. Splenectomy was performed at 12 to 24 hours of age, and 5×10^8 spleen cells and antigen transferred 24 hours after surgery. Serum titers on the 6th day were compared by the rank test with those of an untreated control group in a representative experiment (Text-fig. 5) and proved to be significantly higher ($p = 0.005$).

TABLE IV

Antibody Production of Recipients in Serial Passage Studies with Antigenic Stimulation between Transfers

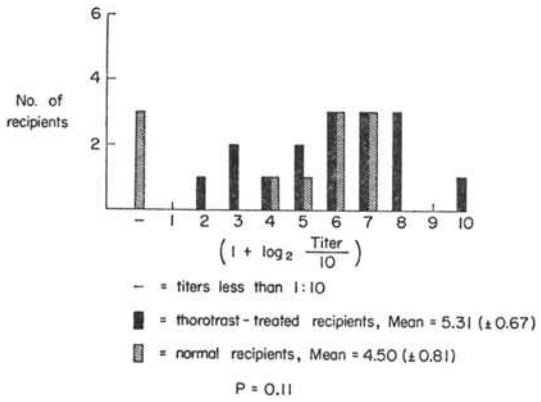
Titer of primary recipient*	Titer of secondary recipient*	Titer of tertiary recipient*
<10, <10, <10, 160	1280	<10
5120	160	<10
2560	160	<10
640	160	<10
<10	160	<10
1280	80	<10
<10	40	<10
2560	20	<10
320, 320, <10	<10	<10
1280	<10	<10
640	<10	<10
640	<10	<10
<10	<10	<10
<10	<10	<10
<10	<10	<10

* Reciprocal of the greatest dilution showing agglutinin activity.

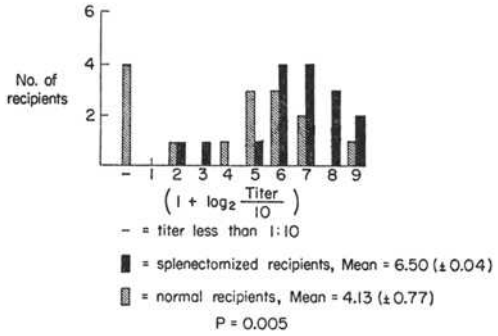
3. Treatment with 19-Nortestosterone.—Prior reports (26, 32) have indicated that 19-nortestosterone profoundly depresses the development and function of the lymphoreticular system of chickens and renders them incapable of producing antibody. Such chicks do not grow normally, have only remnants of thymus

and very small spleens, and lack the bursa of Fabricius. The mortality rate is high, with few surviving more than 2 weeks.

Chicks hatching from eggs injected with 19-nortestosterone on the 5th day of incubation, control chicks hatching from eggs injected with corn oil only, and



TEXT-FIG. 4. Antibody-producing capacity of donor cells in normal and thorotrast-treated recipients. All recipients were injected with 5×10^8 adult spleen cells mixed with 10^9 *B. abortus* organisms. Thorotrast (2.5 ml/kg) was given 24 hours before transfer in the experimental group.

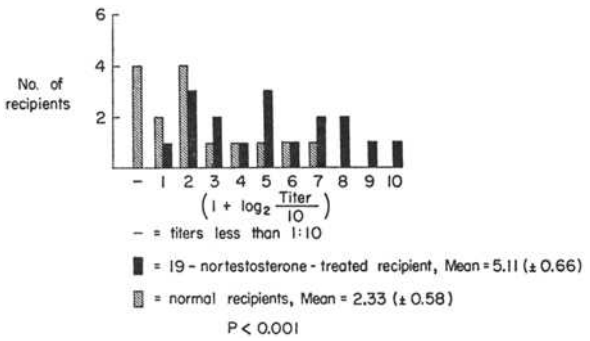


TEXT-FIG. 5. Antibody-producing capacity of donor cells in normal and splenectomized recipients. All recipients were injected with 5×10^8 cells mixed with 10^9 *B. abortus* organisms. Splenectomy was performed 24 hours prior to transfer in the experimental group.

normal chicks were given 10^8 adult spleen cells stimulated *in vitro* with *Brucella* antigen 24 to 48 hours after hatching. This smaller number of cells was used in the expectation that the profound immunologic depression characteristic of 19-nortestosterone–treated chicks would greatly facilitate donor cell survival and function. Comparative titers in one experiment are shown in Text-fig. 6. The enhancing effect of 19-nortestosterone treatment is illustrated on one hand by the highly significant differences in geometric mean titer in the two groups,

and on the other hand by the over-all increase in the proportion of recipients with detectable antibody.

