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Studies on the Thymic Dependence of the Immunoglobulin Classes of the Mouse[1] (38570)

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(Introduced by S. Tokuda)

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The thymus plays an important role in the antibody secreting capacity of the bone marrow-derived (B) lymphocyte lineage to many types of "thymus-dependent" antigens (1, 2). IgG production to such antigens is highly dependent on thymus-derived (T) lymphocytes whereas substantial early IgM production can occur in the presence of very low numbers of T lymphocytes (3, 4). The influence of the thymus on the immunoglobulin (Ig) class distribution of antibodies would presumably be reflected in serum Ig levels (5). Several studies using neonatally thymectomized (6, 7) or congenitally athymic (*nu/nu*) mice (8, 9) have shown that there is generally a marked decrease in the serum concentrations of IgA and IgG. The present study was undertaken to extend these observations and identify all thymus-dependent Ig classes in the mouse using animals with markedly depleted T lymphocyte populations. Accordingly serum Ig concentrations were measured in *nu/nu* mice and irradiated, adult thymectomized mice reconstituted with anti-Thy-1 (anti-theta) treated bone marrow cells (ATx + B) and compared with control animals which possessed T lymphocytes.

Methods and Materials. Animals. C3H female mice were obtained from Charles River (France). Congenitally athymic, nude female mice (*nu/nu*) and normal male littermates (*nu/+* or +/+) were obtained from G. L. Bomholtgård (Ry, Denmark). These *nu/nu* mice and normal littermates were 6 wk of age and had been backcrossed 3–5 generations onto a Balb/c background.

Preparation of irradiated reconstituted mice. The adult thymectomized mice reconstituted with either bone marrow (ATx + B) or bone marrow and thymus (ATx + BT) were prepared essentially according to the method of Feldmann and Basten (10). Adult thymectomy was performed on 5–6-wk old female C3H mice. The mice were irradiated 2 wk after thymectomy with 850 rads (Theratron 80, Cobalt 60 source at 65 rads/min). The irradiated mice were reconstituted within 3 hr with either 6×10^6 bone marrow cells (ATx + B) or the same number of bone marrow cells plus 50×10^6 thymus cells (ATx + BT). The bone marrow cell suspension in all cases was incubated with anti-Thy-1 antiserum and agarose adsorbed guinea pig complement under conditions known to cause 100% lysis of an identical number of thymus cells. The mice were bled 4–6 wk after irradiation. The absence of a thymus was verified in the thymectomized mice by autopsy after completion of the experiment.

Preparation of specific antisera. Antisera to mouse Igs were raised in rabbits by three or more intramuscular injections of 0.5–1.0 mg of purified myeloma protein in complete Freud's adjuvant given at 2–3-wk intervals. The animals were bled one week following the last immunization. The following purified myeloma proteins were used as immunogens: IgG1 = MOPC 71 (Balb/c), IgG2a = 5563 (C3H), IgG2b = MOPC 141 (Balb/c), IgG3 = J 606 (Balb/c), IgM = MOPC 104E (Balb/c), IgA = MOPC 315 (Balb/c). The original IgG3 tumor was kindly supplied by Dr. Howard Grey. The purified myelomas were isolated from serum globulin fractions or tumor extracts by passage over DEAE-cellulose columns using a continuous gradient elution (0.01–0.30 *M* phosphate buffer, pH 8.0).

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The rabbit anti-mouse Ig antisera were made specific by passage over solid phase immunoadsorbent columns (Sepharose 4B) to which purified myeloma proteins were attached (11). The adsorptions which each antiserum underwent to render it monospecific were as follows: Anti-IgG1 = adsorbed with MOPC 141 (IgG2b); anti-IgG2a = adsorbed with MOPC 71 (IgG1); anti-IgG2b = adsorbed with MOPC 71 (IgG1) and 5563 (IgG2a); anti-IgG3 = adsorbed with MOPC 71 (IgG1) and MOPC 141 (IgG2b); anti-IgM = adsorbed with MOPC 315 (IgA) and MOPC 71 (IgG1); anti-IgA = adsorbed with MOPC 104E (IgM) and MOPC 71 (IgG1).

In each case the monospecific antiserum produced a single line after electrophoresis of whole serum and formed a precipitin line only with the appropriate purified protein and not the others. In addition the adsorbed antisera produced agglutination only of sheep red cells coated with the corresponding protein (12).

