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Thymic hormone activity and spontaneous autoimmunity in dwarf mice and their littermates

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Summary. Serum thymic hormone activity (TA) was determined in hereditary hypopituitary dwarf mice (dw/dw) and their littermates (+/dw or +/+). It was found to be very low in the dwarf animals in comparison to their littermates. At 14 weeks of age, the dwarf littermates exhibited significant glomerular lesions characterized by deposits of IgG, IgG1, IgG2, IgA, IgM and C3, which were augmented by thymectomy of adult females. In contrast, hypopituitary dwarf mice had minimal glomerular deposits of immunoglobulins. Unlike these animals, their littermates showed antinuclear antibodies (ANA) and anti-deoxyribonucleic acid (DNA) antibodies in their serum. The present findings are discussed in relation to recent hypotheses on: (1) the role of the hypophysis in thymus-dependent immunological functions; and (2) the significance of T-cell deficiency in the development of autoimmunity.

INTRODUCTION

It has been shown that a relationship exists between the adenohypophysis and the maturation of lymphoid tissue on the one hand and thymus-dependent functions on the other (Pierpaoli and Sorkin, 1967, 1969). Experiments with hypopituitary dwarf mice have demonstrated that they possess a hypotrophic thymus (Baroni, Fabris and Bertoli, 1969; Pierpaoli, Baroni, Fabris and Sorkin, 1969; Fabris, Pierpaoli and Sorkin, 1971a), a decreased number of circulating lymphocytes (Duquesnoy, Kalpaktsoglou and Good, 1970), and lymphocytic depletion in the thymus-dependent areas of their peripheral lymphoid organs (Duquesnoy *et al.*, 1970; Fabris *et al.*, 1971a). In comparison to phenotypically normal mice, their response to some T-dependent antigens is diminished (Baroni *et al.*, 1969; Pierpaoli *et al.*, 1969; Duquesnoy *et al.*, 1970; Fabris *et al.*, 1971a) or delayed (Baroni, Pesando and Bertoli, 1971). These dwarf mice have deficient cell-mediated immune reactions in that allograft is prolonged (Pierpaoli, Fabris and Sorkin, 1970; Fabris *et al.*, 1971a) and the graft-versus-host (GVH) reactivity of their spleen cells is reduced (Duquesnoy *et al.*, 1970). We wish to report in this paper the results of our studies on serum thymic hormone activity (TA) and the development of spontaneous autoimmunity in dwarf mice and their littermates.

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MATERIALS AND METHODS

Inbred Snell–Bagg hypopituitary dwarf mice (dw/dw) and their littermates (+/dw or +/+) were provided by the Centre d'Elevage des Animaux de

Laboratoire du C.N.R.S. (Orléans, La Source, 45, France). These animals were reared in our laboratory from the age of 3 weeks; they were maintained in an isolated room with a minimum temperature of 20°, exposed to artificial daylight for 14 h, and had unlimited access to tap water and standard laboratory chow. The dwarf mice were also fed Swiss cheese. Thymectomy by suction (Miller, 1960) or sham-thymectomy was performed at the age of 4 weeks. The animals were killed when they were 14 weeks old, and blood was collected from the orbital sinus, with the kidneys being fixed immediately for light and immunofluorescence microscopy.

Serum TA was determined in the dwarf mice and their littermates at various intervals from 4 to 14 weeks. The serum was filtered through Amicon Centriflo membranes CF 50 A (molecular weight cut-off: 50,000). Serum TA was immediately detected according to a previously described method (Bach and Dardenne, 1973). The filtered serum was incubated with 3×10^6 spleen cells from adult C57Bl/6 mice (thymectomized 10–15 days earlier) for 90 min at 37° in the presence of 10 μg/ml azathioprine. After incubation, 12×10^6 sheep red blood cells (SRBC) were added, and the mixture was then centrifuged at 200 *g* for 5 min (4°). Rosette-forming cells (RFC), counted in a haemocytometer, numbered 1000×10^6 in the absence of TA, and $200–400 \times 10^6$ in its presence.

For light microscopy, renal tissue was fixed in Dubosq-Brasil solution (Bouin's alcoholic fluid), embedded in paraffin, and cut in 4-μm sections, which were stained with Masson's trichrome or periodic acid silver methenamine. For immunofluorescence studies, the kidneys were frozen in liquid nitrogen and kept at − 70°. Sections were cut at 4 μm on a CTI model international cryostat. Fluorescein-conjugated goat anti-mouse total IgG, IgG1, IgG2, IgA, and IgM (Meloy Laboratories, Incorporated, Springfield, Virginia) were used after their specificity was verified by immunoelectrophoresis. Rabbit anti-mouse C3 (Nordic Immunological Laboratories, The Netherlands) was conjugated to fluorescein isothiocyanate according to a technique described by Oldstone and Dixon (1969). The slides were examined under a Leitz fluorescent microscope using Ug5 and Bg38 filters.

