ONTOGENY OF BURSAL FUNCTION IN CHICKEN

III. Immunocompetent Cell for Humoral Immunity*

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During our studies on the progenitor or stem cell responsible for humoral immunity (1, 2) it became evident that the organ distribution of the stem cell does not parallel the distribution of immunocompetent cells. In the present work, we have studied the occurrence of immunocompetent cells in different chicken tissues during different stages of ontogeny. For this purpose, immunocompetent cells for humoral immunity are considered those cells which react with antigens and produce antibodies, without defining how many cell types are involved.

Previously, different methods have been applied to studies of the immunocompetent cells in avian tissues. Studies by several investigators (3–5) have demonstrated that spleen cells of immunized adult and young chickens adhere and lyse sheep red blood cells (SRBC),[1] whereas cells taken from bursa of Fabricius are inactive in this respect. The earliest studies on antibody response by transferred cells suffered from allogeneic rejection (6, 7). To avoid such rejection, newly hatched and embryonic recipients have been used to demonstrate antibody production by spleen, bone marrow, and thymus cells from immunized and normal donors (8–16). In these models, however, the transplanted cells induced a graft-vs.-host reaction in the young recipients. Graft-vs.-host reactions were also obtained when hormonally bursectomized recipients were used (13, 17). In surgically bursectomized chickens, bursal transplants, as such or in a diffusion chamber, were found to increase antibody synthesis if the chickens were stimulated within 3 days after transplantation (18–21).

The histoincompatibility reactions can be mostly avoided by having birds isogeneic at the *B* locus. Using this model, Orlans and Rose (22) showed that preimmunized

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[1] *Abbreviation used in this paper:* SRBC, sheep red blood cells.

cells taken from cecal tonsil, blood, and peritoneal exudate produced antibodies to human serum albumin when transferred into adult chickens. Gilmour et al. (23) transferred bursa cells into newly hatched irradiated chicks and found that these cells produced antibodies to *Brucella abortus* but not to SRBC; when spleen cells were used, antibody response to both *Brucella* and SRBC was obtained. Similar findings with bursa cells were reported by Weber and Weidanz (24) using 4-wk old irradiated recipients treated in ovo with testosterone and cyclophosphamide. After transplantation of spleen cells, they could demonstrate a response to SRBC but not to *Brucella*.

In the present work, the occurrence of immunocompetent cells for humoral immunity has been studied in embryonic liver, yolk sac, bursa, bone marrow, spleen, and thymus. Cyclophosphamide-treated immunoincompetent chicks have been injected with cells from normal donors identical with recipients at the *B* locus. The same period of ontogeny has been considered as in prior studies with the bursal stem cell (1, 2). The results obtained reveal a different distribution of immunocompetent cells for responses to SRBC and *Brucella*. The distributions observed are compared with the distribution of the stem cells for humoral immunity, and it becomes evident that the presence of immunocompetent cells in an organ does not indicate the presence of stem cells in the same organ or vice versa.

Materials and Methods

Experimental Design.—The major experiments on ontogeny of antibody production by cells from different organs were carried out by injecting 4-day old cyclophosphamide-treated chicks intravenously with lymphoid cells together with SRBC and *Brucella abortus*. 4 days later the chicks were stimulated with the same antigens intraperitoneally. For the cellular cooperation experiments, only SRBC were used as antigen. Antibody titrations were always carried out from samples taken 4 days after the second stimulation.

Chickens.—In the experiments concerning antibody response by transferred cells, the recipients and donors were White Leghorn F_1 hybrids homozygous at the major histocompatibility locus, blood type B^2B^2. All donors were normal unimmunized chickens. Cells taken from a specific organ were pooled before transfer; at least three donors were used for each experiment. In the experiments on graft-vs.-host activity, embryos and chickens of a White Leghorn line, uniformly heterozygous F_1 hybrids B^2B^{15}, and embryos of a random-bred White Leghorn line were also used. Hatching eggs for the lines defined at the *B* locus were obtained from the Basic Research Laboratory, Hy-Line Poultry Farms (Johnston, Iowa) and for the random-bred line from the Ghostley Hatcheries (Anoka, Minn.). The specific details for the incubation of eggs as well as for the housing and care of the chickens have already been given (1).

