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Serum Immunoglobulin Levels in Thymus-Deficient Pituitary Dwarf Mice

P. C. WILKINSON, HARMINDER SINGH AND E. SORKIN

Department of Bacteriology and Immunology, University of Glasgow;*
Schweiz. Forschungsinstitut, Medizinische Abteilung, 7270 Davos-Platz, Switzerland

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Summary. Hypopituitary dwarf Snell–Bagg mice have previously been shown to have a reduced capacity to produce antibody to sheep red cells and to reject foreign skin grafts. Their thymuses, spleens and lymph nodes are hypoplastic. However, quantitative estimation of their serum immunoglobulin levels reveals no significant difference from the values seen in normal litter-mates. Immunoelectrophoretic analysis shows no overall differences in immunoglobulin pattern between normal and dwarf mouse serum except a possible alteration in distribution of the IgG subtypes.

INTRODUCTION

Mutant pituitary dwarf Snell–Bagg mice (genetic symbol dw) have very low levels of growth hormone and thyrotropic hormone. In addition, they show involution of the central and peripheral lymphatic tissue, a reduced antibody response to challenge with sheep red cells and a retarded rejection of skin allografts (Pierpaoli, Baroni, Fabris and Sorkin, 1969; Fabris, Pierpaoli and Sorkin, 1969). Recent experiments (Baroni, 1967; Pierpaoli *et al.*, 1969) in which such mice were reconstituted with hormones suggested a crucial link between the endocrinological and the immunological deficiencies inasmuch as hormone treatment restored the immune response against sheep red cells and reduced the rejection time of allografts to that observed in normal litter-mate controls. Such hormonal treatment caused reversion of the morphology of the thymus and peripheral lymphoid tissues to normal and accelerated the growth of the animals.

The high incidence of infection, lymphoid depletion and early death in untreated dwarf mice suggested to us that their serum immunoglobulin levels might be low. The study reported here which investigates this possibility shows, in fact, that there is no gross quantitative abnormality of serum immunoglobulin levels of dwarf mice as compared with normal litter-mate controls.

MATERIALS AND METHODS

Mice

Male and female, 40–50-day-old dwarf Snell–Bagg mice and normal litter-mates were used as serum donors. Details of the origin of the dwarf mouse stock are given by Pierpaoli *et al.* (1969). Blood was collected by intracardiac puncture or from the retro-orbital venous plexus.

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Sera

All individual sera from single mice were lyophilized and stored at +5°. When needed for the tests, they were reconstituted with double distilled water to the original volume.

Antisera

Goat anti-mouse 7S γ-globulin (Hyland Laboratories Inc., Los Angeles, U.S.A.) was used for immunoelectrophoretic analysis of mouse 7S γ-globulins and for incorporation in agar gel plates for quantitative estimation of serum γG-globulin levels. This antiserum was monospecific for mouse γG-globulin as judged by immunoelectrophoresis against whole mouse serum, giving a single precipitin line with a spur indicating a reaction against both γG_1 and γG_2 globulins (see Fig. 1). The total immunoglobulin content of normal and dwarf mouse sera was examined by immunoelectrophoresis against a polyvalent antiserum prepared in our laboratories by injection of a whole mouse serum globulin fraction into rabbits in Freund's complete adjuvant.

Immunoelectrophoresis of mouse sera was achieved by standard techniques in agar gel incorporating barbitone buffer, pH 8·4.

Quantitative estimation of serum γG levels

The single radial immunodiffusion method, adapted from that of Mancini, Carbonara and Heremans (1965) was used to assay serum γG levels in dwarf and normal litter-mate Snell–Bagg mice. Ionagar (2 per cent), incorporating barbitone buffer (pH 8·6) was mixed at 48° with antiserum against mouse γG-globulin. The antiserum–agar mixture was poured into polystyrene Petri dishes and allowed to set. Each dish contained 10 ml of this mixture made up of 9·5 ml agar and 0·5 ml antiserum. Circular wells were punched in the gel using a template and each well was filled with 2 μl of a 1 : 10 dilution of mouse serum delivered from a micropipette. Twenty-one mouse sera were assayed in this way on a single plate. As controls, weighed amounts of lyophilized mouse γG-globulin, obtained from pooled mouse serum by salt precipitation and DEAE-cellulose chromatography, were dissolved in physiological salt solution at strengths of 2, 1, 0·5 and 0·25 mg/ml. Wells were filled with 2 μl of each of these control preparations. The plates were left for 48 hours to allow ring-shaped precipitates to develop around the wells. The diameter of the rings was measured and a graph was prepared in which ring diameter of the control solutions was plotted against antigen concentration. A straight-line curve was obtained and γG-globulin levels in each of the test sera were determined by comparison with this graph.

