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Abnormal response to minor histocompatibility antigens in Obese strain chickens

(autoimmunity/thyroiditis/thymus)

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ABSTRACT **Obese strain chickens, which spontaneously develop autoimmune thyroiditis, were tested for their ability to tolerate skin allografts. Several procedures known to prolong graft survival in normal strains were employed. These included the use of skin matched at the major histocompatibility locus, grafting on the day of hatching, thymectomy, and x-irradiation.**

A dramatic difference between the Obese and the normal Cornell strain (the strain from which Obese was derived) was detected when both were thymectomized and grafted at hatching. Under these conditions eight of 13 normal but only one of 16 Obese strain birds retained their grafts for 50 days. This suggests the presence of an abnormal thymus or thymus-derived suppressor T cells in Obese strain chickens.

Obese strain (OS) White Leghorn chickens develop a hereditary form of thyroiditis resembling Hashimoto's disease in man, which offers a useful model for studying spontaneous autoimmune disease (for review, see ref. 1). The pathogenesis of the disease is still largely unknown. There are three possible explanations for the development of an immunological reaction against the thyroid gland: (*a*) a normal immunological system responds to altered thyroid antigens; (*b*) an over-responsive immunological system reacts against unaltered thyroid antigen; or (*c*) both phenomena occur. The observation of functional changes in thyroid glands of OS chickens at hatching, before the onset of any demonstrable thyroiditis (2), favors the first hypothesis. The findings in OS chickens of antibodies with an increased titer and incidence reacting with extracts of liver and kidney (3) and erythrocyte nuclei (4) and an increased rejection rate of skin grafts with minor histoincompatibilities (5) support the second hypothesis.

To test the possibility that OS chickens respond to self antigens with an unusually vigorous immunological response, OS and normal Cornell strain (CS) chicks were given dermal allografts differing only in minor histocompatibility antigens. Minor, rather than major, histocompatibility differences were selected because they might be more comparable to an autoimmune response. Within each strain skin grafts were exchanged among birds matched at the major (*B*) histocompatibility locus. Some transplants were given shortly after hatching, since previous studies (6) had shown that transplantation at this time results in significantly longer survival of skin than grafting at 2 weeks. The effects of thymectomy and x-irradiation on skin graft survival were evaluated, since these treatments are known to have pronounced effects both on thyroiditis [increasing the severity of disease (7–9)], and on skin allograft survival [prolonging survival of grafts (10–12)].

In the studies to be described, CS and OS chicks grafted at hatching or at 2 weeks rejected almost all grafts with similar median survival times (MST). X-irradiation, either alone or combined with thymectomy, prolonged graft survival in both strains to a similar degree. In contrast, thymectomy alone significantly prolonged survival of grafts only in CS chicks. This differential effect of thymectomy may be due to abnormal maturation of the OS thymus gland. Several mechanisms are proposed by which thymic development could affect both skin graft rejection and thyroiditis.

MATERIALS AND METHODS

Animals. CS chickens have been maintained as a closed flock at Cornell University since 1935. The incidence of thyroiditis is less than 1%. OS chickens were developed with an incidence of spontaneous autoimmune thyroiditis of over 90% (13) by selection of individuals among the CS chickens with thyroiditis. Serologic typing of the major histocompatibility complex antigens of OS and CS chickens by Bacon *et al.* (5) has enabled Dr. R. K. Cole at Cornell University to set up pen matings of OS B^1B^1 and CS B^1B^1. The diet of OS chickens is supplemented with Protamone®, a thyroxin-like substance which stimulates egg production. B^1B^1 chicks were used as both donors and recipients of skin grafts. Only intra-strain grafts were made in this study and chickens of both sexes were randomly used. Minor histocompatibilities are expected to be similar in these two strains. The OS is reproduced with fewer breeders than the CS, but inbreeding has been purposely avoided in both strains.

Grafting. Within each strain two 1 cm^2 sites were prepared on the back of each recipient, one receiving an allograft, the other an autograft. Grafting was done as described previously (14, 15). Following bandage removal on day 6, the grafts were observed daily for 5 weeks, and then every other day. Grafts were scored by gross examination using the method of Polley *et al.* (15). The median survival time (MST) was calculated by the method of Litchfield (16). All autografts were permanently accepted.

Thymectomy. Thymectomy was performed on the day of hatching by an established procedure (7, 10). Completeness of thymectomy was checked at the time of sacrifice, and one bird with 400 mg of thymus tissue remaining was excluded. Four birds which had between 11 and 65 mg of thymus tissue remaining were included in this study, along with the other operated birds having no detectable thymic tissue.