Cyclophosphamide Treatment.—Cyclophosphamide (Cytoxan; Mead Johnson Laboratories, Evansville, Ind.) was injected intraperitoneally as freshly prepared aqueous solution 2 mg/0.1 cc per chick daily on 4 consecutive days starting on the day of hatching.

Preparation and Injection of Single Cell Suspensions, Antibody Titrations, and the Statistics.—Methods employed in this investigation have also been described (1).

Antigenic Stimulation.—In the experiments on the ontogeny of antibody production by cells from different organs, 0.1 cc of packed SRBC and 0.1 cc of 1:50 dilution of *Brucella abortus* (U.S. Department of Agriculture) were given simultaneously (2×10^9 SRBC and 5×10^8 *Brucella* organisms per chicken). The antigens were administered intravenously with

lymphoid cells 1 day after the last cyclophosphamide injection in a total volume of 0.5 cc. 4 days later, the same amount of both antigens in 0.5 cc was given intraperitoneally. The same schedule was followed in the experiments on cellular cooperation, except that 0.1 cc of packed or 20% SRBC were used instead of SRBC and *Brucella* together. The animals were always bled by heart puncture 4 days after the second stimulation. This scheme has been applied earlier in mice by Claman et al. (25–27).

Assay of Graft-Versus-Host Activity.—Spleen cells and heparinized (5 units/cc) heart blood taken from cyclophosphamide-treated and from normal, untreated chicks at various ages were injected intravenously into 12-day old embryos. Spleen cells from several donors were pooled before transfer; peripheral blood from each donor was used individually. The eggs were opened 7 days later, and spleen indices were determined as usual (28). As donors and recipients, a parental-F_1 hybrid combination with respect to the *B* locus was used: B^2B^2 line as donors and B^2B^{15} as recipients. Also, embryos of a random-bred line were used as recipients; they were injected with cells taken from B^2B^2 and B^2B^{15} lines.

TABLE I

Antibody Response of Cyclophosphamide-Treated 4-Day Old Chicks and of Normal, Untreated Chicks at Different Ages

Age at first stimulation	Treatment	SRBC			*Brucella*	
		No. of responders/ total	Mean ± SD $\log_2$ titer of responders		No. of responders/ total	Mean ± SD $\log_2$ titer of responders
			Direct	Indirect		
days						
4	CY	0/65	—	—	0/65	—
4	CY	0/47*	—	—	0/42*	—
4	None	15/15	3.7 ± 1.7	5.9 ± 1.5	5/15	3.2 ± 1.3
11	"	10/10	4.9 ± 1.5	7.7 ± 2.0	4/10	3.8 ± 2.2
18	"	12/12	4.7 ± 0.8	10.7 ± 1.3	10/12	4.6 ± 1.3
25	"	10/10	4.8 ± 0.9	9.5 ± 1.9	10/10	6.8 ± 0.9
32	"	12/12	6.2 ± 1.6	10.1 ± 1.1	12/12	9.2 ± 1.0

Cyclophosphamide (CY) was given 2 mg/chick per day on 4 consecutive days beginning on the day of hatching.

Both antigens (0.1 cc of packed SRBC and 0.1 cc of 1:50 *Brucella abortus*) were given together in 0.5 cc, intravenously 1 day after the final injection with cyclophosphamide, and intraperitoneally for the second stimulation 4 days later. Bleeding was carried out 4 days after the second stimulation.

* These birds were stimulated with either SRBC or Brucella alone.

RESULTS

Immunocompetence of Cell Recipients.—To ascertain that antibody responses were due to the function of transferred cells and not to the immune reaction of the recipients, humoral and cellular immunocompetence of the hosts was evaluated.

It has been previously reported that chickens treated in the newly hatched period with cyclophosphamide are severely deficient in humoral immunity when tested at the age of a month or later (1, 2, 29–31). Cyclophosphamide-treated controls included in the present study and injected only with the antigens indi-

TABLE II

Graft-Versus-Host Activity of Lymphoid Cells Taken From Cyclophosphamide-Treated Chickens

