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# Antinuclear Antibodies in Mice

## II. TRANSMISSION WITH SPLEEN CELLS; INHIBITION OR PREVENTION WITH THYMUS OR SPLEEN CELLS

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**Summary.** Seven-week-old and 16-week-old A/Jax mice were injected with viable spleen cells or homogenates of spleen cells obtained from older syngeneic mice which either had autoimmune anti-deoxyribonucleoprotein (DNP) antibody in their sera or lacked this activity. None of the 7-week-old recipients developed detectable anti-DNP antibody. However, most of the animals in the 16-week-old group developed this autoantibody. The viability of the cells and the presence of or absence of anti-DNP antibody in the donor's sera did not appear to influence the autoimmune response of these recipients. When viable thymus cells which were obtained from young A/Jax mice were transferred to groups of older syngeneic animals that had developed anti-DNP antibody spontaneously, the anti-DNP decreased or disappeared from the sera of most recipients. Untreated controls did not show this variation. When 36-week-old A/Jax mice which lacked anti-DNP antibody were injected with thymus or spleen cells obtained from young donors, none of the recipients or untreated controls developed anti-DNP antibody. After specific immunization with DNP, however, the control animals began to produce autoimmune anti-DNP antibody while the animals treated with thymus or spleen cells remained unresponsive. These observations support the hypothesis that in A/Jax mice: (1) autoimmunity to DNP may result from failure of normal homeostasis mechanisms which allow proliferation of autoimmune cells; (2) the number of cells with autoimmune potential may increase during ageing; (3) the efficiency of the homeostasis system may decrease during ageing as the result of microbial or genetic factors; and (4) cells which participate in homeostasis are found in the thymus and spleen of young mice and may be the thymus dependent lymphocytes.

## INTRODUCTION

During ageing some members of several strains of mice begin to spontaneously produce autoimmune antinuclear antibodies with specificity (Friou and Teague, 1963, 1964; Barnes and Tuffrey, 1967; Teague, Friou and Myers, 1968), for deoxyribonucleoprotein (DNP). Many of these animals have positive lupus erythematosus (LE) cell tests. Later in life, strain A/Jax mice also produce antibodies with specificity for deoxyribonucleic acid

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(DNA) (Teague *et al.*, 1968). Extensive studies with ageing A/Jax mice revealed that anti-DNP or anti-DNA antibodies did not appear spontaneously before 22 weeks of age. The incidence increased almost linearly from 48 weeks of age to approximately 90 per cent in 92-week-old animals (Teague *et al.*, 1968). The capacity to produce anti-nuclear antibodies appears to be influenced by genetic factors. A number of other strains of mice (Friou and Teague, 1964) and (A/Jax × DBA/1J)$F_1$ hybrids kept under similar environmental conditions to the A/Jax strain did not produce anti-DNP antibody spontaneously during ageing (Teague *et al.*, 1968). Additional findings have demonstrated that older A/Jax mice (32 weeks of age) but not younger animals (16 weeks of age) could be induced to produce anti-DNP antibody following immunization. DBA/1J and (A/Jax × DBA/1J)$F_1$ hybrids were also unresponsive to immunization (Teague and Friou, 1964; Teague *et al.*, 1968).

The present investigation was intended to determine if passively acquired autoimmunity to DNP could be observed in A/Jax mice injected with syngeneic spleen cells. The results of this study demonstrate that neither the presence nor the absence of anti-DNP antibody in the spleen cell donor's sera, nor the viability of the injected cells, was a critical factor which determined whether or not anti-DNP antibody appeared in the recipient's sera. The more important factor appeared to be the age of the recipient and donor. Data will also be presented which suggests that some constituent of the thymus and spleen is intimately associated with the control or inhibition of autoimmunity to DNP.

## EXPERIMENTAL

### *Mice*

The A/Jax strain mice used in these experiments were obtained from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. These animals are highly inbred after many generations of brother–sister matings, readily accepting skin grafts from other individuals of the strain. Production stock young mice (virgins) were purchased when they were 4–6 weeks of age. Retired-breeder animals were 8 months old when purchased and had recently been removed from breeder colonies. All animals were housed in the same air-conditioned room and maintained until the appropriate age.