X-irradiation. Chicks were exposed to whole body x-irradiation (810 rads, 8.1 J/kg) within 24 hr of hatching. The x-ray source was a Picker Vanguard 270 with a 100 inter-cavitary probe.

Thyroid Pathology. Thyroid glands were fixed in 10% buffered formalin, embedded, examined, and scored by standard methods (17).

Abbreviations: effector T cell, thymus-dependent lymphocyte that upon exposure to the proper antigen promotes an immune response; suppressor T cell, thymus-dependent lymphocyte that suppresses an immune response; B cell, bursa-dependent lymphocyte; OS, Obese strain; CS, Cornell strain; MST, median survival time.

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Table 1. Skin allograft survival in CS and OS chicks

Group	Strain	Age of grafting	Number of recipients	Treatment of recipients	Number accepted/ total	MST (days)*	Limits of MST†
1	CS	2 weeks	16	None	0/16	14.6	12.5–17.0
	OS		15	None	0/15	15.0	12.6–17.8
2	CS	At hatching	9	None	2/9	19.7	16.0–24.2
	OS		7	None	0/7	18.0	14.8–22.0
3	CS	At hatching	13	Thymectomized	8/13	>50‡	
	OS		16	Thymectomized	1/16	25.0	18.9–33.0
4	CS	At hatching	10	X-irradiated	1/10	29.0	21.8–38.6
	OS		5	X-irradiated	1/5	31.0	20.0–48.0
5	CS	At hatching	8	Thymectomized + X-irradiated	3/8	42.0	34.1–51.7
	OS		9	Thymectomized + X-irradiated	5/9	>50‡	

CS and OS chicks were grafted with intrastrain B-compatible skin and the grafts were examined daily for 35 days and then every other day up to 50 days.

* Median survival time calculated by the method of Litchfield (15).

† Within 95% confidence limits.

‡ Since more than half of the grafts survived 50 days, the median survival time could not be calculated.

Thyroid Antibody Titers. Antibody was detected by means of a passive hemagglutination test, coupling thyroglobulin to chicken erythrocytes by means of $CrCl_3$ (18).

Treatment Groups. CS and OS chickens were treated in the following ways: Group 1, grafted at 2 weeks; Group 2, grafted at hatching; Group 3, thymectomized and grafted at hatching; Group 4, x-irradiated and grafted at hatching; Group 5, thymectomized, x-irradiated, and grafted at hatching.

RESULTS

All allografts performed on 2-week-old chickens (Group 1) were rejected at approximately the same time in both strains (Table 1). Since the birds were matched at the major histocompatibility locus, these findings support the supposition that the degrees of histoincompatibility due to minor alloantigens of the two strains were similar. When newly hatched chicks (Group 2) were used as recipients, seven of nine CS chickens rejected allografts within 50 days and the MST of the nine grafts was 19.7 days, which was comparable to the MST of 18.0 days for the seven grafts on OS chickens.

When newly hatched chickens were grafted immediately after thymectomy (Group 3) there was a significant difference in the survival of the grafts between the two strains. Eight of 13 CS chickens accepted allografts with only slight or no signs of rejection for the 50-day observation period. Only one of the 16 similarly treated OS chicks accepted a skin graft for 50 days. Serum thyroglobulin antibody titers for nine of the thymectomized CS and seven of the thymectomized OS chicks were determined at 50 days. The mean $\log_2$ titer for the CS was 3.2 and the OS, 9.9. Although thyroid glands of seven of the nine CS chickens were devoid of lymphoid infiltrations, the two birds with some infiltration (scored 0.4 on a scale 0 to 4) were found to have the highest antibody titers ($\log_2$ titers of 9 and 5). Both of these birds rejected their skin grafts, in contrast to only three rejections by the other seven CS thymectomized birds assayed for thyroid antibody. All of the seven thymectomized OS birds had severe thyroiditis, judged by the absence of thyroid tissue at the time of hatching.

Almost all newly hatched CS and OS chickens grafted after whole body x-irradiation (Group 4) rejected their grafts by about 30 days after grafting, but when thymectomy was combined with x-irradiation (Group 5) approximately half of the OS and CS chickens retained their grafts for the 50-day period of observation and for several additional weeks. There was no significant difference in the survival of the grafts between strains in Group 5.

