TRANSFER OF ANTIBODY PRODUCTION WITH CELLS FROM BURSA OF FABRICIUS*

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The development of full immunological responsiveness in chickens is dependent upon the presence of both central lymphoid organs—the thymus and the bursa of Fabricius. It has been amply demonstrated (1–7) that in the absence of the bursa after either hormonal treatment in ovo or surgery, the chicken is unable to produce normal levels of circulating antibody in response to antigenic stimulation. Since homograft reactivity is reduced in neonatally thymectomized chickens but is unimpaired in bursaless birds (4, 8), it has been suggested that there are two separate immune mechanisms, thymus-dependent cell-associated immunity and bursa-dependent humoral antibody production (4).

The separate nature of the effects of the two central lymphoid organs during ontogenesis has been confirmed by the results of a combined morphological and functional study (6). After surgical thymectomy and irradiation of chicks at hatching, there was a deficit in cell-associated immune reactions such as graft rejection, delayed hypersensitivity, and graft-*versus*-host splenomegaly. This deficit was accompanied by a lack of a dense population of small lymphocytes in the spleen and other peripheral lymphoid tissue. In contrast, the lack of antibody response to antigenic stimulation after bursectomy and irradiation was accompanied by a lack of germinal centers and plasma cells in the same tissues.

The mechanism by which the two central lymphoid organs function during maturation of the lymphoid tissue has not been established. Neither organ produces antibody in response to systemic immunization in young adult chickens (9). It has been postulated that the thymus and bursa act as sites of cell maturation, and are thus the primary source of potential immunologically competent cells which then migrate and populate the peripheral lymphoid tissue (10). It has, however, been shown that cells taken from the bursa as early as the 18th day of embryonic development are capable of synthesizing immunoglobulin in short-term in vitro cultures, and the capacity of bursal cells to secrete both IgM and IgG in such cultures precedes the capacity of

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splenic cells by about 1 wk (11). Since bursal cells from germfree chicks also have the capacity to synthesize immunoglobulins (11), it remains to be established whether cells capable of specifically responding to antigen are present in the neonatal bursa.

The absence of detectable antibody-synthesizing cells in the bursa after systemic immunization (9) may be the result of poor penetration of antigen into this organ and does not necessarily reflect the absence of antigen-sensitive cells. The difficulties posed by a physical blood-organ barrier can be avoided by performing experiments in which dispersed cells are transferred to nonreactive recipients, with antigen subsequently injected by the same route. In experiments of this kind with outbred chicks, it has been shown that embryos can be used as recipients, but that neonatal chicks give unsatisfactory results because they react against transferred cells (12). Little more success is obtained if the neonatal recipients are bursectomized (13), or irradiated as in preliminary studies in this laboratory. On the other hand, much better results have been obtained in the present experiments, by using F_1 chicks derived from two considerably inbred lines which are each isogenic at the major histocompatibility locus (14). As will be shown, bursal cells are more effective than splenic cells in transferring a primary immune response to *Brucella abortus* from 4-wk-old donors to irradiated neonatal recipients, while thymic cells are much less effective. In contrast, only splenic cells can transfer a response to sheep erythrocytes.

Materials and Methods

Animals.—Donors and recipients were White Leghorn F_1 hybrids obtained as hatching eggs from the Basic Research Laboratory, Hy-Line Poultry Farms (Johnston, Iowa), through the courtesy of Dr. G. R. J. Law. They came from matings of H1 ♂ × H36 ♀ (15), the parents being chosen from segments of these inbred lines homozygous respectively for alleles B^2 and B^{14} (15) of the major blood-group/histocompatibility locus (14). Eggs were incubated in a Jamesway model 252 incubator (James Manufacturing Co., Ft. Atkinson, Wis.). The chicks were kept in a tier-brooder until 6 wk old, and afterwards in cages, with free access at all times to feed and water.

Hormonal Bursectomy.—Embryos were injected into the allantoic cavity on the 12th day of incubation with 3.7 mg (0.15 ml) of testosterone propionate in corn oil.