### *Serum*

Blood was obtained from the ventral tail vein (Teague *et al.*, 1968) after clotting, the tubes were centrifuged, and the portion of the tube containing serum was used immediately in serological tests or stored at $-10°$.

### *Serological test for anti-DNP antibody*

An indirect fluorescent antiglobulin technique was utilized for the detection of antibodies in undiluted mouse serum which reacted with spots of purified calf thymus DNP (Friou and Teague, 1964; Teague *et al.*, 1968). The intensity of fluorescence was rated on a negative (−) to three plus (+++) scale in comparison with controls. Antibody to mouse immunoglobulins was obtained from Antibodies Incorporated, Davis, California and was labelled with fluorescein isothiocyanate by standard methods (Coons and Kaplan, 1950; Riggs, Siewald, Burckhalter, Downs and Metcalf, 1958; Marshall, Eveland and Smith, 1958). Immunoelectrophoretic analysis against serum revealed that this antiserum contained only antibodies for mouse immunoglobulins.

*Cell transfers*

In all experiments, donors and recipients were A/Jax mice of the same sex. Donor mice were selected on the basis of the presence or absence of anti-DNP antibody. They were killed by cervical dislocation and cardiac blood was collected and tested for anti-DNP antibody to confirm previous serological findings. Viable cell suspensions were prepared under sterile conditions by a modification of the method of Howard and Woodruff (1961). Two spleens or four thymuses were obtained from each group of donor mice. Similar organs were pooled in 0·5 ml of antibiotic free medium 199 tissue culture solution (Microbiological Associates, Bethesda, Maryland) and disrupted with a glass homogenizer equipped with a loose fitting piston. The resulting cell suspension was passed through an 80 mesh stainless steel wire screen into 1 ml of medium 199. A count of viable cells was made with 0·9 per cent trypan blue. Suspensions were diluted with medium 199 to contain approximately $4–8 \times 10^6$ viable mononuclear cells per 0·1 ml. Disrupted cells were prepared by subjecting 0·5–1 ml of viable cell suspension ($4–8 \times 10^6$/0·1 ml of fluid) to three alternate cycles of freezing in a carbon dioxide–ethanol bath and thawing at room temperature. The homogenate was centrifuged at 1000 ***g*** for 10 minutes and the supernatant used for injection. The sediment and supernatant did not contain intact cells when examined microscopically. Recipient mice were injected intraperitoneally with 0·1 ml volumes of cell preparations or 0·1 ml of medium 199 using a sterile plastic tuberculin syringe and a 25 gauge needle (Becton, Dickinson and Company, Rutherford, New Jersey). Injections were made as soon as possible after the viable cells and cell homogenates were prepared.

Groups of 7- and 16-week-old female or male A/Jax mice were injected with spleen cell preparations derived from retired-breeder mice. The spleen cells were obtained from mice which either possessed or lacked detectable anti-DNP antibody activity. The members of each group were injected with either: (1) viable cells from donors with anti-DNP antibody, (2) cells homogenate from donors with anti-DNP antibody, (3) viable cells from donors which lacked anti-DNP antibody, (4) cell homogenate from donors which lacked anti-DNP antibody, or (5) medium 199. Each animal was bled on a weekly schedule for 7 weeks after injection, and each serum sample was tested for anti-DNP antibody.

In another experiment, thymus cells derived from 7-week-old female donors were injected into female retired-breeder A/Jax mice which had been determined to have spontaneously developed anti-DNP antibody activity. Control animals were injected with medium 199. Each animal was bled weekly for 6 weeks, and the serum samples were evaluated for anti-DNP antibody.