DISCUSSION

The most interesting new finding in this investigation is that eight of 13 skin grafts placed on neonatally thymectomized CS chicks survived for at least 50 days while only one of 16 grafts on comparably treated OS chicks survived that long. To explain this phenomenon, we have considered two possibilities. The first is that the degree of "foreignness" of the grafts on OS chicks was greater than on CS chickens. Since birds were matched at the *B* locus, both strains were responding only to minor histocompatibility antigens present in the OS or CS flocks. When unoperated OS and CS chicks were grafted at hatching or at 2 weeks, no differences in time of rejection could be detected between the two strains (Groups 1 and 2). Furthermore, x-irradiation, alone or coupled with thymectomy, similarly failed to reveal a significant difference in the time of graft rejection by OS and CS chickens.

The second explanation for the differential effect of thymectomy is that the thymus of OS chickens develops differently from the thymus of normal CS chickens. Significant numbers of OS effector T (thymus-dependent) cells‡ might have already migrated from the OS thymus to the periphery prior to surgical extirpation at hatching. The T cell subclass of the earliest migrating cells is unknown, although some recent experiments in mice (19) suggest that suppressor T cells normally leave the thymus before helper T cells. This finding is compatible with "suppressor cell" theories of self-tolerance which require that the first peripheral lymphocytes appearing in ontogeny recognize self-antigens and function by suppressing the immunologic responsiveness of the later developing T [and possibly B (bursa-dependent)] cells. We speculate that prolonged survival or "tolerance" of skin in thymectomized CS chickens is caused by the initial contact between suppressor cells and skin alloantigens. More direct support for the role of suppressor cells

‡ The definition of "effector T cell" for purposes of this discussion is a specific antigen-reactive T cell that upon exposure to the proper antigen is activated to proliferate and promote an immune response (e.g., by the release of lymphokines or by helping B cells to produce antibody). Conversely, a "suppressor T cell" reduces an immune response (i.e., prolongs allograft survival or diminishes autoimmune disease).

in prolonging graft survival comes from recent experiments of Droege, who showed that syngeneic thymocytes transferred to 400 roentgen (0.10 coulomb/kg) sublethally x-irradiated chicks actually prolonged allograft survival (20). Graft prolongation in his system was presumably due to the greater relative effectiveness of the transferred suppressor T cells compared to the effector T cells in the same inoculum. If, during ontogeny, sufficient suppressor cell activity develops before effector cells migrate from the thymus, then effector cells might be suppressed even after contact with antigen. In the case of OS chickens, it is possible that accelerated or abnormal thymus development results in a much greater effector to suppressor cell ratio in the periphery at the time of hatching. Consequently, thymectomy did not prolong skin graft survival.

This theory accounts in part for the spontaneous development of thyroiditis in OS chickens, since insufficient numbers of suppressor T cells persist and the remaining cells therefore fail to interrupt the response of autoreactive B cells. In fact, Richter and Wick (21) have found increased numbers of thyroglobulin-specific rosette-forming B cells in young OS chicks. Patients with Hashimoto's thyroiditis and even healthy individuals have circulating B cells that bind thyroglobulin (22). In the normal CS chick, thymectomy performed at hatching was probably early enough to reduce significantly the large-scale release of effector T cells. This assumption is supported by a report (23) that thymectomy of normal chicks within 24 hr of hatching significantly reduces the total number of circulating lymphocytes at 2 weeks of age.

Whole body x-irradiation, which kills peripheral lymphocytes as well as those in the central lymphatic organs, can be expected to deplete lymphocytes to a similar extent in both strains, but probably did not completely eliminate effector T cells. This treatment therefore resulted in prolonged skin graft survival in both strains, but by the time the thymus tissue regenerated, more effector T cells were produced, and most of the grafts were eventually rejected. The x-ray dose selected, 810 rad, is large enough, when combined with thymectomy, to impair significantly but not eliminate T helper cell function (24) and T-cell-dependent rejection of skin grafts in normal chickens (11). In mice T suppressor cells are sensitive to 800 roentgen (0.21 coulomb/kg) (25) x-irradiation, whereas T cells active in helping B cells are insensitive (26). However, 810 rad is well below lethal levels for OS chickens, since 16 of the 19 OS chicks thymectomized and exposed to 900 rad shortly after hatching (not reconstituted with bone marrow) have survived until they were sacrificed at 7 weeks (Sundick and Bacon, unpublished results).

CS and OS chicks in treatment Groups 1 and 2 rejected B-compatible grafts at comparable times, in contrast with the earlier results of Bacon *et al.* (5). However, in the earlier study both B-incompatible and B-compatible allografts were placed on each chick. Schierman and McBride have shown that concurrent B-incompatible grafts augment B-compatible graft rejection (27). Thus, augmentation of B-compatible graft rejection by B-incompatible grafts was possible in the first experiment, and may have been more extensive in OS than CS chicks.

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