Preparation of Cell Suspensions.—Donor chicks were killed by cervical dislocation at various ages from 4–11 wk. The bursa of Fabricius, spleen, and thymus were dissected out into ice-cold Hanks' balanced salt solution (BSS), and single cell suspensions were prepared as described previously (16). The cells were washed three times in BSS containing 5% chicken serum, and finally resuspended in the same medium at the cell concentration required for injection in 0.5 ml.

Irradiation.—Recipient chicks were irradiated within 24–48 hr of hatching. They usually received 600 r, at a dose rate of 275 r/min as measured in air, and at a target distance of 50 cm. The X-irradiation unit (Maxitron, General Electric Co., Long Island City, N.Y.) was run at 300 kv, 20 ma, and with 2.0 mm of copper external filtration.

Injections.—Recipient chicks were injected intraperitoneally on day 2 or 3 after hatching with $0.5–3 \times 10^8$ cells. In a few experiments injection was intravenous, into the jugular vein. Antigenic challenge was made intraperitoneally either immediately or at varying time intervals, and used either 0.1 ml 20% sheep erythrocytes ($\sim 2 \times 10^8$ cells), 0.1 ml killed *Brucella*

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abortus, as supplied by the United States Department of Agriculture for the ring test, diluted 1:1 (~6 × 10^8 organisms), or both of these together.

The same antigenic doses as above were used in primary immunizations of unirradiated chicks at ages up to 3 wk, either for measuring the antibody response or for providing pre-immunized cell donors. Chickens aged 4 wk received a double dose.

Bleedings and Titrations.—In all cases, chicks were bled by cardiac puncture 7 days after antigen challenge. Titrations of serum agglutinins to sheep erythrocytes (SE) or *Brucella abortus* (BA) were made at room temperature. Some of the more potent sera were also titrated for mercapto-ethanol-resistant antibodies, after dilution 1:5 and incubation with an equal volume of 0.2 M 2-mercapto-ethanol for 3 hr at room temperature. This treatment has been shown to produce near-maximal inactivation of 19S antibody with little loss of 7S (17).

RESULTS

Effect of Irradiation on Normal Primary Immune Response.—As shown in Table I, the capacity of intact chicks to produce a primary immune response

TABLE I

Effect of Age on Primary Immune Response to Brucella abortus in Nonirradiated Chicks

Age at injection	*Brucella abortus*	
	No. recipients responding/No. injected	Mean $\log_2$ titer of responders*
2 days	1/8	3.0
1 wk	8/8	4.6
2 wk	5/5	10.0
4 wk	5/5	15.6

* Mean of $\log_2$ reciprocal of serum agglutinin titer in positive recipients 1 wk after immunization.

to BA developed soon after hatching. A similar rate of development has been reported (18) for the primary immune response to SE, although somewhat lower 4-wk titers were found. In the transfer experiments, all recipient chicks were irradiated 1–2 days after hatching to suppress their immune responses. The effectiveness of such treatment was checked in each experiment by including a group of six to eight irradiated controls receiving antigen but no cells. None of a total of 67 such controls injected once with BA on either day 2 or 7 posthatching showed any response; the corresponding ratio for SE was 0/68.

Histocompatibility and Transfer.—In early experiments, attempts were made to equip 2-day-old irradiated outbred recipients with the capacity to produce a primary immune response, by transfer of lymphoid cells from 4-wk-old outbred donors. The proportion of recipients developing a primary immune response was low, even when spleen cells were transferred. These poor results may have been due to cell damage resulting from reactions caused by the strong histocompatibility differences known to occur between most outbred chickens. In further work, transfers were therefore made between F_1 chicks derived from a

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cross of two considerably inbred lines isogenic for the strong *B* blood-group/histocompatibility locus.

