Immunological Studies of the Snell-Bagg Pituitary Dwarf Mouse[1] (34440)

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The relationship between the pituitary gland and the thymus has recently received renewed interest. Pierpaoli and Sorkin have described the induction of wasting disease and lymphoid organ degeneration after administration of antisera to pituitary extracts (1) or growth hormone (2). They also have observed degranulation of pituitary acidophils after neonatal thymectomy (3) and have postulated a direct pituitary control over thymus function, possibly mediated by growth hormone (2).

Baroni has studied some immunological characteristics of the autosomal recessive dwarf mutant of the Snell-Bagg (SB) strain of mice (4,5). This dwarf mouse has shown diminished antibody production against sheep erythrocytes and hypoplasia of lymphoid tissues. Treatment of the dwarf mouse with growth hormone and thyroxine resulted in a reconstitution of the immune apparatus (6).

In our laboratory we have studied the immunological recovery after sublethal irradiation of the hypophysectomized rat and found that such a recovery was diminished as indicated with decreased leukocyte count, decreased hemagglutinating antibody response to sheep erythrocytes, and increased allogeneic skin graft survival as compared with nonoperated rats (7). The present study deals with a further characterization of the pituitary dwarf SB mouse. We have confirmed Baroni's observations (8) and we have also concluded that the pituitary dwarf SB mouse is immunologically deficient. The deficiency could well be limited to the thymus and thymus-dependent system.

Materials and Methods. Dwarf SB mice (genetic symbol dw) were obtained from the Jackson Laboratory (Bar Harbor, Maine) or from breeding of normal SB mice, which were heterozygous for the dwarfing gene. The life span of the dwarf mouse was limited to about 50–80 days. All experiments were carried out with 8- to 10-week-old mice. Control groups consisted of heterozygous and homozygous normal SB mice of the same age.

Normal and dwarf mice were bled from the tail and the peripheral white blood cell count was determined. Serum immunoglobulins IgG_1, IgG_2, IgM, and IgA were determined with immunoelectrophoresis. (For method, see P. Kalpaktsoglou, R. Hong, and R. A. Good: to be published.) Groups of eight normal and dwarf mice were immunized with one intraperitoneal injection of 0.5×10^8/10 g body weight of washed sheep erythrocytes or 25×10^9/10 g body weight of washed heat-killed Brucella abortus cells (strain 1119-3, obtained from U. S. Department of Agriculture, Washington, D. C.). All groups were bled 1 week after injection and the plasma was assayed for agglutinating antibody using the microtiter technique. The graft-versus-host reactivity of spleen cells from normal and dwarf mice was determined with the assay of Simonsen (9) whereby 10 million spleen cells suspended in 0.2 ml of Hanks' balanced salt solution were injected intraperitoneally in 8-day-old $(A \times SB)F_1$ hybrid mice. Eight days later the F_1 hybrids were killed, their relative spleen weights determined, and the spleen indices were calculated by dividing the relative spleen weights of the experimental groups by the mean of the relative spleen weight of the uninjected con-

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TABLE I. White Blood Cell Count of 10-Week-Old Normal and Dwarf Snell-Bagg Mice.

	PMN	Lymphocytes
Normal	2105 ± 349	9361 ± 751
Dwarf	2352 ± 368	810 ± 222

trols. Morphological studies were carried out on lymphoid tissue and other organs employing the usual histological procedures.

Results. Ten-week old dwarf SB mice were shown to be severely lymphophenic with normal levels of polymorphonuclear leukocytes (Table I). Both hemagglutinating antibody titers to sheep erythrocytes and agglutinating antibody titers to Brucella antigen (Table II) were significantly lower ($p < .001$) in the dwarf SB mouse than in the normal SB mouse. However, four out of eight dwarfs died within 1 week after injection of Brucella antigen, possibly due to toxicity of the preparation of Brucella antigen. As shown with qualitative immunoelectrophoresis the immunoglobulin concentration of IgG_1, IgG_2, IgM, and IgA were approximately the same in the dwarf as in the normal SB mouse (Fig. 1). The graft-versus-host reactivity of dwarf SB spleen cells was somewhat depressed in the dwarf as indicated by a lower mean spleen index ($p < .01$). Absence of splenomegaly (spleen indices less than 1.30) was seen in three out of ten young (A × SB)F_1 hybrids after injection of dwarf spleen cells while normal spleen cells uniformly induced significant splenomegaly (Table III).