Further evidence of the effect of 19-nortestosterone on the homograft reaction in chicks was obtained from a study of the graft *versus* host reaction at 12 days of age. At this age many chicks respond to antigenic stimulation (21). When 12 day old 19-nortestosterone–treated chicks were injected with large numbers of adult spleen cells, they developed severe signs of the graft *versus* host reaction and weight loss characteristic of the runting syndrome. Only occasional normal recipients showed slight splenomegaly.



TEXT-FIG. 6. Antibody-producing capacity of adult donor cells in normal and 19-nortestosterone–treated recipients. All recipients received 10^8 cells mixed with 2×10^8 *B. abortus* antigen *in vitro*. The experimental group was treated with 19-nortestosterone on the 5th day of incubation.

Morphologic Studies

At autopsy, 60 per cent of normal primary recipients of 5×10^8 or more adult spleen cells showed changes characteristic of the GVHR (27, 28, 33–36). The spleen was enlarged to two to five times its normal weight and was occasionally studded with white nodules. The liver was also enlarged, and at the border occasional thick, hyaline growths were seen which made the liver adherent to the overlying peritoneum and underlying mesenteries. White nodules were present over the mesenteries and serosal surface of the bowel and gizzard in some recipients. In those severely affected, weight loss was evident, and a subcutaneous effusion of thick, yellow, viscous material, resembling lymph, first described by Simonsen (27), was noted over the abdominal wall.

These changes were exaggerated in 19-nortestosterone–treated recipients. When injected with one-half the usual number of spleen cells (10^8), hepatosplenomegaly was the only gross finding in normal 1 day old recipients, while 1 day old 19-nortestosterone–treated recipients showed thickened white nodular growths at the liver border, over the mesenteries, gizzard, and throughout the peritoneal cavity, as well as subcutaneous, yellow effusions in the abdominal wall.

Microscopic examination of methyl green–pyronin–stained sections and Wright-Giemsa–stained imprints of spleen revealed many large basophilic reticular cells (hemocytoblasts) and cells of the plasmacytic series in recipients showing gross signs of the graft *versus* host reaction (Fig. 1). Many presumably immunologically competent plasma cells were found in the liver, lung, peripheral blood, bone marrow, muscle, peritoneum, and mesenteries, as well as in the spleen in recipients showing gross signs of the GVHR.

Sections from the fibrotic hyaline areas of the liver border and through the white nodules studding the mesenteries demonstrated a peripheral zone of fibroblasts, an inner mantle of lymphocytes and macrophages, and a central zone of necrosis, as described by Holub (34) and Biggs and Payne (36).

DISCUSSION

The experiments reported in this paper were originally undertaken to study the effect of primary *in vitro* antigenic stimulation on a population of immunologically competent cells. The homologous chicken system, consisting of adult donor spleen leukocytes transferred to newly hatched recipients, was chosen because of the ready availability of adult donors and large numbers of newly hatched recipients. Thus, it seemed to be suited to study of proliferating cells and serial transfer of cells from one recipient to another.

In the analysis of variability of titer in primary recipients, three factors were considered: the variable antibody-producing capabilities of individual populations of donor cells; the homograft reaction of recipients (22); and the physiologic adequacy of the neonatal environment for supporting the growth of adult antibody-producing cells (37). Statistical analysis comparing antibody titers of random donor-host combinations with those of single donor-host subgroups indicated that the variable capacity of the individual donor cell population to respond to antigenic challenge was a major source of recipient titer variability. We have interpreted this as an indication that the primary source of variability was the genetic capacity of the donor cell population to respond to the antigen.

Since each donor cell population appeared to represent a given level of antibody-producing capacity, it seemed reasonable to postulate that such characteristic levels would be perpetuated in serial transfer studies. Experiments showed, however, that the initial level was not maintained; antibody was not detectable after the second transfer in the series, and evidence for the GVHR disappeared after the third passage. A similar loss of antibody-producing capacity was noted when antigen was given with the spleen cells at each transfer.