Donors				Cells injected	Recipients			P*
Strain	Treatment	Age	Number		Strain	Number	Spleen index Mean ± SD	
		days						
B^2B^2	None	5	11	7×10^5 spleen cells	B^2B^{15}	8	1.36 ± 0.38	
"	CY	5	11	7×10^5 " "	"	5	1.02 ± 0.19	
"	None	5	4	0.2 cc blood	"	6	1.35 ± 0.12	<0.001
"	CY	5	4	0.2 " "	"	4	0.97 ± 0.09	
"	None	13	6	7×10^5 spleen cells	"	6	2.02 ± 0.27	<0.01
"	CY	13	9	7×10^5 " "	"	7	1.38 ± 0.29	
"	None	13	6	7×10^6 spleen cells	"	7	2.22 ± 0.42	<0.01
"	CY	13	9	7×10^6 " "	"	7	1.41 ± 0.39	
"	None	13	3	0.2 cc blood	"	5	3.90 ± 0.86	<0.05
"	CY	13	3	0.2 " "	"	5	2.50 ± 0.53	
"	None	21	6	7×10^5 spleen cells	Random bred	12	3.33 ± 1.62	<0.01
"	CY	21	6	7×10^5 " "	" "	12	1.87 ± 0.77	
"	None	21	6	7×10^6 spleen cells	" "	7	3.40 ± 0.78	
"	CY	21	6	7×10^6 " "	" "	13	2.94 ± 1.06	
"	None	21	3	0.1 cc blood	" "	13	5.12 ± 1.29	
"	CY	21	3	0.1 " "	" "	9	4.37 ± 1.79	
B^2B^{15}	None	8	5	7×10^5 spleen cells	" "	10	2.86 ± 0.27	<0.001
"	CY	8	15	7×10^5 " "	" "	12	1.16 ± 0.31	
"	None	8	5	2×10^6 spleen cells	" "	8	3.24 ± 0.71	<0.001
"	CY	8	15	2×10^6 " "	" "	9	1.65 ± 0.78	
"	None	8	7	0.1 cc blood	" "	18	4.68 ± 2.26	<0.001
"	CY	8	5	0.1 " "	" "	26	2.24 ± 1.45	
Controls								
—	—	—	—	None	B^2B^{15}	13	1.01 ± 0.22	
—	—	—	—	None	Random bred	6	0.90 ± 0.26	
B^2B^2	None	5 + 13	11 + 6	7×10^5 or 7×10^6 dead‡ spleen cells	B^2B^{15}	20	1.00 ± 0.25	
B^2B^2	None	21	6	7×10^6 dead spleen cells	Random bred	13	1.00 ± 0.29	
B^2B^{15}	None	8	5	2×10^6 dead spleen cells	" "	13	1.00 ± 0.22	

* Only values <0.05 are given.

‡ 1 hr at 56°C.

cate that cyclophosphamide-induced immunological unresponsiveness begins immediately after the treatment (Table I).

Table I shows also the development of antibody responses in intact untreated chicks. At the age of 4 days, 15 of 15 birds responded with moderate antibody titers to SRBC, whereas only 5 of 15 birds responded to *Brucella*. When stimulated at the age of 25 days, 10 of 10 normal birds produced also *Brucella* antibodies.

Cellular immunocompetence of cyclophosphamide-treated chicks was determined by means of the graft-vs.-host reaction. The capacity of spleen cells and peripheral blood to induce splenomegaly in 12-day embryos was determined (Table II). When cells from 5-, 13-, and 21-day B^2B^2 donors were injected into B^2B^{15} embryos, the cyclophosphamide-treated donors were deficient in graft-vs.-host activity when compared with normal untreated donors. The difference was clearer with cells from 5- and 13-day old donors than with cells from 21-day donors. This finding cannot, however, be taken as evidence of absolute recovery of immunocompetence by 21 days, since even in the earlier stages use of a too large cell dose for testing can obscure the differences between treated and untreated birds.

It has been demonstrated that graft-vs.-host competence of B^2B^2 lymphoid cells is less than that of lymphoid cells with other genotypes (32, 33). To determine whether this phenomenon might have influenced our results, 8-day old B^2B^{15} donors were used to assess the graft-vs.-host activity of cyclophosphamide-treated chicks. The results (Table II) confirm those obtained when cells of B^2B^2 donors were used.

These findings indicate that chickens treated in the newly hatched period with cyclophosphamide are inferior in cellular immunity to normal chicks of the same age. At the age of 4 days (time of lymphoid cell transfer used in this study) the cyclophosphamide-treated animals are completely immunoincompetent, These findings also indicate the inferiority in cellular immunity up to the age of 21 days, and data published by others (29, 34) reveal it also later in life.