Morphology. The darf thymus was characterized by atrophy and a marked loss of lymphocytes in both medulla and cortex. In approximately 25% of the dwarf thymuses, we observed a specific absence of lymphocytes in the dwarf thymus cortex of dwarf mouse, similarly to the so-called cortical inversion of the preleukemic AK mouse thymus as described by Metcalf (10). The presence of PAS-positive cells in the thymus cortex was prominent. The peripheral lymphoid organs were also small in size. An average spleen of a 5- to 6-g dwarf SB mouse

TABLE II. Hemagglutinating Antibody Titer to Sheep Erythrocytes and Agglutinating Antibody Titer to Brucella Antigen 7 Days after ip Injection into Normal and Dwarf Snell-Bagg Mice.

	Log_2 antibody titer	
	SRBC	Brucella
Normal	8.4 ± 0.3	7.3 ± 0.2
Dwarf	3.2 ± 0.4	2.7 ± 0.1

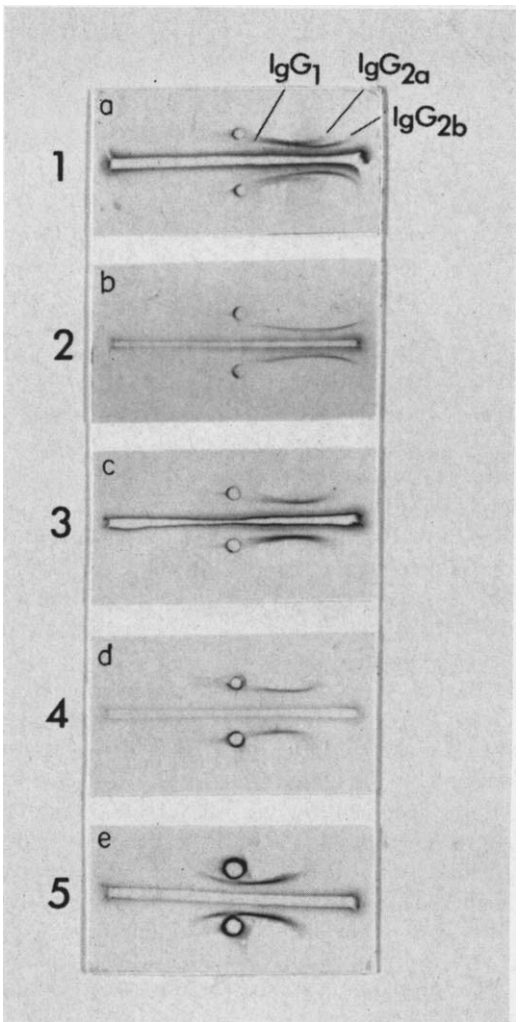

FIG. 1. *Results of the immunoelectrophorectic analysis of normal and dwarf Snell-Bagg mouse serum.* Top wells: Normal SB mouse serum. Bottom wells: Dwarf SB mouse serum. Troughs: Goat antisera directed against mouse immunoglobulin constituents. 1 = anti-Fc of IgG; 2 = anti-Fc of IgG_1; 3 = anti-Fc of IgG_{2a}; 4 = anti-IgM; 5 = anti-H chain of IgA. Precipitin lines of: *a*: IgG_1, IgG_{2a}, and IgG_{2b}; *b*: IgG_1; *c*: IgG_{2a}; *d*: IgM; *e*: IgA.

TABLE III. Results of Graft-Versus-Host Assay of 10×10^6 Normal or Dwarf Snell-Bagg Spleen Cells Injected into 8-Day-Old (A × SB)F_1 Hybrids.