Loss of activity upon serial transfer of immunological competent cells has been reported by Nossal (5) and Burnet and Boyer (9), but Simonsen (27) carried out successive transfers for more than nine passages. Dilution of cells undoubtedly accounts for some of the loss of activity. Our morphologic studies indicated that large, pyroninophilic mononuclear cells, among them mature plasma cells, not ordinarily present in newly hatched chicks and presumably

of donor origin, were widely distributed through the host (Fig. 1). Under proper conditions of recipient environment, these cells should have proliferated to yield sufficient numbers of cells for further successful transfers, as in Simonsen's studies (27). However, in our serial transfer experiments, recovery of spleen cells from secondary recipients was limited to 10^7 cells or less, and greatly reduced numbers were recovered following the third passage. Nossal (5) and Urso and Makinodan (13) have shown that repeated contact with antigen is necessary for immunologically competent cells to proliferate. Moreover, recent studies have demonstrated that the generation time of cells producing antibody in the presence of secondary stimulation and in the absence of destructive factors is less than 12 hours (12–14). In our studies there were two forms of antigenic stimulation: the added exogenous antigen, *Brucella abortus*, and the endogenous isoantigens and transplantation antigens in the chick host, but all immunologic activity was lost on serial transfer.

Serial passage studies by Nossal (5) showed loss of immunologic activity, as noted above; however, when he used inbred mice rather than non-inbred strains, he was able to continue transfers for more than 8 months.

Taking all this evidence into account, and acknowledging that mechanical failure of cell transfer might have accounted for some of the loss of activity, we concluded that the host environment in the chick recipients not only could not sustain proliferation but was actively destructive of adult antibody-producing cells. Another factor which might have been operative is the phenomenon termed "exhaustive sensitization" by Simonsen (38). Massive amounts of foreign antigen representing histocompatibility differences might have induced tolerance on the part of the transferred adult cells to host antigens. Induction of tolerance in adult cells has been demonstrated repeatedly in the experiments of Martinez, Good, and others (39–41) in mice. Tolerance to host transplantation antigens might have been a factor limiting perpetuation of the graft *versus* host reaction in this study and in the experiments of Burnet and Boyer (9).

The outcome of the studies of Trnka and Riha (22) and of our studies suggested a major role for the homograft reaction, particularly in the chicks forming no measurable antibody following transfer. It will be recalled that the plot of antibody titers in random donor-host pairs was bimodal, with the highest peak in the "no measurable antibody" category (Text-fig. 3). Prior manipulation of the host's lymphoreticular system by administration of thorotrast, splenectomy, or treatment with 19-nortestosterone, eliminated this initial peak. This suggests that the homograft reaction of the newly hatched recipients masked the response of donor cells. When the homograft reaction was reduced or absent, the antibody titer distribution was markedly altered, reflecting to a greater extent the inherent variation in the capacity of discrete donor cell populations to respond to antigen.

Donor spleen cells functioned most effectively in the 19-nortestosterone–

treated group which represented failure of normal development of the lymphoid tissue of the host. This, in turn, appears to be related to the inhibition of development of the bursa of Fabricius (26, 32). Similar interference with development of immunologic and morphologic integrity of the lymphoid tissue in mammals seems to result from removal of the thymus at an early age (42).

It seems clear that the ability to reject adult cells was established to some extent in many chicks at hatching. This is consistent with the more direct studies of homograft reactivity in newly hatched chicks by Cannon *et al.* (43). Porter (44) observed that neither tolerance nor the runting syndomre could be achieved in rabbits unless adult homologous cells were injected into recipients before the 28th intrauterine day. Apparently homograft immunity develops earlier than the ability to synthesize antibody, since none of our control chicks (receiving antigen alone) produced measurable antibody before the 12th day after hatching. In Trnka's (21) studies chicks showed antibody production to *B. abortus* beginning between the 12th and 20th day of life. These results might have been influenced by lack of sensitivity of the agglutination technique and the amount or type of antigen. Harris *et al.* (45) have reported antibody to bovine gamma globulin on the 8th day in neonatal rabbits following immunization on the 5th day of life. Riha (46) using red cell antigens and Condie (47) using hemocyanin have demonstrated antibody on the 8th day of life, in rabbits, following immunization on the day of birth. Smith (48) has reported agglutinins to antigens of *S. typhosa* in both the neonatal rabbit and the human newborn after intensive antigenic stimulation. In all these experiments the amount of antibody was low; however, they emphasize that the immunologic unresponsiveness of the newborn animal, as discussed by Bridges *et al.* (49) and by Dixon and Weigle (37), is relative rather than absolute.