ANA were detected in the serum by the usual indirect immunofluorescence method. Sections of frozen normal rat liver were cut at 4 μm on a cryostat, incubated with the serum at room temperature for 25 min, rinsed in phosphate-buffered saline, pH 7·3, for 10 min, and finally re-incubated with fluorescein labelled rabbit anti-mouse immunoglobulins (Institut Pasteur, Paris) for 25 min. The sera used were diluted 1/10.

Anti-DNA antibodies were detected by radioimmunoassay (Farr and Bloch, 1960). Briefly, 0·1 ml (200 μg) of ^{125}I-labelled DNA was mixed with 0·1 ml of decomplemented serum diluted 1/10 with sodium borate buffer. The mixture was incubated at 37° for 30 min and at 4° for 24 h. Immunoglobulins were then precipitated by adding ammonium sulphate at a final concentration of 50 per cent. After centrifugation, the pellets were resuspended in 1 ml of 0·1 *N* HCl, added to 10 ml of Instagel (Packard), and counted. The specific precipitation of ^{125}I-labelled DNA was calculated and corrected after non-specific precipitation was estimated with control sera. Precipitation values higher than 30 per cent were considered to be positive.

RESULTS

Serum TA

Hypopituitary dwarf mice showed lower serum TA than their littermates, with a progressive decrease being seen during the study which resulted in a striking fall by the 14th week. During the same period, the TA remained stable in the control mice (Fig. 1).

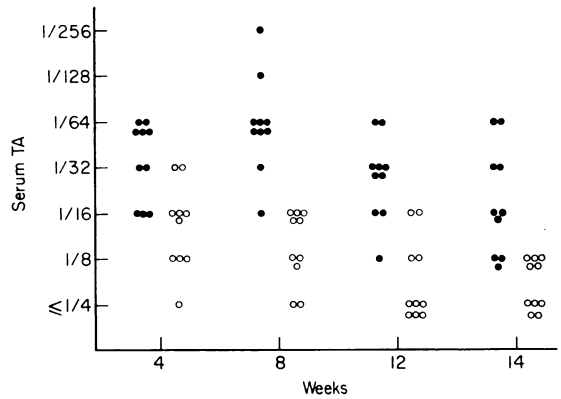

Figure 1. Comparison of serum TA in dwarf mice (dw/dw) and their littermates (dw/+ ; +/+). Each circle represents one mouse: (●) normal; (○) dwarf.

Light microscopy

The kidneys of dwarf mice were histologically

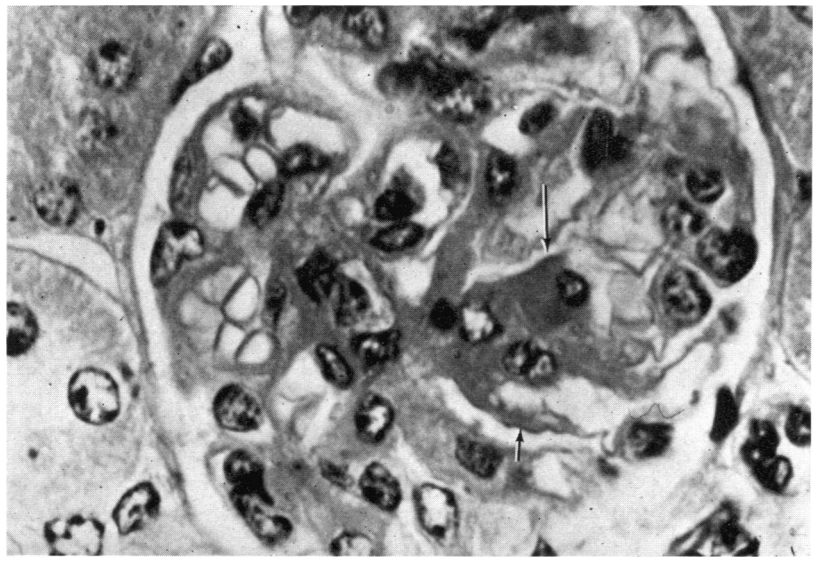

Figure 2. Glomerulus from a dwarf littermate showing enlarged mesangial area and the presence of a large intercapillary deposit (long arrow). A small subendothelial deposit is also visible on a capillary basement membrane (short arrow). (Trichrome stain; magnification × 3000.)