Group	Spleen indices					Mean ± SE	Positive
Normal SB	1.86	2.08	2.24	2.31	2.35	2.50 ± 0.11[a]	10/10
	2.56	2.71	2.82	2.87	3.16		
Dwarf SB	0.96	1.13	1.23	1.65	2.15	1.90 ± 0.18[a]	7/10
	2.16	2.33	2.42	2.43	2.54		

[a] $p < .01$.

contained only approximately 10–15 million lymphoid cells as compared with 180–200 million lymphoid cells in an average spleen of a 20- to 25-g normal SB mouse or 80-100 million cells in a 5- to 6-g 7-day-old SB mouse. The peripheral lymphoid tissues show hypocellularity, in particular in the so-called thymus-dependent areas, *i.e.*, the paracortical or deep cortical regions of lymph nodes and the perifollicular sheaths in the spleen to be deficient in cellularity. The lymphoid follicles of the lymph nodes and spleen were small in size, but apparently structurally similar to the lymphoid follicles of the normal SB mouse. The number of plasma cells was, however, somewhat decreased. Adrenal glands of the dwarfs were relatively small. The ratio of cortex-medulla was lower than in the normal. Also the cells of the zona fasciculata were eosinophilic as compared with normal clear foamy cells.

Discussion. Our results have shown that the pituitary dwarf SB mouse is immunologically deficient, an observation which is in agreement with Baroni's findings (4–6). The pituitary dwarf SB mouse is characterized by a lack of pituitary acidophils, and growth hormone is virutally absent (11, 12). The genetic effect is located in the anterior pituitary and not in its hypothalamic control (13). Both prolactin and TSH content of the dwarf pituitary is somewhat decreased (12). ACTH is also lower, while gonadotrophins are normal in the dwarf in spite of underdeveloped gonads and sterility of the dwarf (14).

While it was apparent that because of the deficient hormonal status of the SB dwarf mouse, we could expect generally lower metabolic activities of most tissues, including lymphoid tissues, our observations indicated that a distinct immunologic deficiency was present in the dwarf mouse. The lymphopenic state, with normal levels of polymorphonuclear leukocytes and the decreased graft-versus-host reactivity of spleen cells in the dwarf, suggested a selective deficiency of the thymus-dependent lymphoid system. Also the response of dwarf spleen cells to phytohemagglutinin has been markedly lower than normal SB spleen cells (8). The hemagglutinating antibody response to sheep erythrocytes, which appears to be an antibody response which is thymus-dependent (15), was greatly diminished in the dwarf mouse. On the other hand, immunoglobulin levels IgG_1, IgG_2, IgM, and IgA in the dwarf mouse all seemed to be within the normal range. Histological studies of the thymus-independent areas of the spleen, lymph nodes, and Peyer's patches demonstrated a lower number of plasma cells. The lymphoid follicles were generally small, but not much different in structure from normal follicles. Germinal centers were present in lymph nodes especially after antigenic stimulation.

The 10-week-old dwarf thymus was histologically atrophic and hypoplastic. In a number of dwarf thymuses a phenomenon was observed similar to the "cortical inversion" present in preleukemic AK mice as described by Metcalf (10). The thymus cortex was entirely depleted of lymphoid cells, while the medulla appeared to be normal or nearly normal in cellularity. With respect to a possible association between immunological deficiency and malignant adaptation (16), these findings could well indicate that the SB dwarf mouse was in a preleukemic or a precancerous state. However, we have not yet observed any

cancer in the dwarf mouse, probably because the limited life span of the dwarf mouse did not allow a full development of malignant adaptation.

The thymus-dependent areas, *i.e.*, the paracortical areas of lymph nodes and the perifollicular areas of the splenic lymphoid follicles, were depleted of small lymphocytes.