Distribution of Immunocompetent Cells Responsible for Humoral Immunity.— 2×10^7 lymphoid cells from bursa, bone marrow, spleen, and thymus from donors of different ages were injected intravenously together with SRBC and *Brucella abortus* into cyclophosphamide-treated 4-day old chicks. A second antigenic stimulation was given intraperitoneally 4 days later. Antibodies to both antigens were determined from samples taken 4 days after the second stimulation.

The antibody responses to SRBC are illustrated in Fig. 1. With increasing age of donors, spleen cells are the first to respond to SRBC in 100% of the recipients; these cells were taken from 3½-wk old donors. Thymus and bone marrow cells elicit a similar response when obtained from 7- and 12-wk donors, respectively. With all of these three cell types, the number of responding recipients increases with the age of donors, and is maintained at 80–100% after reaching that level. Bursa cells also give an antibody response in an increasing number of recipients, reaching a 45% level at the donor age of 12 wk. Contrary to the results with other cells studied, bursa cells do not form antibodies to SRBC when taken from 17-wk donors. Direct and indirect hemagglutinin titers with all cell types parallel roughly the number of responding hosts except when cells taken from 17-wk donors were used. In such samples a slight reduction, especially in the direct titers, was found when comparison was made with the response by cells taken from younger animals.

The antibody responses to *Brucella* are shown in Fig. 2. Bursa cells, from chicks aged 3½ wk, are the first to respond in 100% of the recipients. By 12 wk of age, spleen and thymus cells also elicit a similar response. For bone marrow cells, such a response is obtained at 17 wk of age. Agglutinin titers parallel the number of responders and rise steadily with the increasing age of donors. Con-

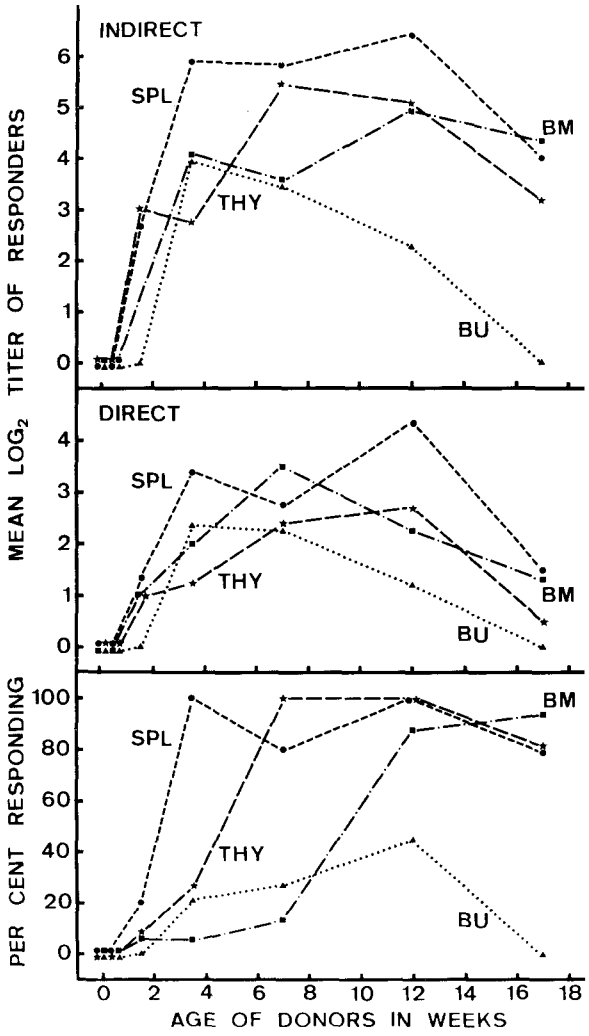

FIG. 1. Antibody response to SRBC by 2 × 10^7 lymphoid cells injected into cyclophosphamide-treated 4-day old chicks. SRBC were given intravenously together with *Brucella* and lymphoid cells; for the second stimulation both antigens were given intraperitoneally 4 days later. Bleeding was carried out 4 days after the second stimulation. Each dot represents 14-24 recipients. *SPL* = spleen cells, *THY* = thymus cells, *BU* = bursa cells, *BM* = bone marrow cells.

trary to the findings with SRBC antibodies, anti-*Brucella* response by bursa cells does not significantly decline at 17 wk of age. These observations indicate a different distribution of immunocompetent cells for anti-SRBC response and for anti-*Brucella* response.