The extent of histocompatibility differences due to loci other than the major *B* locus was investigated by a series of skin grafts. 20 chicks were grafted at 18 days of age, using a previously described technique (19). The donors and recipients were arranged in a ring design, 1-to-2, 2-to-3, etc., ending with 20-to-1. The strongest reactions occurred in the five female-to-male pairings. In these, a mild reaction was first seen variously on days 12–19 after grafting, and total rejection occurred only several weeks later. These weak responses, representing mainly the effect of the heterogametic (or W) antigen (19), are in marked contrast to the well-known vigorous responses to a *B* locus difference (onset on

TABLE II

Primary Immune Response to Brucella abortus and Sheep Erythrocytes in Irradiated Chicks Receiving 2 × 10⁸ Lymphoid Cells Intraperitoneally

Cells transferred	Age of donor	*Brucella abortus*		Sheep erythrocytes	
		No. recipients responding/No. injected	Mean $\log_2$ titer of responders*	No. recipients responding/No. injected	Mean $\log_2$ titer of responders*
	wk				
Bursa	4	11/13	10.5	0/27	—
Spleen	4	12/14	8.6	26/36	5.4
Thymus	4	2/8‡	5.0	1/22	3.0
Bursa	10–11	18/19	11.5	2/20	1.5
Spleen	10–11	30/32	12.4	30/31	5.8
Thymus	10	6/7‡	9.3	2/15	3.0

* Mean of $\log_2$ reciprocal of serum agglutinin titer in positive recipients.

‡ Results of a single experiment; in all other cases, the values are pooled from two to five experiments with similar results.

days 3–4, total rejection by days 7–8). The responses in the 15-male-to-female or like-sex pairings were even weaker. Five had shown no rejection reaction at the end of observations (54 days), while the other 10 showed onsets at times up to 50 days. None of these reacted as early or as strongly as in the female-to-male pairings. It, therefore, seems unlikely that there were any histocompatibility differences among these F_1 chicks strong enough to affect the lymphoid cells transferred.

Transfer of Primary Immune Response.—Irradiated chicks at 2–3 days of age were injected intraperitoneally with suspensions of lymphoid cells, and challenged with antigen by the same route either immediately or 2 hr later. Their primary immune responses are shown in Table II. With 4-wk-old donors, bursal cells were more effective than splenic cells in transferring a response to BA, while thymic cells were much less active. Splenic cells from 10–11-wk-old donors gave a somewhat higher response to BA than bursal cells, and at this age thymic

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cells were only slightly less active. The responses to SE were quite different. At both donor ages splenic cells appeared capable of transferring a response to this antigen, whereas neither bursal nor thymic cells showed any appreciable capacity to form antibody to SE after transfer.

Where transfer was successful, the level of antibody response was dependent upon the cell dose. For example, when only 1×10^8 bursal or splenic cells were transferred instead of the usual 2×10^8, the mean titers of agglutinins to BA were lower than those given in Table II by 1–2 logs, but almost all recipients still responded. In contrast, increasing the number of bursal cells to 3×10^8 did not produce any appreciable response to SE, even when 10-wk-old donors were used.

Synergism between Cell Types.—One possible reason for the difference between the results obtained with BA and SE is that synergism between cell types might be required for the response to SE, but not for that to BA. Such synergism has been reported in mice, where it was found that the immune response to SE depends on a cooperation between thymus-derived and bone marrow–derived cells (20, 21). Representative tests of synergism in the response to SE are recorded in Table III. With 4-wk-old donors, a dose of 2×10^8 splenic cells produced somewhat higher titers when mixed with an equal number of bursal cells than when given alone (Exp. 1 and 2). In addition, a total of 2×10^8 equally mixed bursal + splenic cells was as effective as 2×10^8 splenic cells when 10-wk-old donors were used (Exp. 4), and only slightly less effective with 4-wk-old donors (Exp. 2). There results are suggestive, but hardly sufficient to warrant a definite conclusion of synergism. With thymus + spleen, the results were even less convincing (Exp. 2, 3). No evidence for synergism was obtained in the thymus and bursa combination (Exp. 3, 5, 6).

From the representative results recorded in Table IV, there was clearly no evidence at all for synergism between bursa and spleen in the response to BA. The titers produced by cell mixtures were either similar to the titers produced by either cell population alone (Exp. 7–9), or were intermediate between them (Exp. 10, 11).