Table 1. Immunofluorescence and serological findings in 14-week-old dwarf mice

Mouse no.	Sex	IgG	IgG1	IgG2	IgA	IgM	C3	ANA	Anti-DNA antibody binding (per cent)
1	M	± M	–	± M	–	–	–	–	30·6
2	M	–	–	–	–	–	–	–	20
3	M	± M	–	± M	± M	± M	–	–	23·5
4	M	± M	–	± M	–	± M	–	–	n.d.
5	M	± M	–	± M	–	± M	–	–	n.d.
6	F	± M	–	± M	± M	± M	–	–	23·7
7	F	± M	–	± M	± M	± M	–	–	23·4
8	F	–	–	–	–	± M	–	–	n.d.
9	F	± M	–	± M	± M	± M	–	–	n.d.
10	F	–	–	–	–	–	–	–	n.d.

– = No and ± = minimal immunoglobulin deposits; M = mesangial area; n.d. = not determined.

normal. However, their littermates exhibited glomerular lesions characterized mainly by enlargement of the mesangial area without cellular proliferation but with intercapillary deposits that could be visualized with Masson's trichrome (Fig. 2). In one mouse, the lesions were more severe: almost all of the glomeruli displayed segmentary proliferative damage with mesangial and subendothelial deposits on the peripheral capillary loops.

Immunofluorescence microscopy

There were minimal deposits of IgG, IgG2 and IgM in the mesangial areas of dwarf mice glomeruli and no C3 deposits could be seen (Table 1). On the other hand, significant granular deposits of all the immunoglobulins as well as C3 were visible in the glomeruli of the control littermates (Table 2). These deposits were located mainly in the mesangial

Table 2. Immunofluorescence and serological findings in 14-week-old littermates

Mouse no.	Sex	IgG	IgG1	IgG2	IgA	IgM	C3	ANA	Anti-DNA antibody binding (per cent)
Sham-thymectomy*									
1	M	+++M	+M	++M	+++M	++M	+M	+	46·1
2	M	++M	+M	++M	+++M	++M	±M	+	42
3	M	++M	±M	+M	++M; ++C	+++M	±M	+	42
4	M	++M	+M	++M	+++M; +C	++M	±M	—	n.d.
5	M	++M	+M	++M	+++M	++M	±M	+	51
6	M	+++M	+M	++M	+++M	++M	+M	+	n.d.
7	F	++M	±M	+M	++M; +C	++M	+M	+	44
8	F	++M; +C	+M	++M	+++M; +C	++M; +C	+M	-	n.d.
9	F	+M	−	+M	++M	++M	±M	+	42·4
10	F	++M	+M	+M	++M	+++M; +C	+M	+	45·1
11	F	++M	+M	+M	++M	++M; ±C	+M	+	42
Thymectomy*									
12	M	++M	±M	+M	++M	+++M; ±C	+M	+	38
13	M	+M	±M	±M	+M	+M	−	+	37·9
14	M	++M	+M	+M	++M; ±C	++M	++M	+	41·4
15	M	++M	+M	++M	+++M	++M	±M	+	64·1
16	M	++M	±M	+M	++M	++M	±M	+	42·8
17	M	+M	−	±M	++M	+M	±M	+	n.d.
18	F	+++M; +C	++M; ±C	++M; +C	+++M; +C	+++M; +C	++M	+	45·9
19	F	+++M; +C	+++M; +C	+++M; ±C	+++M; +C	+++M; +C	+++M; +C	−	n.d.
20	F	++M	+M	+M	+++M	+++M	+M	+	43·7
21	F	+++M; +C	+++M; ±C	+++M; ±C	+++M; ±C	+++M; ±C	+++M; +C	+	47·6
22	F	++M	+M	++M	++M	++M	+M	−	n.d.
23	F	+++M	++M	++M	++M	+++M; +C	+M	−	33·9

− = No, ± = minimal, + = small, ++ = moderate, +++ = extensive immunoglobulin deposits; M = mesangial area; C = along capillary basement membrane; n.d. = not determined.

* All mice were thymectomized or sham-thymectomized at 4 weeks of age.