These observations suggest that the immunologic deficiency of the pituitary dwarf could be rather limited to the thymus and the thymus-dependent lymphoid system. The question arises whether the deficiency is the result of deficient pituitary function or a consequence of stress. Although the dwarf mice were kept carefully under optimal conditions with easy access to food and water, they might have been more susceptible to such stress-inducing factors as competition with normal littermates during weaning, temperature changes, and other undetermined environmental conditions. The high toxicity of Brucella antigen and also of pentobarbital for the dwarf animals are examples of the increased susceptibility to toxic influence. The lymphoid organ atrophy as a result of stress, however, is generally attributable to an increased activity of the pituitary-adrenal axis (17). The dwarf pituitary ACTH content has been shown to be decreased (12), while the adrenal glands were small in size. Also, the cortex-medulla ratio was lower in the dwarf than the normal (12). The eosinophilic cells of the zona fasciculata of the dwarf adrenal cortex, as compared with the clear foamy cells of the zona fasciculata in the normal adrenal cortex, suggested a lower metabolic activity.

Although the lymphoid organ atrophy in the dwarf would be compatible with the appearance after stress, the activity of the pituitary-adrenal axis appeared to be low; two observations which led us into a paradoxical situation. The lymphoid organs looked as though the animal was under stress whereas no evidence of stress was found in either morphological or hormonal studies of the pituitary-adrenal axis (12).

If we do not consider stress as a major cause of the apparent lymphoid organ atrophy and deficient function, but consider rather the existence of a direct pituitary control of the thymus and thymus-dependent lymphoid system, failure of the pituitary would be expected to lead to maldevelopment or atrophy and dysfunction of that segment of the lymphoid system and immunological function attributable to the thymic influence. The noticeable lack of growth hormone in the dwarf mouse suggests that the particular lack of this hormone will lead to immunologic deficiency, especially of the thymus and thymus-dependent system. Treatment with bovine growth hormone, especially when combined with thyroxin, prevented thymus atrophy and the cellular depletion of lymphoid tissue, and normalized the antibody response to sheep erythrocytes (6). Baroni suggested that the primary site of action of these hormones is in the bone marrow (6). In a series of unpublished experiments in which we lethally irradiated normal and hypohysectomized rats, we found that irradiated hypophysectomized rats could not be protected with administration of bone marrow. The function of the thymus in immunologic recovery after irradiation and injection of bone marrow cells is that the undifferentiated bone marrow cells migrate to the thymus where they, under specific microenvironmental conditions, differentiate to immunocompetent cells (18). In the hypophysectomized rat the microenvironment of the thymus was apparently deficient and differentiation of bone marrow cells into immunocompetent cells under thymic influence did not occur for this reason.

From these observations we concluded that the microenvironment of the thymus, where the immunological maturation of bone marrow cells takes place to immunologically competent cells capable of expressing the cell-mediated immunities and which probably is under influence of the thymus epithelium, is under pituitary influence (R. J. Duquesnoy and R. A. Good, unpublished observations).

Growth hormone has been postulated to be the mediator of pituitary control of the thymus (2, 6). Pierpaoli and Sorkin observed wasting disease and lymphoid organ atrophy in mice after the administration of antisera to bovine growth hormone, and the wasting dis-

ease observed had much in common with that observed after neonatal thymectomy (2). The immunologically deficient SB dwarf mouse has almost no growth hormone secretion (12). However, the possibility of a distinct thymus-acting or thymotropic hormone, secreted by the pituitary cannot be excluded. We have injected newborn rats and mice with antisera raised in monkeys against rat growth hormone preparations from birth to 3 weeks of age. Although we observed a considerable inhibition of total body growth, in a few instances resulting in wasting and consequent death possibly due to infection, we did not observe significant changes in various immunologic criteria, such as peripheral lymphocyte levels, antibody response to sheep erythrocytes and lymphoid tissue histology, up to 2 months after the injections were discontinued (R. J. Duquesnoy, A. F., Parlow and R. A. Good, unpublished observations). Considering the important role of the thymus in the immunological maturation early in life, administration of antiserum to specific thymotropic factors should have lead to an impairment of the thymus and thymus-dependent system.