With a dose of 2×10^7 lymphoid cells, no antibody response was obtained with cells from newly hatched and 4-day old donors (Figs. 1 and 2). Therefore,

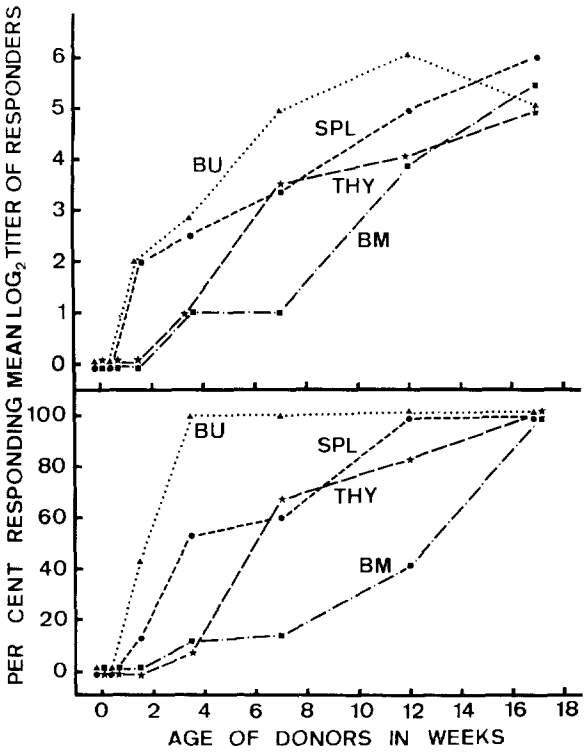

FIG. 2. Antibody response to *Brucella abortus* by 2×10^7 lymphoid cells injected into cyclophosphamide-treated 4-day old chicks. Titrations were done from the same samples as those presented in Fig. 1.

4- and 11-day cells were also used in a 4×10^7 dose (Table III). The results with cells from 11-day old donors confirm those obtained with the smaller cell dose: to SRBC the best responses were given by spleen and thymus cells, and to *Brucella* by bursa and spleen cells. With cells from 4-day old donors, only spleen cells induced a response to SRBC; neither bone marrow, thymus, nor spleen cells responded to *Brucella*. With 4 day bursa, a debris-free suspension for an intravenous injection was not achieved with a sufficiently high (4×10^7) concentration of bursa cells; therefore, no data are presented.

Antibody production by cells taken from yolk sac and embryonic liver was also studied. A similar schedule for cell transfer and antigenic stimulation was employed as in studies with bursa, bone marrow, spleen, and thymus cells. Yolk sac or liver preparations did not induce antibody response to SRBC or *Brucella* (Table IV).

TABLE III

Antibody Response by 4 × 10^7 Lymphoid Cells Injected into Cyclophosphamide-Treated 4-Day Old Chickens

Cells transferred	SRBC			*Brucella*	
	No. of responders/ total	Mean ± SD $\log_2$ titer of responders		No. of responders/ total	Mean ± SD $\log_2$ titer of responders
		Direct	Indirect		
4 day bone marrow	0/19	—	—	0/19	—
4 day thymus	0/17	—	—	0/17	—
4 day spleen	4/13	0.2 ± 0.5	2.2 ± 0.5	0/13	—
11 day bone marrow	1/17	—	1.0	1/17	3.0
11 day thymus	4/17	1.0 ± 1.0	2.8 ± 1.5	2/17	4.0 ± 0.0
11 day bursa	1/14	—	4.0	7/14	3.1 ± 0.7
11 day spleen	8/19	1.0 ± 0.8	3.0 ± 1.7	6/19	4.5 ± 0.6

Both antigens were given intravenously together with the cells, and again 4 days later intraperitoneally. Bleeding was carried out 4 days after the second stimulation.

TABLE IV

Summary of Experiments Revealing No Antibody Response to SRBC and Brucella by Cells Transferred to Cyclophosphamide-Treated 4-Day Old Chicks

Cells transferred	× 10^7	No. of recipients
7 day yolk sac	1	13
11 day " "	4	7
11 day embryonic liver	1	13
14 day " "	1	14

Both antigens were given intravenously together with the cells, and again 4 days later intraperitoneally. Bleeding was carried out 4 days after the second stimulation.

