

Serum IgA Levels and Antinuclear Antibody Formation in Mice¹ (39566)WAYNE D. MERCER, JUDITH A. PACHCIARZ,² AND PERRY O. TEAGUE³*Department of Pathology, University of Florida, Medical School, Gainesville, Florida 32610*

Some forms of autoimmune disease in humans are characterized by IgA deficiency and the appearance of antinuclear antibodies (1-3). By contrast, however, in aged humans the presence of autoantibodies is associated with an IgA hypergammaglobulinemia (4). This suggests that the relationships between the appearance of autoimmune antibodies and deficiencies or elevation of serum IgA levels may be due to different mechanisms which may be age dependent. We have investigated mice for age-associated changes in thymus and bone marrow function as they relate to serum IgA levels and the appearance of antideoxyribonucleoprotein (anti-DNP) antibodies in autoimmunity.

Materials and methods. A/J and DBA/1J mice were obtained from Jackson Laboratories (Bar Harbor, Me.) and were either 4-6 weeks of age (young mice) or 18-month-old retired breeders (old mice) when used. Only female mice were used in these experiments.

Thymectomy was performed in young animals using a standard suction technique under Nembutal (Abbott Labs) anesthesia (5). Thymus grafts were obtained from 6-week-old (YT) or 18-month-old (OT) syngeneic donors, irradiated, and placed on the cut surface of the recipient's kidney (6). All mice were examined for completeness of thymectomy and/or graft acceptance at autopsy by both visual and histologic techniques. Representative tissue was taken from the site of thymectomy or thymus graft, sections were prepared using hema-

toxylin and eosin and examined microscopically. The data obtained from studies of animals with incomplete thymectomy or lack of graft acceptance are not included in these results.

Irradiation was performed using a ¹³⁷Cs source in a Model M Gammator (Radiation Research Corp., Parsippany, N.J.) at a dose rate of 110 R/0.1 min. Thymus grafts were irradiated to kill thymocytes at 550 R (7). Total body irradiation to induce IgA deficiency (8, 9) and destroy hemopoietic and lymphoid tissues was accomplished at a dose of 880 R. Irradiation was performed 1 week after thymectomy. Each mouse was irradiated individually in a perforated Plexiglas cylinder while rotating at 4.5 rpm.

Bone marrow cells were obtained from syngeneic 6-week-old (YBM) or 18-month-old (OBM) donors and diluted such that each recipient received 20×10^6 cells *iv*. Bone marrow reconstitution was performed within 2 hr following irradiation:

Blood was obtained from the periorbital sinus. Serum was separated and stored at -20° until testing for serum IgA or anti-DNP antibodies. Anti-DNP antibodies were determined by indirect immunofluorescence using undiluted serum (10). Each sample was independently evaluated by three observers and the average reading used (- or ± readings were considered as negative). All other results are recorded as positive. DBA/1J mice were not tested for anti-DNP antibodies since they have previously been shown not to develop anti-DNP antibodies spontaneously (11).

Serum IgA levels were determined using radial immunodiffusion plates and standards (Melyo Labs).

Statistical tests were performed using Student's *t* test.

Results. Serum IgA levels increased with age in both A/J and DBA/1J mice in all test groups with one exception (Table I). The oldest DBA/1J group had lower IgA levels

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TABLE I. SERUM IgA LEVELS IN NORMAL A/J AND DBA/1J MICE.

Strain	Age in weeks	Mean IgA (mg/dl)	Range (mg/dl)	Number of mice tested
A/J	8	21	18-24	3
	12	34	18-66	10
	25	158	59-256	9
	80	212	70-367	9
	97	371	175-553	7
DBA	8	N.T. ^a		
	12	68	32-90	7
	25	146	82-195	10
	80	252	118-330	10
	97	193	128-256	6

^a N.T., Not tested.

than one of the younger DBA/1J test groups. In addition, the oldest DBA/1J mice had significantly lower IgA levels compared to the oldest A/J group ($P < 0.01$).

Thymectomy of young adult A/J mice had no significant effect on IgA levels at any age tested even though there was an accelerated appearance of anti-DNP antibodies by 21 weeks after treatment (Table II).

The reconstitution treatments varied considerably in their effectiveness to restore serum IgA levels to normal for age values and to prevent detectable anti-DNP antibody formation. Animals reconstituted with YT and YBM had normal or elevated IgA levels and normal anti-DNP antibody frequency at all ages tested.