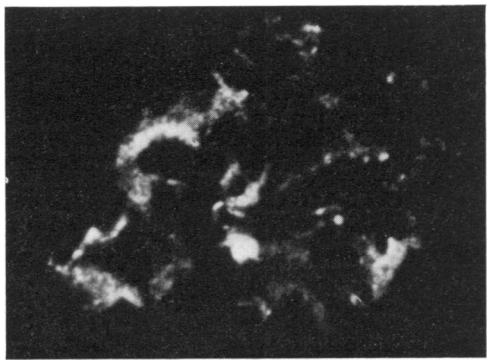

Figure 3. Immunofluorescence micrograph of glomerulus from a dwarf littermate showing deposits of IgG in the mesangium and along the capillary basement membrane. (Magnification × 1600.)

areas but, in twelve mice, there were some along the capillary basement membranes (Fig. 3). No differences in the composition or density of the deposits were noted between thymectomized and sham-thymectomized males. However, thymectomized females had more deposits, especially of IgG1 and C3, than controls of the same sex (Table 2).

Serological studies

ANA and anti-DNA antibodies in the serum of dwarf mice were always found to be negative (Table 1). However, the ANA were positive in eighteen out of twenty-three normal littermates. Anti-DNA antibodies were verified in seventeen of these animals and were always positive (Table 2).

DISCUSSION

Our results showing a decrease of TA in the serum of hereditary hypopuitary dwarf mice provide additional evidence of some immunological deficiency in these animals, affecting principally their thymus-dependent immunological functions. The association between decreased thymus activity and the spontaneous development of autoimmunity is now well recognized (Talal and Steinberg, 1974). It has been shown that normal strains of mice develop circulating autoantibodies with age (Teague, Yunis, Rodey, Frish, Stutman and Good, 1970; Marckham, Sutherland and Mardiney, 1973) and that this phenomenon is enhanced by neonatal thymectomy (Teague *et al.*, 1970). New Zealand Black mice, well known for their spontaneous autoimmunity, exhibit deficient T-cell functions (Talal *et al.*, 1974) and decreased circulating thymic hormone since the age of 1 month (Bach, Dardenne and Salomon, 1973). Moreover, congenitally athymic nude mice develop spontaneous circulating autoantibodies (Monier, Sepetjian, Ortonne and Thivolet, 1973) and glomerular lesions (Pelletier, Hinglais and Bach, 1975) early in life. It is thus surprising that our hypopituitary dwarf animals showed neither circulating autoantibodies nor glomerular lesions while their littermates exhibited circulating ANA and anti-DNA antibodies with glomerular damage as early as 14 weeks after birth, suggestive of an immune-complex type of glomerulonephritis. One explanation may be that dwarf mice possess mature lymphocyte precursors which can, under humoral or adjuvant influences, differentiate into fully immunocompetent cells. Some experimental data suggest that there is an almost normal percentage of theta-bearing lymphocytes in the thymus and peripheral lymphoid organs of these mice, as compared to their littermates (Baroni and Doria, 1973). Dwarf mice also display a significant contact sensitivity response to picryl chloride (Baroni and Doria, 1973), and a normal reaction to BSA in Freund's complete adjuvant (Fabris *et al.*, 1971a). More recently, their thymus cells were found to exhibit a greater *in vitro* blastogenic reaction to phytohaemagglutinin and a better GVH reactivity than corresponding cells of littermates (Duquesnoy and Ahrens, 1973). Moreover, some T-dependent immune responses can be restored in these mice by somatotrophic hormone and thyroxine (Pierpaoli *et al.*, 1969; 1970; Fabris, Pierpaoli and Sorkin, 1971b). Indeed, the action of growth hormone (GH) on lymphoid organs is not elucidated. However, it has been established that GH can stimulate lymphocyte transformation *in vitro* (Astaldi, Burgio, Astaldi, Yalcin, Moardi and Gatti, 1972) and enhance the rate of RNA and DNA synthesis in lymphoid structures (Pandian and Talwar, 1971). Consequently, the absence of GH and the secondary decrease of intracellular synthesis in dwarf mice may result in a deficient immunological response to foreign and indigenous antigens.

Although further investigation of immunological responsiveness in the presumably normal littermates is necessary, our results indicate that they precociously acquire hyperreactivity to autoantigens. According to some recent hypotheses on interaction between cell-mediated and humoral immunity, one can speculate that there is a precocious decrease of

'suppressor T cells', that is, a subpopulation of thymus-derived cells capable of inhibiting the production of antibodies to indigenous and foreign antigens (Morse, Steinberg, Schur and Reed, 1974; Miller, 1975; Allison, Denman and Barnes, 1971). The possible enhancement of autoimmune disease that we observed in female thymectomized littermates is in agreement with a previous report (Teague *et al.*, 1970) suggesting that adult thymectomy may aid the development of autoimmunity.

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