Cellular Cooperation.—During ontogeny, spleen cells were the first to produce antibodies to SRBC (Fig. 1, Table III). The spleen is considered a peripheral lymphoid organ receiving its immunocompetent lymphoid cell population from thymus and bursa. The early vigorous response to SRBC by spleen cells may indicate a cellular cooperation by different cell types, similarly to that observed with thymus and bone marrow in mice and less clearly in other rodents (25–27, 35, 36). Consideration of a synergistic action is also provoked by the vigorous anti-SRBC response by 4-day old normal intact birds (Table I), when com-

pared with the minimal responses produced by 4-day spleen cells in the transfer studies (Fig. 1, Table III). Yet we repeatedly found that the spleen at 4 days contained less than 2×10^7 cells, which was the usual number of cells transferred per chick.

To study possible synergistic influences of thymus and bone marrow cells, 2×10^7 lymphoid cells from each organ were injected intravenously together with 0.1 ml of packed SRBC into 4-day old cyclophosphamide-treated recipients. Otherwise, the same scheme as was employed for antibody production by cells from a single organ was followed. This protocol includes an intraperitoneal stimulation 4 days after the initial injection, and a bleeding after an additional 4 days. The controls received 4×10^7 thymus or bone marrow cells with the antigen. Each group consisted of 9–17 cell recipients. Neither direct or indirect antibody titer assays obtained revealed evidence for synergism between thymus and bone marrow cells. Equally negative results were obtained using donors of different ages, as well as using a smaller dose of the antigen (0.1 ml of 20% SRBC). Altogether, 4- and 11-day, 3-, 6-, 9-, and 15-wk old donors were separately used in these experiments.

Using a similar experimental design, attempts were made to demonstrate synergism between thymus and bursa cells in antibody response to SRBC. 11-day and 3- and 6-wk old cell donors were used. None of the experiments revealed any sign of cellular cooperation between thymus and bursa cells.

DISCUSSION

It has been previously reported that cyclophosphamide treatment in the newly hatched period leads in chicken to a severe and permanent deficiency in humoral immunity (1, 2, 29–31). Our data indicate that cellular immunity is also affected. The presence of an intact cellular immunity in chickens treated in the newly hatched period with cyclophosphamide would be indeed surprising, because normally thymus cells, like bursa cells, are proliferating vigorously during this period. Also, 30% of hormonally bursectomized chickens show varying degrees of thymic atrophy (37). Seto (34) has demonstrated that treatment of chick embryos with cyclophosphamide on the 17th or 18th day of incubation reduces the posthatching graft-vs.-host competence of lymphoid cells; the influence of cyclophosphamide on graft-vs.-host activity was considerable up to the age of 6 wk and could still be detected at 11 wk. A similar effect is reflected in the study of Lerman and Weidanz (29). These investigators measured graft-vs.-host activity of peripheral blood from chickens treated in the newly hatched period with cyclophosphamide. Calculation of their data reveals a difference between the cyclophosphamide-treated donors and normal untreated donors ($P < 0.025$, Student's t test). Further, at the time of the test (at 8 wk of ages) less than 30% of the original group were alive; these chickens probably represented the population least thymic deficient. When evaluating graft-vs.-host reactions, it is important to determine the effect of cell dose. High doses induce

strong reactions which may mask differences detectable only if smaller number of cells are employed. This hazard was clearly shown in our experiments in which 21-day old donors were used (Table II).

For the purpose of the present study, the profound dual immunoincompetence of cyclophosphamide-treated chicks makes them especially suitable as cell recipients. Indeed they serve as the equivalent of living test tubes having little background immunity of their own during the test period. When this model is used with chicks isogeneic at the major histocompatibility locus, both cell rejection by the host and graft-vs.-host reaction which has complicated many of the earlier studies (6–17) can be avoided.

Using this model, a systematic study of the ontogeny of immunocompetent cells in different chicken tissues has been possible. Our results show that during ontogeny, immunocompetent cells appear first in the spleen with respect to an anti-SRBC response. For anti-*Brucella* response, immunocompetent cells are found in significant numbers first in the bursa. Bursa cells are quite poor in their anti-SRBC response; this is the only immune response by any of the cell types studied where the response decreases with increasing age of donors. At 17 wk, when bursa is not more than a rudiment, bursa cells do not form antibodies against SRBC, but their response to *Brucella* is nearly as vigorous as it was at 7 wk of age. Our findings together with prior studies (23, 24, 38, 39) indicate that *Brucella* and some other bacterial antigens are to be considered as bursa-dependent or thymus-independent antigens.