In contrast, animals treated with OT and OBM failed to redevelop normal serum IgA levels, this being particularly noticeable at 21 weeks after treatment. These animals also had an accelerated appearance of anti-DNP antibodies.

Other experiments were performed using donor tissues of varying ages to determine whether the age of either the thymus, bone marrow, or both is the critical factor in reconstitution in terms of serum IgA levels and anti-DNP antibody. Animals grafted with OT and YBM had an increased frequency of anti-DNP antibody at 21 weeks after treatment. An initial high frequency of anti-DNP antibodies was observed in animals treated with YT and OBM but a reduction in frequency occurred by 21 weeks. The frequency at 21 weeks was still higher than normal but was lower than age-matched ani-

mals receiving OT and OBM or OT and YBM.

Reconstitution with YT and OBM was not followed by a recovery of normal for age IgA values. In contrast, animals receiving OT and YBM had normal or elevated IgA levels after reconstitution with the exception of those tested 21 weeks after treatment ($P < 0.025$). The critical factor determining the extent of recovery of IgA levels was the age of the bone marrow donor since both groups receiving OBM (OT and OBM; YT and OBM) failed to recover normal IgA levels by the end of the experiment. Conversely, both groups receiving YBM had normal or elevated IgA levels after reconstitution with the exception of the 21-week OT and YBM recipients previously noted.

TABLE II. SERUM IgA LEVELS AND ANTI-DNP ANTIBODY FREQUENCY IN A/J MICE AFTER ADULT THYMECTOMY, LETHAL IRRADIATION, AND RECONSTITUTION WITH THYMUS AND BONE MARROW FROM DONORS OF VARYING AGES.^a

Treatment	Number of weeks after treatment ^b	Mean IgA (mg/dl)	Anti-DNP antibody (% positive)
Young thymus, young bone marrow	4	22	0
	8	175	0
	21	168	0
Old thymus, old bone marrow	4	15	20
	8	19	80
	21	22	80
Young thymus, old bone marrow	4	20	50
	8	32	33
	21	18	20
Old thymus, young bone marrow	4	48	0
	8	57	0
	21	86	33
Thymectomized	4	N.T. ^c	N.T.
	8	52	0
	21	113	33
Normal young (no treatment)	4	21 ^d	0
	8	34	0
	21	158	0

^a Individual determinations were made in each test group, which consisted of four to six animals.

^b Treatment at 4-6 weeks of age.

^c N.T., Not tested.

^d Age-matched values from normal animals (Table I).

Thymectomy had no significant effect on IgA levels at the ages tested in DBA/1J mice. The effects of the reconstitution treatments in this strain were not as pronounced as in A/J mice (Table III). The YT and YBM treatment was the most effective in restoring IgA levels although both the 8- and 21-week treatment groups had lower values than normal animals. The values obtained with OT and OBM and those obtained with YT and OBM reconstitution were lower than normal values. Those animals receiving OT and YBM had nearly normal IgA levels 8 weeks after treatment, but at 21 weeks after treatment the IgA level was markedly lower than normal levels and lower than those obtained in the YT and YBM group.

Discussion. Certain strains of mice such as A/J and NZB provide good experimental models of autoimmune disease. Anti-DNP antibody production in A/J mice is deter-

mined by age and genetic factors (10, 11) and is associated with a decrease in thymus-dependent immune functions with age (15-18). Thymectomy accelerates the appearance of anti-DNP antibody (15) and the formation of anti-DNP antibody can be reversed or delayed by treatment with thymus or spleen cells from young syngeneic donors (12, 19, 20).

Studies have also revealed that DBA/1J mice do not develop anti-DNP antibodies nor do they experience a decline in thymus-dependent immunity with age (11, 17). Such findings suggest that the thymus or T-dependent lymphocytes have a critical role in regulating the formation of anti-DNP antibodies (12, 15, 17). In addition, a loss of suppressor T-cell function occurs with age and this is a critical factor determining the appearance of spontaneous autoimmunity (12-14).