Delayed Injection of Antigen.—When SE were injected 5 days after cell transfer (Table III, Exp. 4), bursal cells from 10-wk-old donors were effective in transferring a response. Similar cells were quite ineffective in the same or higher dose (Exp. 5, 6), if SE were injected shortly after cells. In another experiment, the same difference was obtained with 6-wk-old donors and a 4 day delay. In either case, the immune response of splenic cells was little affected by antigen delay. These results may indicate that bursal cells need a period either for maturation or for interaction with cells in the recipient spleen, before they can react to the SE antigen.

Delay in injecting BA (Table IV, Exp. 10) led to some lowering of the response of transferred bursal cells, possibly owing to attrition of effective cells.

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Modification of Transferred Cell Activity in the Hosts.—In a few experiments, the cells were transferred intravenously rather than intraperitoneally. This led to some increases in their response to BA (e.g., Table IV, Exp. 9), but synergism was still absent.

An increased level of irradiation of recipients, e.g., 900 r (Table IV, Exp 11) instead of 600 r, also led to marked increases in titers to BA, although only about half of the recipients survived long enough to be bled. These higher titers

TABLE III

Effect of Cell Mixture on Primary Immune Response to Sheep Erythrocytes in Irradiated Chicks Receiving Lymphoid Cells Intraperitoneally

Exp. No. & Donor age	Cells transferred	No. of cells	No. recipients responding/No. injected	Mean $\log_2$ titer of responders*
		$\times 10^8$		
1. 4 wk	Bursa	2	0/9	—
	Spleen	2	9/9	4.6
	Bursa + spleen	2 + 2	8/8	6.9
2. 4 wk	Bursa	2	0/3	—
	Spleen	2	4/7	4.0
	Bursa + spleen	2 + 2	4/8	5.2
	Bursa + spleen	1 + 1	3/6	3.3
	Thymus	2	1/8	3.0
	Thymus + spleen	2 + 1	7/9	3.4
3. 4 wk	Bursa	2	0/7	—
	Spleen	1.5	5/10	5.0
	Thymus	1.5	0/6	—
	Thymus + spleen	0.75 + 0.75	3/5	4.0
	Thymus + bursa	0.75 + 1	2/7	2.0
4. 10 wk‡	Bursa	2	8/11	2.9
	Spleen	2	11/11	5.9
	Bursa + spleen	1 + 1	10/11	6.0
5. 10 wk	Bursa	3	0/9	—
	Spleen	3	8/8	5.1
	Thymus	3	2/7	3.0
	Thymus + bursa	1.5 + 1.5	0/9	—
6. 10 wk	Bursa	2	0/8	—
	Spleen	2	4/8	2.2
	Thymus	2	0/8	—
	Thymus + bursa	2 + 2	0/8	—

* Mean of $\log_2$ reciprocal of serum agglutinin titer in positive recipients.

‡ Antigen injected 5 days after cells; in all other experiments, 2–5 min after.

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may have resulted from either a better penetration ("homing") of the donor cells in the lethally irradiated recipients, or inhibition of a weak host-*versus*-graft histocompatibility reaction.

The possible involvement of radio-resistant host cells was tested by comparing the responses to BA or SE in hormonally bursectomized vs. intact recipients, with both groups irradiated as usual, and using 5-wk-old donors. The

TABLE IV

Effect of Cell Mixture on Primary Immune Response to Brucella abortus in Irradiated Chicks Receiving Lymphoid Cells Intraperitoneally

Exp. No. & Donor age	Cells transferred	No. of cells	No. recipients responding/ No. injected	Mean $\log_2$ titer of responders*
		$\times 10^8$		
7. 4 wk	Bursa	2	8/9	10.2
	Spleen	2	7/9	9.6
	Bursa + spleen	1 + 1	9/9	9.0
8. 5 wk	Bursa	1	5/19	7.4
	Spleen	1	19/22	8.2
	Bursa + spleen	0.5 + 0.5	8/21	6.4
9. 5 wk‡	Bursa	1	6/6	10.2
	Spleen	1	3/6	11.0
	Bursa + spleen	0.5 + 0.5	6/6	10.7
10. 10 wk§	Bursa	2	4/11	4.8
	Spleen	2	11/11	10.5
	Bursa + spleen	1 + 1	11/11	7.2
11. 11 wk‖	Bursa	2	11/11	11.6
	Spleen	2	4/5	15.1
	Bursa + spleen	1 + 1	4/4	14.6

* Mean of $\log_2$ reciprocal of serum agglutinin titer in positive recipients.
‡ Cells injected intravenously; in all other experiments, intraperitoneally.
§ Antigen injected 5 days after cells; in all other experiments, 2–5 min after.
‖ Recipients irradiated 900 r; in all other experiments, 600 r.

titers obtained in the two groups were closely similar to each other, whether bursal or splenic cells were transferred.