Earlier studies using adult cell transfers to the newborn yielded inconsistent conclusions with regard to the newborn's capability for supporting maturation of immunologically competent cells (7, 20–23, 27, 37, 50). These have been reviewed by Cochrane and Dixon (18), and can now be understood to be related to strain differences, variable forms and amounts of antigenic stimulation of donor cells, and particularly the presence of homograft immunity at birth in most species. When the proportions of antigen and cells are appropriate, and when the homograft reaction is absent, newborn recipients seem to provide an environment conducive to the growth and maturation of adult, immunologically competent cells.

The control studies with transfer of normal cells only and of heated cells mixed with antigen, as well as histologic study of chicks receiving viable adult spleen cells, indicated that antibody to *B. abortus* could only be detected following transfer of adult cells mixed with antigen. This offers presumptive evidence for antibody formation by the donor cells residing in newly hatched recipients. Recent studies by Trentin (51) using chromosomal identification,

by Urso and Makinodan (13) with diffusion chambers, by Doria *et al.* (52) using serologic tests for isoagglutinins, and by others (18) have shown clearly that antibody production or the GVHR, as described here, can be attributed directly to donor cells and not to recipients.

The studies reported here emphasize the difficulties in using a homologous cell transfer system for experiments on the population dynamics of immunologically competent cells. Because of variability in host reaction and lack of sensitivity in antibody measurements, our experiments were inconclusive in distinguishing between an instructional or selective role for the antigen. Studies similar to some of those reported here, utilizing histocompatibility differences in inbred strains of chickens, have been carried out by Burnet and Boyer (9) and by Burnet and Burnet (11). Their studies are also inconclusive, since acquistion of tolerance by adult donor cells to histocompatibility antigens of the foreign embryo might have influenced their results. Further efforts to separate inductive and selective effects of antigen using population dynamic methods and cell transfer systems would appear to require exogenous antigenic stimulation with isologous recipients.

SUMMARY

1. To evaluate the effect of primary *in vitro* antigenic stimulation on a population of immunologically competent cells, a homologous cell transfer system was used, with adult chickens as the spleen cell donors, killed *Brucella abortus* as the antigen, and newly hatched chicks as the recipients.

2. The distribution of antibody titers in recipients of cells from random donors was bimodal, with about 30 per cent showing no detectable titer and the remainder distributed normally.

3. Variability of titer was reduced significantly in subgroups receiving cells from a single donor, indicating that the primary source of variability was the genetic capacity of discrete cell populations to respond to antigen.

4. In serial passage studies, activity of donor cell populations was lost rapidly: antibody was not demonstrated after the second passage, and the graft *versus* host reaction (splenomegaly) was not demonstrated after the third passage. Results were similar with *in vitro* antigenic stimulation at the time of the first passage only and with additional stimulation at the time of subsequent transfers.

5. The thesis that the homograft reaction of the newly hatched recipients had contributed significantly to the variability in the single transfer studies and to the rapid loss of activity in the serial transfer experiments was confirmed by the results of transfers following alteration of the lymphoreticular system of the host by thorotrast administration, splenectomy, and treatment with 19-nortestosterone during embryogenesis. All three favored the survival and function of transferred cells, raising the average antibody titer and virtually

eliminating the no-response category. The inhibition of homograft immunity was most pronounced in the 19-nortestosterone group.