Our data suggest that the loss of thymic suppressor T-cell function may be involved in the appearance of anti-DNP antibodies in A/J mice. Irradiated young thymus grafts are effective in preventing or reducing anti-DNP antibody formation in young mice, but grafts from aged donors are not as effective. It is of particular importance to note that the irradiated thymus grafts we used consisted largely or entirely of thymic epithelial cells. There were significant differences in the anti-DNP antibody frequency in those animals receiving YT (YT and YBM) or OT (OT and YBM). Apparently, age-related involution of thymus function is reflected at least in part by changes in the thymic epithelial cells. It is significant that pathologic changes have been observed in thymic epithelial cells of New Zealand mice with autoimmune disease (21). In addition, thymic hormone preparations, which represent a humoral secretion of thymic epithelial cells (22), have been demonstrated by immunofluorescent techniques in thymic epithelial cells (23). A loss of thymic hormone function has been postulated as a significant cause of the immunologic abnormalities observed in New Zealand mice (24). Whether changes in thymic hormones are responsible for the age-dependent changes in thymus function in A/J mice remains to be determined.

Our data also suggest that a loss of sup-

TABLE III. SERUM IgA LEVELS IN DBA MICE AFTER ADULT THYMECTOMY, LETHAL IRRADIATION, AND RECONSTITUTION WITH THYMUS AND BONE MARROW FROM DONORS OF VARYING AGES.^a

Treatment	Number of weeks after treatment ^b	Mean IgA (mg/dl)	Range (mg/dl)
Young thymus, young bone marrow	8	48	29-87
	21	88	59-116
Old thymus, old bone marrow	8	34	29-41
	21	40	32-49
Young thymus, old bone marrow	8	37	21-52
	21	43	21-64
Old thymus, young bone marrow	8	61	52-74
	21	48	21-116
Thymectomized	8	110	35-182
	21	178	52-256
Normal young ^c (no treatment)	8	68	32-90
	21	146	82-195

^a Individual determinations were made in each test group, which consisted of four to six animals.

^b Treatment at 4-6 weeks of age.

^c Age-matched values from Table I.

pressor cell function or potential to develop suppressor cells occurs within the bone marrow cell population with age. We have already noted that those mice receiving YT and YBM had normal for age anti-DNP antibody frequency. However, those mice receiving YT and OBM had increased anti-DNP antibody. Similarly, those receiving OT and YBM had a lower frequency of anti-DNP antibody when compared with those receiving OT and OBM. Since there are no suppressor cells in an irradiated thymus graft, the suppressor cell or its stem cell (or both) presumably originated from the donor bone marrow. This conclusion is supported by studies in which lethally irradiated mice given bone marrow from aged donors experienced impaired thymic regeneration capability (25).

The thymus has an important role in the establishment of normal serum IgA levels in mice. Several studies have indicated that there is a marked IgA deficiency in athymic nude mice in comparison to normal mouse strains (26-29). Treatment of nude mice which have a marked IgA deficiency with thymus cells or graft restores their IgA levels to nearly normal (28). Thymectomy had no significant effect on serum IgA levels in either A/J or DBA/1J which suggests that thymus function may not be as important in controlling IgA levels in aged animals as it is in younger or athymic mice.

Certain aged humans develop increased IgA levels associated with an increased frequency of autoantibodies and a decreased response of peripheral blood lymphocytes to PHA (4). IgA levels in the oldest A/J mice tested were elevated in comparison to DBA/1J mice of the same age and were associated with a high frequency of anti-DNP antibodies. In addition, previous studies have established that spleen cells of A/J mice develop a decreased response to PHA during aging (17, 18).

We observed that the age of the bone marrow donor was a critical factor in determining the effectiveness of our reconstitution procedures in terms of restoring IgA levels. Those animals which received bone marrow from aged donors had pronounced IgA deficiency at the end of the experiment. The reduced ability of bone marrow from

aged donors to reconstitute thymectomized, irradiated mice may be due to a relative inability of aged bone marrow cells to proliferate to generate functional B cells (30-33).

Summary. Aged A/J mice, an autoimmune strain, develop increasing levels of serum IgA compared to aged DBA/1J mice, a nonautoimmune strain.

Adult thymectomy did not affect IgA levels in A/J and DBA/1J mice but accelerated anti-DNP antibody formation in A/J mice. These results can be explained by spontaneous loss of suppressor T cells which prevent the formation of anti-DNP antibodies in A/J mice.

Experiments involving thymectomized, irradiated mice reconstituted with irradiated thymus and bone marrow from donors of varying ages suggested that there are intrinsic changes and thymic epithelium function with age. Bone marrow cells from aged donors were not as effective in restoring IgA levels and suppressing anti-DNP antibody as those from young donors.

Thymic epithelium from aged donors was not as effective as that from young donors in suppressing the formation of anti-DNP antibodies.

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