Gilmour et al. (23), using irradiated chicks as recipients of cells isogeneic at the *B* locus, could not demonstrate anti-SRBC response by transferred bursa cells. Their approach was different from ours in that the cells were given intraperitoneally and stimulated only once, immediately after the transfer. However, if the stimulation of 2×10^8 10-wk bursa cells was delayed by 5 days and bleeding done 7 days thereafter, 8 of 11 recipients responded with low titers (23). This and the observations herein suggest that the anti-SRBC response by bursa cells may not be due to the presence of immunocompetent cells in the bursa, but rather to the development of immunocompetent cells from their progenitors, i.e., from the bursal stem cells or their immediate descendants. The curves showing the anti-SRBC response by bursa cells (Fig. 1) reflect quite accurately previously described maturational events and the presence of stem cells in the bursa (2). By contrast, the corresponding curves for spleen, thymus, and bone marrow, and also for bursa in anti-*Brucella* response (Fig. 2) are quite different. We conclude that the bursa of Fabricius does not contain immunocompetent cells for the anti-SRBC response, whereas respective cells for the production of *Brucella* antibodies are present in the bursa in significant numbers beginning as early as the 2nd wk after hatching.

According to our data, the chicken thymus contains cells capable of responding to SRBC and to *Brucella*. This is contrary to that inferred from studies of mammalian thymus when unimmunized donors are used (25–27, 35, 36), and

reveals again the complex cellular composition of the avian thymus. As previouslv discussed (2), the literature documents that cells of bursal origin are found in the chicken thymus (40–44). However, these reports do not give any suggestions about the functional cell types present. Findings in the present study indicate that immunocompetent cells responsible for humoral antibody production are present in the chicken thymus. These cells probably represent the bursa-derived population in the thymus.

It has become generally accepted that a cooperation between thymus-derived and bone marrow-derived cells occurs in the antibody response to SRBC and some other thymus-dependent antigens (25–27, 35, 36). We also considered that a cellular cooperation might explain the early occurrence of immunocompetent cells in the chicken spleen. However, none of our efforts to establish the existence of synergism between cell types were successful even though we studied both thymus-marrow and thymus-bursa combinations. Using a model similar to that employed here, Jeejeebhoy (36) demonstrated in mice a synergism between thymus and marrow cells in the production of SRBC agglutinins; thus our failure probably is not attributable to the model used. Experience of Thorbecke and coworkers (23, 45) with the response to SRBC of thymus-bursa combinations in irradiated chicks agrees with ours. Recently, McArthur et al. (46) have been able to demonstrate a thymus-marrow synergism in response of chicken cells to SRBC. For a cooperative effect, bone marrow cells had to be used in numerical excess over the thymus cells. It seems likely to us, that cellular cooperation is not absolutely necessary for a normal antibody response and may only be active in exceptional situations (46–52).

A general scheme for the ontogeny of the bursa-dependent immune system is shown in Table V on the basis of the observations presented in this and two of our papers published elsewhere (1, 2). It is clear that stem cells and immunocompetent cells have different distributions. The stem cells, characterized by their ability to confer a long-term reconstitution of immunodeficient chicks, are found in the bursa during embryonic development and during the 1st few wk after hatching. The stem cells appear in bone marrow, thymus, and spleen only after the bursa has begun to involute. Furthermore, the early bursal stem cell and that occurring later in both the bursa and the other organs differ in one important feature: only the embryonic or early bursal stem cell is able to, and must, enter the bursa and proliferate in the bursal follicles. By 3–4 wk of age a shift in the degree of maturation of the bursal stem cell population occurs. After this age stem cells of the bursa-dependent line can proceed to their ultimate destination and develop without local bursal influence. Perhaps they already carry membrane markers (53–56) reflecting a differentiative step which restricts their traffic pattern. The negative results obtained with yolk sac and fetal liver cells indicate that the bursal reticulum of 4-day old chicks does not any more possess the essential environment for maturation of the primitive stem cells (1). In other words, development of the bursa-dependent cell line

seems to proceed stepwise from the very first multipotential stem cell. Each succeeding phase can only be entered when the preceding is completed.