RESULTS

SERUM γG GLOBULIN LEVELS

Quantitative estimation of the γG-globulin levels in sera from ten normal and eleven dwarf Snell–Bagg mice by the single radial immunodiffusion method gave the following results: Mean ring diameter for normal mouse sera 4·41±0·58 mm: mean ring diameter for dwarf mouse sera, 4·28±0·67 mm. Mean concentration of γG-globulin in normal mouse sera, 7·0±2·8 mg/ml; mean concentration of γG-globulin in dwarf mouse sera, 6·4±3·2 mg/ml. Individual results are shown in Table 1. There was no significant difference between serum γG-globulin levels of normal Snell–Bagg mice and those of dwarf mice.

TABLE 1

SERUM γG LEVELS IN NORMAL AND DWARF MICE

Normal mouse serum		Dwarf mouse serum	
Precipitin ring diameter (mm)	Concentration γG (mg/ml)	Precipitin ring diameter (mm)	Concentration γG (mg/ml)
5·4	11·8	5·5	12·2
5·2	10·8	5·2	10·8
4·8	8·8	4·9	9·4
4·7	8·4	4·9	9·4
4·5	7·4	4·1	5·5
4·3	6·4	3·9	4·6
4·0	5·0	3·9	4·6
3·8	4·0	3·8	4·0
3·7	3·6	3·7	3·6
3·7	3·6	3·6	3·2
		3·6	3·2
Mean 4·41 ± 0·58	7·0 ± 2·8	4·28 ± 0·67	6·4 ± 3·2

IMMUNOELECTROPHORESIS

These observations were borne out by immunoelectrophoretic analysis of sera from twenty-one dwarf Snell–Bagg mice and nineteen litter-mate controls using a monospecific anti γG-antiserum. Individual sera within the two groups showed differences in sharpness of the γG precipitin arc, its extent and its distance from the antigen well but no overall difference could be shown between the two groups. One qualitative difference was, however, seen in that the γG precipitin arcs from dwarf mouse sera showed a spur at their cathodal end—the type of spur associated with γG_1 globulin—while those from normal mice showed a spur of the type given by γG_2 globulin at their anodal end (Fig. 1). This finding might indicate a difference in concentration of the two γG subtypes in the two groups of mice, but subtype-specific antisera were not available to test this.

Fig. 2 shows the immunoelectrophoretic pattern of serum from a dwarf Snell–Bagg mouse compared with that from a litter-mate control. The precipitin arcs representing γG, γA and γM appear to be similar in strength and position in both sera. This was true of all sera tested from the two groups. Although we were unable to assay γA and γM levels

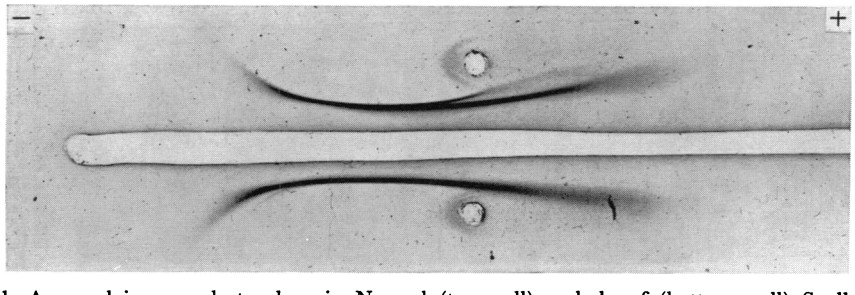

FIG. 1. Agar gel immunoelectrophoresis. Normal (top well) and dwarf (bottom well) Snell–Bagg mouse sera against anti-mouse γG (trough). Note similarity of strength and position of the arcs given by both sera but that a spur is seen near the starting well in the top arc which is not present in the lower one and that a fine spur is present at the cathodal end of the lower arc which is not seen in the upper arc.

quantitatively this immunoelectrophoretic analysis is sufficient to indicate no gross deficiency of serum γA and γM levels in dwarf mice in comparison with their normal litter-mate controls.