Bursa-Dependence of Transferred Cells.—When hormonally bursectomized chicks were used as 4-wk-old donors, 2×10^8 splenic cells were quite ineffective in transferring a response to BA (no titer in nine out of nine recipients). Even when the bursectomized donors had been injected with either BA or SE 1 wk before transfer, there was no response to BA in five out of five recipients of 10^8 splenic cells, nor to SE in four out of four recipients of 2×10^8 splenic cells.

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Effect of Preimmunization of Donors.—Unirradiated chicks were given one injection of antigen at either 11 or 21 days of age, and used at 4 wk of age as cell donors in transfers. The results are given in Table V. As expected, the splenic cells gave higher titers against both antigens than in previous transfers (Tables II and IV). Both bursa and thymus were also affected by the preimmunization, since their cells could now transfer a response to SE, although they could not do so before (Table II). The augmentation of their response to BA was much more marked with thymic cells (see Table II) than with bursal cells (see Tables II and IV). Indeed, it appeared that there was a depletion of bursal reactivity when the interval between preimmunization and transfer was 7 days, and an augmentation when it was 17 days.

TABLE V

Effect of Time of Preimmunization of Donors on Immune Response to Brucella abortus and Sheep Erythrocytes in Irradiated Chicks Receiving Lymphoid Cells Intraperitoneally

Time between immunization and transfer	Cells transferred	No. of cells	*Brucella abortus*		Sheep erythrocytes	
			No. recipients responding/ No. injected	Mean $\log_2$ titer of responders*	No. recipients responding/ No. injected	Mean $\log_2$ titer of responders*
days		$\times 10^8$				
7	Bursa	1	8/10	5.2		
17	Bursa	1	10/10	12.0	5/10	2.0
7	Spleen	2			8/8	10.3
7	Spleen	1	8/8	16.3		
17	Spleen	1	8/8	13.6	8/8	7.9
7	Thymus	1	10/10	12.7		
17	Thymus	1	10/10	12.7	10/10	3.4

Donors were 4 wk old at transfer, and had received one injection of antigen 7 or 17 days previously.

* Mean of $\log_2$ reciprocal of serum agglutinin titer in positive recipients.

Classes of Antibody Made by Transferred Cells.—In order to test their relative content of 19 and 7S (IgM and IgG) antibodies, some of the more powerful anti-BA sera were retitrated after incubation with mercapto-ethanol. Of 13 primary immune sera from chicks receiving bursal cells, with an original mean $\log_2$ titer of 10.8, none showed any residual activity. Of 10 similar sera (original mean, 10.6) from recipients of splenic cells, only four showed residual 7S titers, ranging from 1:10 to 1:80 (mean $\log_2$ titer, 4.1).

The secondary immune sera made by transferred preimmunized cells were also tested for the presence of mercapto-ethanol–resistant antibody. The results, given in Table VI, involve all the positive anti-BA sera listed in Table V, except for the group of weak sera made by 7-day "immune" bursal cells, and one other serum of which there was not enough left for retesting. All the secondary sera

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had a 7S component, and the ratio of 7S:19S in sera from recipients of splenic cells was much higher when the interval between immunization and transfer was 17 rather than 7 days. This difference was less for thymic cells. Sera from bursal cell recipients had a much higher over-all titer when the interval was 17 rather than 7 days, as well as an appreciable 7S component.