Clinically, patients with immunological deficiency disease often show growth retardation and retarded bone age as well as the specific immunologic deficiencies. Ammann, Duquesnoy, and Good have demonstrated an abnormal pituitary growth hormone response in some patients with ataxia-telangiectasia These patients lack a normal immunologic development and often have a morphologically underdeveloped thymus which may be due to a defective hypothalamic-pituitary control (19). On the other hand, no immunologic abnormality has yet been described in patients with hypopituitary dwarfism. One patient with sex-linked lymphopenic immunologic deficiency syndrome also had a normal growth hormone secretion. This immunologic deficiency disease is probably the result of an abnormality of the lymphoid stem cell which is unable to differentiate along either the thymus-dependent or the thymus-independent lymphoid cell line. Two patients with the Bruton-type sex-linked agammaglobulinemia had also a normal growth hormone response to insulin-induced hypoglycemia (19).

At the present it is difficult to differentiate between growth hormone and a specific thymotropic hormone as a pituitary mediator of thymus function.

From experiments thus far carried out one could conclude that thymotropic activity contaminating growth hormone preparations, rather than growth hormone itself, is responsible for the influence on the lymphoid tissues. This possibility seems especially likely in work done across species barriers. The similarity between prolactin and growth hormone originally caused much confusion when initial experiments revealed lactogenic activity of growth hormone (20). At the present time we could be in a similar situation, *vis a vis* the thymotropic activity and growth hormone activity of pituitary preparations of growth hormone.

Summary. The Snell-Bagg dwarf mouse was immunologically deficient as shown by severe lymphopenia, decreased agglutinating antibody response to sheep erythrocytes and Brucella antigen, and decreased graft-versus-host reactivity of dwarf spleen cells. The lymphoid organs of the dwarf were small, atrophic, and generally depleted of small lymphocytes. Serum immunoglobulins appeared to be within normal ranges. These findings suggested a selective deficiency of the thymus-dependent lymphoid system of the dwarf, caused by a defective thymotropic pituitary control.

1. Pierpaoli, W. and Sorkin, E., Nature **215**, 834 (1967).

2. Pierpaoli, W. and Sorkin, E., J. Immunol. **101**, 1036 (1968).

3. Pierpaoli, W. and Sorkin, E., Brit. J. Exptl. Pathol. **48**, 627 (1967).

4. Baroni, C., Experientia **23**, 282 (1967).

5. Baroni, C., Fabris, N., and Bertoli, G., Experientia **23**, 1059 (1967).

6. Baroni, C. D., Fabris, N., and Bertoli, G., Immunology **17**, 305 (1969).

7. Duquesnoy, R. J., Mariani, T., and Good, R. A., Proc. Soc. Exptl. Biol. Med. **131**, 1176 (1969).

8. Duquesnoy, R. J., Rodey, G. E., Holmes, B., and Good, R. A., Federation Proc. **28**, 276 (1969).

9. Simonsen, M., Progr. Allerg. **6**, 349 (1962).

10. Metcalf, D., "The Thymus," p. 103. Springer,

New York (1966).

11. Bartke, A., Anat. Record **149**, 225 (1964).

12. Lewis, U. J., Mem. Soc. Endocrinol. **15**, 179 (1967).

13. Carsner, R. L. and Rennels, E. G., Science **131**, 829 (1960).

14. Bartke, A., Mem. Soc. Endocrinol. **15**, 193 (1967).

15. Miller, J. F. A. P. and Osoba, D., Physiol. Rev. **47**, 437 (1967).

16. Good, R. A. and Finstad, J., J. Natl. Cancer Inst. **31**, 41 (1969).

17. Dougherty, T. F., Physiol. Rev. **32**, 379 (1952).

18. Harris, J. E. and Ford, G. E., Nature **201**, 884 (1964).

19. Ammann, A. J., Duquesnoy, R. J., and Good, R. A., Clin. Expt. Immunol. Immunochem. (1970) in press.

20. Segaloff, A., *in* "Methods in Hormone Research" (R. I. Dorfman, ed.) Vol. 2, p. 609. Academic Press, New York (1962).

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