Determination of serum Ig. This was done by means of a single radial immunodiffusion method (13). Results for IgG1, IgG2a, IgG2b, and IgM were expressed in terms of mg/ml when the test sera were compared with a reference serum of known Ig concentration. The remainder of the Ig levels were expressed as percentages of normal or control Balb/c sera. Statistical tests of significance were carried out using the Student's *t* test.

Preparation of anti-Thy-1 (anti-theta) antiserum. Rabbit anti-Thy-1 antiserum was prepared by the immunization of rabbits with mouse brain according to the method of Golub (14).

Results and Discussion. A first approach to the assessment of the influence of the thymus on Ig production is to compare serum Ig levels in T lymphocyte deficient mice versus normal controls. The homozygous *nu/nu* mouse is markedly deficient in Thy-1 positive lymphocytes (15) whereas the littermate control mice have normal numbers of T lymphocytes. Table I shows that there is a significant decrease ($P < 0.05$) in 3 of the 6 Ig classes of *nu/nu* mice when compared with littermate normal mice. The values of IgG1, IgA, and IgG2a were respectively 8, 17, and 31% of normal. IgM was increased (340% of normal). Depressed IgG1, IgA, and IgG2a levels in *nu/nu* mice have been reported by Pritchard *et al.* (8) and Bloemmer and Eyssen (9). The latter study also reported a decrease in IgG2b which was not seen in the present study. Crewther and Warner (16) reported a marked reduction in IgA but found only a moderate, if any, reduction in IgG1 and IgG2. In two of the reports alluded to above (9, 16) IgM levels were normal. However, Pritchard *et al.* (8) found that although the mean percentage of IgM was 107% of normal in 15 7- to 8-wk-old *nu/nu* mice, eight out of 41 *nu/nu* mice of various ages had elevated IgM levels. Elevated IgM levels were also found in some C3H mice of various ages which had undergone neonatal thymectomy (6). Fahey *et al.* (7) showed an elevation of mean IgM levels in 6-wk-old neonatally thymectomized C3H mice although approximately normal levels were seen in older mice. Balb/c mice thymectomized at a later age (21 days) showed subnormal IgA but normal levels of IgM and IgG (17). However, peripheral migration of thymus-derived cells during the 21 days before thymectomy probably occurred and may account for the difference between the neonatal and adult mouse thymectomy studies. Overall there is consistent agreement

TABLE I. SERUM IMMUNOGLOBULIN LEVELS[a] OF CONGENITALLY ATHYMIC NU/NU/MICE.

Class of Ig	Nude (*nu/nu*)	Control
G1	0.15 ± 0.18[b] (8)[c]	1.87 ± 0.22
G2a	0.75 ± 1.04[b] (31)	2.47 ± 0.92
G2b	1.55 ± 0.42	1.58 ± 0.94
G3	134 ± 22%	100 ± 20%
A	15 ± 2%[b] (17)	89 ± 12%
M	8.30 ± 2.31[b] (340)	2.40 ± 2.25

[a] Expressed as mg/ml or percentage of a reference or control sera. In the case of IgG3 the control sera mean was taken as 100% whereas in the case of IgA another normal serum was used as a reference and the control and nude sera expressed as a percentage of this.

[b] Statistically significant from control, $P < .05$.

[c] The figure in parenthesis is the percentage of the control where a significant difference exists.

TABLE II. SERUM IMMUNOGLOBULIN LEVELS[a] OF RECONSTITUTED C3H MICE.

Class of Ig	ATx + B	ATx + BT	Control[b]
G1	0.27 ± 0.16[c] (11)[d]	1.54 ± 1.52	2.58 ± 0.79
G2a	6.31 ± 2.53	6.21 ± 4.55	4.07 ± 0.29
G2b	3.68 ± 1.78	2.67 ± 1.30	3.14 ± 1.10
G3	73 ± 11%	65 ± 20%	100 ± 33%
A	144 ± 52%	93 ± 32%	99 ± 15%
M	3.71 ± 1.26[c] (55)	3.34 ± 1.28[c] (49)	6.82 ± 1.53

[a] Expressed as mg/ml or percentage of a reference or control sera. In the case of IgG3 the control mean was taken as 100% whereas in the case of IgA another normal serum was taken as a reference and the control and reconstituted sera expressed as a percentage of this.