DISCUSSION

Our main finding was that a primary immune response to a bacterial antigen could be transferred to neonatally X-irradiated recipients by cells from the bursa. This suggests that precursors of antibody-producing cells are present in this organ. It seems likely that there is some relationship between this property

TABLE VI

Effect of Mercapto-Ethanol on Immune Sera to Brucella abortus from Irradiated Chicks Receiving 10^8 Preimmunized Lymphoid Cells Intraperitoneally

Time between immunization and transfer	Cells transferred	No. of sera	Mean $\log_2$ titer*	
			Untreated	Preincubated with Mercapto-ethanol
days				
	Bursa	8	5.2	—
7	Spleen	8	16.3	6.5
	Thymus	8	14.9	6.0
	Bursa	10	12.0	6.5
17	Spleen	7	13.9	11.0
	Thymus	10	12.7	8.0

Donor were 4 wk old at transfer, and had received one injection of antigen 7 or 17 days previously.

* Mean of $\log_2$ reciprocal of serum agglutination titers.

of the bursa and the previously demonstrated capacity of bursal cells to synthesize IgM and IgG in tissue culture, particularly since in both functions the capacity of the bursa precedes that of the spleen. Thus, IgM and IgG production developed earlier in bursa than in the spleen during ontogeny (11), and the capacity to form antibody to BA upon transfer was greater in bursal than in splenic cells of 4-wk-old chicks. Both properties, therefore, appear related to a specific function of the bursa as a central lymphoid organ rather than to a contamination of bursa with cells from peripheral lymphoid tissue. Thymic cells showed much less activity in the transfer of antibody synthesis to *Brucella* antigen, as well as in immunoglobulin production in tissue culture.

Injection of donors with BA 7 days before transfer led to increases in the strength of the response only when splenic or thymic cells were transferred. This suggests, as shown previously (9), that the bursa was not directly affected by the preimmunization. However, between 7 and 17 days after immunization,

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the response following transfer of bursal cells increased appreciably. Thus, when transfer was 17 days after the preimmunization, the bursa could produce almost as strong a secondary response as the thymus, although somewhat weaker than the spleen, especially in the 7S component. It remains uncertain whether part of the sensitization process may have occurred actually within the bursa, or whether the memory cells present in the bursal preparations were derived by immigration from peripheral lymphoid tissue.

As previously briefly reported (22), no antibody response could be transferred with splenic cells from hormonally bursectomized 4-wk-old donors. This was so even if the donors had been previously injected with either antigen. These results confirm that the development of splenic capacity to mount an antibody response is dependent on the presence of an intact bursa during ontogeny. This influence of the bursa may be mediated at least in part by hormonal means, although the data supporting this view have been criticized (23). On the other hand, our finding that bursal cells could themselves transfer antibody formation strongly suggests rather that the mechanism of influence is that the precursors of splenic antibody-forming cells originate in the bursa. These cells may even be already competent within the donor bursa; but if not, they clearly develop competence within the host rapidly enough to produce a primary response within 7 days. Any such development within the irradiated hosts does not require the presence of an intact bursa of Fabricius, since use of bursectomized recipients had no influence on the incidence and level of the response transferred by bursal cells.

At 4 wk of age, the order of effectiveness of donor cells in transferring a primary response to BA was bursa, spleen, thymus; but by 10–11 wk of age the order had changed to spleen, bursa, thymus. This changeover between bursa and spleen parallels the previous findings (11) that, although immunoglobulin production was detectable earlier in bursa than in spleen, the spleen became more active than the bursa within a few weeks after hatching.

It seems strange that bursal cells should be superior to splenic cells in transfer of the response to *Brucella* antigen, but much less active, if at all, in transferring a response to sheep erythrocytes. One possibility is that the ability of splenic cells to transfer a weak response to the latter antigen may be the result of a certain degree of sensitization in the donors, due to the presence of cross-reacting antigens in the intestinal flora. The ability of bursal cells to transfer a response would then remain below detectable levels. Another more likely explanation might be that three cell types are needed for the response to SE, as in mice (24–26), and that at least one of them is lacking in bursa, in thymus, and in the combination from these two, while all three are present in splenic cell suspensions from 4-wk-old donors. Some evidence was obtained in the synergism experiments to suggest that bursal and splenic cells may cooperate in the primary response, but it was not very conclusive. It is possible that the

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intraperitoneal route employed in most of our experiments is not so effective in demonstrating synergism as the intravenous route used in mice (20, 21). In addition, a double exposure to antigen might be required. Attempts to resolve this question might, therefore, involve the use of older recipients where repeated intravenous injections are more feasible.