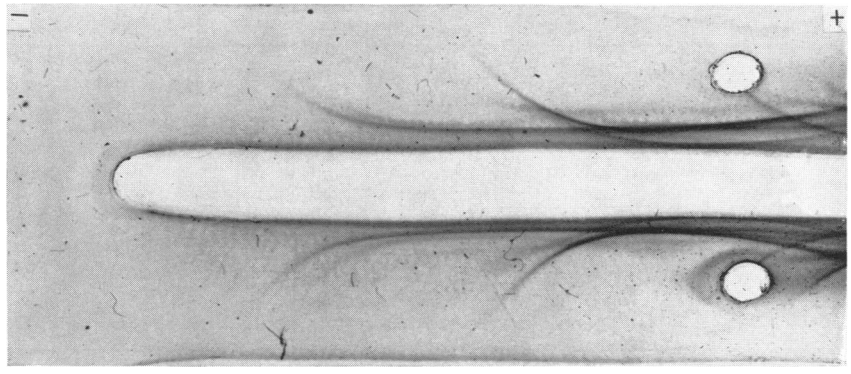

FIG. 2. Agar gel immunoelectrophoresis. Immunoglobulin arcs of normal (top well) and dwarf (bottom well) Snell–Bagg mouse sera developed against rabbit anti-mouse globulin (trough). Note that the immunoglobulin patterns of both mouse sera are essentially identical.

DISCUSSION

The normal immunoglobulin levels in pituitary dwarf mice reported here stand in contrast to the immunological deficiency state present in the same mice as reported earlier (Pierpaoli *et al.*, 1969), i.e. hypoplasia of the thymus and lymphatic tissue and reduction in the circulating lymphocyte count, delayed allograft rejection and poor response to challenge with sheep red cells. These abnormalities in dwarf mice and in mice deprived experimentally of pituitary hormones (Pierpaoli and Sorkin, 1967, 1968, 1969a, b) are dependent on the endocrine system and can be corrected by administration of pituitary somatotropic or other hormones.

Possible explanations for the normal immunoglobulin levels in dwarf mice are as follows:

(1) These mice have a relative deficiency of thymus-dependent immunity but other immunological responses are normal as is seen in di George's syndrome in man. If this is the case, it suggests that thymus-dependent immunity, delayed hypersensitivity and graft rejection are hormone dependent to a greater extent than non-thymus dependent humoral immunity.

(2) Although the serum immunoglobulin level is quantitatively normal, the antibody molecules may be abnormal in respect to their ability to combine with antigen. The response to sheep red cells in our mice was poor at 4 days after challenge, but later measurements showed a day by day increase in the response of dwarf mice at a time when normal littermates had already reached peak antibody levels. The antibody response to sheep cells has been shown previously to be thymus-dependent in mice (Humphrey, Parrott and East, 1964). In recent experiments (Fabris, Pierpaoli and Sorkin, unpublished), it was shown that on primary challenge with a different antigen, bovine serum albumin, dwarf mice achieved a level of antibody as high as their normal litter-mates but they reached maximum values about a week later than the normal controls. These results imply that dwarf mice produce antibody abnormally slowly but do not suggest that this antibody combines poorly with antigen.

(3) Dwarf mice are not entirely deficient of lymphoid cells. The plasma cell content of the spleen and lymph nodes of our dwarf mice is lower than that of normal mice (Fabris, Pierpaoli and Sorkin, unpublished). Although production of lymphoid cells is likely to be hormone-dependent, the capacity of these cells, once produced, to mature into immunoglobulin forming cells may not be hormone dependent to the same extent although their speed of proliferation and division may be slower than in normal animals. Moreover, serum immunoglobulin levels may be maintained in the face of reduced synthesis by slower catabolism of those immunoglobulin molecules which are present in serum.

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