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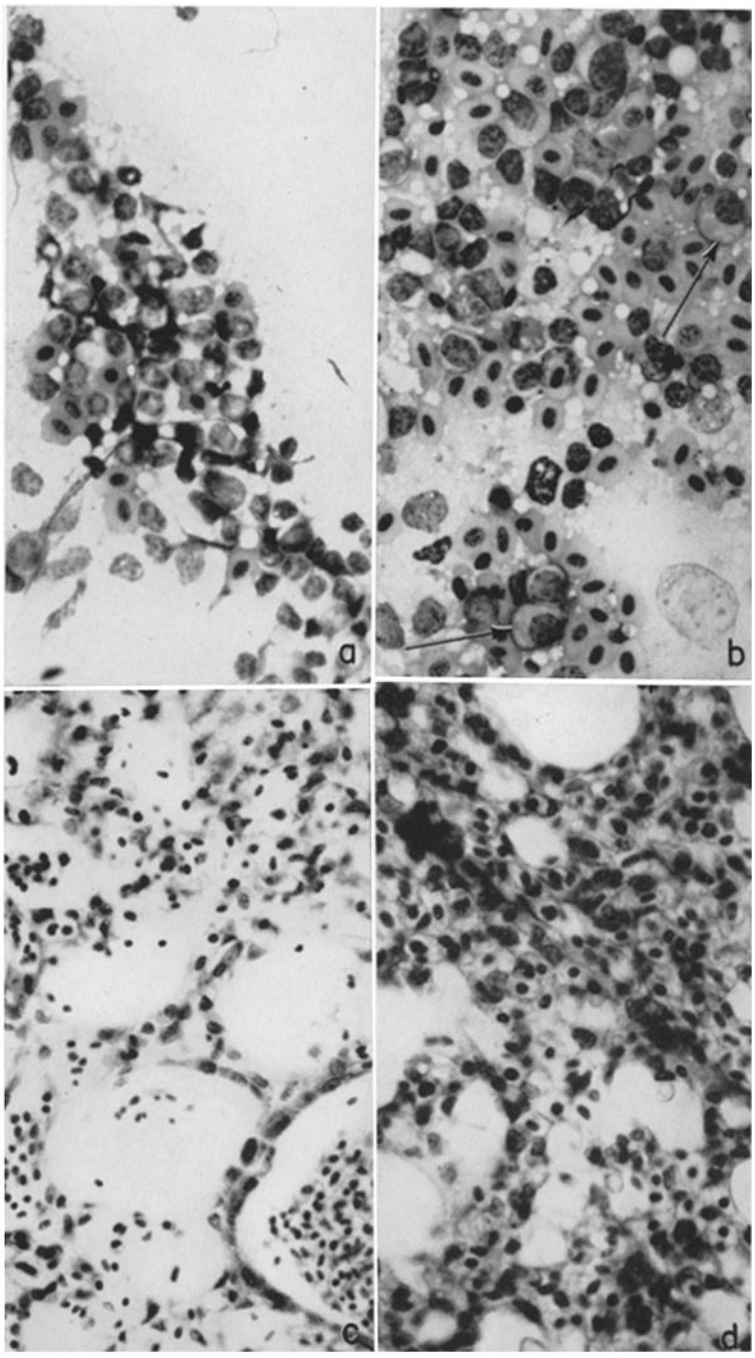
EXPLANATION OF PLATE

EXPLANATION OF PLATE 126

FIG. 1. (*a*) Wright-Giemsa–stained imprint of spleen cells from a normal 6 day old chick. Chicks of the same age given *B. abortus* antigen demonstrated the same morphologic picture. Cells pictured are representative of the spleen cell population: small and large lymphocytes, reticular cells, and nucleated red blood cells. (*b*) Wright-Giemsa–stained imprint of spleen cells from a 6 day old recipient of 5×10^8 adult homologous spleen cells mixed with *B. abortus* antigen. The morphologic picture was similar in recipients getting adult spleen cells only. In addition to the cell types noted in *a*, mature plasma cells (arrows) and other cells of the plasma cell line were abundant. This histologic picture was characteristic of the graft *versus* host reaction. (*c*) Methyl green–pyronin–stained section of lung from a normal 6 day old chick. (*d*) Methyl green–pyronin–stained section of lung from a recipient of 5×10^8 adult homologous cells. Many pyroninophilic plasma cells (cells with dark cytoplasm in photograph), presumably of donor origin, were found scattered throughout the lung, liver, peripheral blood, mesenteries, muscle, and spleen.



(Papermaster *et al.*: Antibody production by adult chicken spleen cells)