[b] Controls are normal, nonirradiated C3H females of the same age.

[c] Significant difference from control ($P < 0.05$).

[d] The figure in parentheses is the percentage of the control where a significant difference exists.

with a depression of IgG1, IgG2a, and IgA in *nu/nu* mice and in thymectomized, nonirradiated mice. The elevated IgM levels seen in our studies are different from the normal levels reported by some (9, 16) but are consistent with the findings of other investigators (6–8). Possibly some of the discrepancies of various Ig levels in *nu/nu* mice from various laboratories could be attributed to the genetic differences which undoubtedly exist in these different colonies throughout the world. Taken at its face value the increase in IgM would suggest an absence of a "suppressor" T lymphocyte population influencing the IgM serum level.

In Table II are listed the results obtained with the reconstituted C3H mice. When ATx + B mice were compared with normal controls a significant depression was noted in the IgG1 and IgM serum levels. The ATx + BT mice in comparison with normal controls had a significant depression in IgM only. The only significant difference in serum Ig levels between Atx + B and ATx + BT mice was in IgG1. The lack of a decrease in IgA between ATx + B and ATx + BT was surprising in view of the very low levels seen in *nu/nu* mice. IgM levels were not different between the ATx + B and ATx + BT mice but they were both approximately one-half the nonirradiated controls. The reason for this difference in IgM levels between the irradiated, reconstituted groups and the nonirradiated controls is uncertain. Levels of all Igs are known to be depressed in lethally irradiated, nonreconstituted mice (18) before their death 7–10 days following irradiation. However, we might expect a depression in other Ig classes besides IgM if irradiation *per se* was an important factor in Ig depression. Such a depression in other classes was not observed. However it is conceivable that reconstitution moderates the effect of irradiation on other classes more so than IgM.

The decreased levels of IgG1, IgG2a, and IgA in the *nu/nu* mice could be the result of decreased synthesis or increased catabolism or loss. Generally, increased catabolism or loss of Ig in neonatally thymectomized mice were detected only when a wasting syndrome was present (7). However, since the *nu/nu* mice no evidence of this syndrome except terminally it seemed unlikely that increased catabolism or loss was the cause of a decreased Ig serum level. Furthermore since unstimulated axenic mice have low serum IgG1 and IgG2 levels which rise in response to antigenic stimulation (5), and since many antigens are thymus-dependent, it follows that a decreased level in any given serum Ig class level in the *nu/nu* mice may reflect the inability of the mice to respond to diverse thymus-dependent antigens. There are numerous reports which show that the IgG response to thymus-dependent antigens is markedly decreased in T lymphocyte deficient mice. For example Pritchard *et al.* showed that there was some early IgM response but minimal or no IgG response when *nu/nu* mice were immunized with sheep red blood cells (8). This is in contrast to their normal response to thymus-independent antigens (19). In addition Torri-

giani (20) attributed most of the decreased response to HSA in neonatally thymectomized mice to a relative decrease in the IgG1 response.

Overall the results reported here support the concept that the thymus has a profound influence on IgG1 serum levels. The decrease in IgG2a and IgA observed in the *nu/nu* mice was not found in the irradiated mice. It is unclear whether this discrepancy might be attributable to a more plentiful residual T lymphocyte population in the latter group rather than an apparent lack of thymic influence. The IgM level differences between the *nu/nu* and irradiated mice might be explained in a similar manner with a relative absence of a suppressor T lymphocyte population in the *nu/nu* group as well as the effect of irradiation in the latter group.

Summary. The influence of the thymus on serum immunogobulin (Ig) concentration was studied by a comparison of serum Ig levels in congenitally athymic (*nu/nu*) mice versus control littermate heterozygotes and adult thymectomized, irradiated, bone marrow reconstituted mice (ATx + B) versus adult thymectomized, irradiated mice reconstituted with bone marrow and thymus (ATx + BT). In the former group IgG1, IgA, and IgG2a were 8%, 17%, and 31% of controls. IgM levels were increased (340%) compared to controls. When ATx + B mice were compared with nonirradiated controls significant depressions were noted in serum IgG1 and IgM. The only significant decrease in serum Ig levels between ATx + B and ATx + BT was in IgG1. These results are discussed in terms of the effects of thymic influence, residual T lymphocyte population differences between the two groups, and the effect of irradiation.

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