Bursal cells did show some capacity to transfer a primary response to SE when antigen injection was delayed 4–5 days after cell transfer. This result may have been dependent upon some degree of maturation of donor bursal cells during this time, or possibly upon a recovery of some cooperating cell types of the recipients, or both. In any event, the bursal cells contributed at least some part of the immune capacity, since no control chick receiving SE after this time interval ever showed any response.

It would be of considerable interest to examine whether the difference in the responses of 4-wk-old bursal cells to BA or SE extends to other antigens. So far, we have found in preliminary experiments that bacteriophage appears more like BA, in that cells from all three organs can transfer a response, while bovine gamma globulin resembles SE, in that only splenic cells are effective.

Our experiments showed more success in achieving transfer of immune responses with dispersed lymphoid cells than any previously reported. This was probably caused by our use of F_1 chicks which were isogenic at the major *B* histocompatibility locus (analogous to H-2 in mice), since we thereby avoided damage to the transferred cells by powerful host-*versus*-graft reactions. Trnka and Riha (12) demonstrated that such reactions were an important factor in reducing the effectiveness of transferred cells. Using outbred chickens they found that adult splenic cells were only moderately effective in transferring a primary immune response to *Brucella suis* when the recipients were 2-day-old chicks (mean $\log_2$ titer, 5), but were highly effective when they were embryos at 18 days of incubation (mean, 12). In the latter cases, but not the former, all responding chicks developed graft-*versus*-host disease and died by 12 days old. Thus, effective antibody production went in parallel with functional survival of the foreign cells as evidenced by their production of graft-*versus*-host disease.

Although our chicks were also relatively homogeneous for other weak histocompatibility loci, presumably because the parental lines were somewhat inbred (15), it is doubtful that this had an influence on the success of cell transfers comparable with that of the major locus. Irradiation of the recipients could also not have been sufficient in itself to ensure acceptance of the transferred cells without the matching at the *B* locus, as shown by the relative failure of our own preliminary experiments using irradiated outbred recipients. On the contrary, we found that in experiments with the F_1 isogenic chicks, the primary response to BA could be successfully transferred to nonirradiated 2-day-old chicks, even with bursal cells.

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SUMMARY

F_1 hybrid chicks isogenic for the strong *B* histocompatibility locus and for most weak *H*-loci were X-irradiated on day 1 after hatching, injected intraperitoneally on day 2 with dispersed cells of bursa, spleen, or thymus from 4- or 10-wk-old F_1 hybrid donors, and immediately challenged by the same route with either *Brucella abortus*, sheep erythrocytes, or a mixture of both together. The agglutinin titers were measured in sera obtained 1 wk later. With 4-wk-old donors, a greater primary response to *Brucella abortus* was obtained after transfers of cells from bursa than from spleen, while thymus was much less effective. With 10-wk-old donors, the decreasing order of response was spleen, bursa, thymus.

Only splenic cells were effective in transferring a response to sheep erythrocytes, at either donor age. In tests of synergism by cell mixtures from pairs of organs, the only positive finding was a modest augmentation of titer against sheep erythrocytes by bursa + spleen as compared with spleen alone. Bursal cells from 6- or 10-wk-old donors were effective in transferring a response to sheep erythrocytes when antigen injection was delayed until 5 days after cell transfer.

Splenic cells from hormonally bursectomized donors were ineffective in transferring a primary response, even when the donors had been injected with antigen 1 wk before transfer. Preimmunization of normal donors led to marked increases in the responses to *Brucella abortus* produced by transferred splenic or thymic cells. With bursal cells, an increased response was obtained only if the interval between preimmunization and transfer was 17 rather than 7 days. With the 17-day interval, both bursal and thymic cells could also transfer a response to sheep erythrocytes.

The primary sera to *Brucella abortus* produced after transfers of bursal or splenic cells contained almost entirely 19S antibodies. A 7S component was found in all the secondary sera tested.

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