The first immunocompetent cells for humoral immunity were encountered in the spleen at the age of 4 days. After the age of 3 wk these cells were present in increasing numbers in all the organs studied, the only exception being the

TABLE V

Comparison of Short-term and Long-term Effects of Different Cell Transplantations to 4-Day Old Cyclophosphamide-Treated Chickens

Cells transferred	Short-term effects		Long-term effects							
	Antibody response to		Antibody response to		Natural rabbit hemag-glutinins	Immuno-globulins	Microscopic structure		Sur-vival	Body weight
	SRBC	*Brucella*	SRBC	*Brucella*			Spleen	Bursa		
17–21 day embryonic bursa	−*	−	++	+	+	++	++	++	++	+
4 day bursa	−	−	+++‡	++	++	++	++	++	++	+
3½ wk bursa	+	++	+	−	+++	++		−	+	−
7–10 wk bursa	+	+++	+++	+++	+++	+++	+	−	+	−
16–17 wk bursa	−	+++							−	−
14–20 day embryonic bone marrow			−	−	−	−	−	−	−	−
4 day bone marrow	−	−	−	−	−	−	−	−	−	−
3½ wk bone marrow	+	+	−	−	−	++	−	−	+	+
7 wk bone marrow	+	+	−	−	++	++	−	−		+
10–17 wk bone marrow	+++	+++	+++	+++	+++	+++	+	−	++	++
4 day thymus	−	−	−	−	−	−	−	−	−	−
3½ wk thymus	+	+	−	−	−	+	−	−	−	−
7 wk thymus	++	+	−	−	++	++	−	−	+	+
14–17 wk thymus	+++	+++	+	+	+++	++	+	−	++	++
14–21 day embryonic spleen	−	−	−	−	−	−	−	−	−	−
4 day spleen	+	−	−	−	−	−	−	−	−	−
3½ wk spleen	+++	+	−	−	−	++	−	−	+	+
14–17 wk spleen	+++	+++	+++	+++	+++	+++	+	−	++	++

In the text, short-term effects are considered a function of immunocompetent cells, and long-term effects a function of stem cells.

* −: Denotes no restoration; indistinguishable from chickens treated with cyclophosphamide alone.

‡ +++: Denotes in long-term effects restoration of the function or structure to the level of normal, untreated controls; in short-term effects the maximal antibody production.

bursa cells involved in the anti-SRBC response. In the bursa the stem cell population is present before immunocompetent cells can be detected. The opposite is true for bone marrow, spleen, and thymus where cells capable of providing short-term antibody-forming capacity are always found some weeks before capacity to achieve a long-term reconstitution of immunodeficient chicks. These findings indicate that all along its postembryonic development bursa seeds out immunocompetent cells but does not release the lymphoid stem cell population before this population has matured to a new level and before the bursa itself, after fulfilling its task, begins to involute.

SUMMARY

To study the occurrence of immunocompetent cells directly responsible for antibody production, cells from yolk sac, embryonic liver, bursa of Fabricius, bone marrow, spleen, or thymus were injected together with SRBC and *Brucella abortus* into 4-day old cyclophosphamide-treated chicks. A second stimulation was given 4 days later, and samples taken 4 days thereafter were used for antibody titrations.

During ontogeny, immunocompetent cells appeared in significant numbers first in the spleen for anti-SRBC responses, but in the bursa for anti-*Brucella* responses. Later these cells were also found in thymus and bone marrow. In the bursa, cells immunocompetent for anti-SRBC response were not encountered in significant numbers. The slight response to SRBC by transferred bursa cells reflects the presence of stem cells and their immediate descendents in the bursa at different stages of development.

These findings are compared with the development and maturation of the stem cell responsible for humoral immunity. In the bursa, development of the stem cell population precedes that of immunocompetent cells. The opposite relationship was found in bone marrow, spleen, and thymus where immunocompetent cells were always present some weeks before the appearance of cells capable of achieving a long-lasting reconstitution of bursa-dependent functions. These observations reveal that the bursa seeds out immunocompetent cells during its entire postembryonic development, but does not release the lymphoid stem cell population before this population has matured sufficiently and before the bursa itself, after fulfilling its function, starts to involute.

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