Additional groups of retired-breeder mice which had not developed anti-DNP antibody spontaneously were injected with either viable thymus or spleen cells obtained from 4-week-old donors, or medium 199. Each animal was bled at 1, 2 and 3 weeks after cell injection and tested for anti-DNP antibody. Immediately after the third bleeding, each animal was immunized with 2 mg of calf thymus DNP in Freund's complete adjuvant as previously described (Teague *et al.*, 1968). At 1 and 2 weeks after immunization, each animal was bled. The serum samples from both of these bleedings were assayed for anti-DNP antibody.

Additional details regarding sources, housing and handling of animals, methods of obtaining and storing serum, as well as immunofluorescent technique have been described (Teague *et al.*, 1968).

TABLE 1

ANTI-DNP ANTIBODY ACTIVITY IN 7-WEEK-OLD (7-WO) AND 16-WEEK-OLD (16-WO) A/Jax MICE AFTER INJECTION OF VIABLE SPLEEN CELLS OR SPLEEN CELL HOMOGENATE DERIVED FROM 36- TO 44-WEEK-OLD SYNGENEIC DONORS*

| Material injected† | Donor and recipient sex | Weeks after injection | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | |
| | | 7-WO | 16-WO | 7-WO | 16-WO | 7-WO | 16-WO | 7-WO | 16-WO | 7-WO | 16-WO | 7-WO | 16-WO | 7-WO | 16-WO |
| Viable cells, donors anti-DNP+ | Female | 0/12‡ | 6/11 | 0/12 | 8/11 | 0/12 | 7/11 | 0/12 | 8/11 | 0/12 | 8/11 | 0/12 | 8/11 | 0/12 | 8/11 |
| | Male | 0/11 | 4/6 | 0/11 | 5/6 | 0/11 | 3/6 | 0/11 | 4/6 | 0/11 | 4/6 | 0/11 | 4/6 | 0/11 | 4/6 |
| Cell homogenate, donors anti-DNP+ | Female | 0/6 | 2/5 | 0/6 | 3/5 | 0/6 | 3/5 | 0/6 | 2/5 | 0/6 | 2/5 | 0/6 | 2/5 | 0/6 | 2/5 |
| Viable cells, donors lacked anti-DNP | Female | 0/12 | 3/11 | 0/12 | 3/11 | 0/12 | 4/11 | 0/12 | 5/11 | 0/12 | 5/11 | 0/12 | 6/11 | 0/12 | 6/11 |
| | Male | 0/11 | 0/6 | 0/11 | 0/5§ | 0/11 | 0/5 | 0/11 | 0/5 | 0/11 | 0/5 | 0/11 | 0/5 | 0/11 | 0/5 |
| Cell homogenate, donors lacked anti-DNP | Female | 0/12 | 5/11 | 0/12 | 4/11 | 0/12 | 4/11 | 0/12 | 4/11 | 0/12 | 4/11 | 0/12 | 4/11 | 0/12 | 4/11 |
| | Male | 0/11 | 1/6 | 0/11 | 1/6 | 0/11 | 1/6 | 0/11 | 1/6 | 0/11 | 1/6 | 0/11 | 1/6 | 0/11 | 1/6 |
| Medium 199 | Female | 0/15 | 0/13 | 0/15 | 0/13 | 0/15 | 0/13 | 0/15 | 0/13 | 0/15 | 0/13 | 0/15 | 0/13 | 0/15 | 0/13 |
| | Male | 0/15 | 0/6 | 0/15 | 0/6 | 0/15 | 0/6 | 0/15 | 0/6 | 0/15 | 0/6 | 0/15 | 0/6 | 0/15 | 0/6 |

* All recipients lacked anti-DNP antibody before injection.
† The dosage ranged from $6{\cdot}6 \times 10^6$ to $7{\cdot}8 \times 10^6$ viable or homogenized cells.
‡ Number of mice with anti-DNP antibody/total number of mice injected.
§ Number of tested mice decreased because one animal died.

## RESULTS

### INJECTION OF YOUNG ANIMALS WITH VIABLE OR LYSED SPLEEN CELLS FROM OLDER MICE

To determine if adoptive or passive transfer of autoimmunity to DNP could be accomplished in A/Jax mice, groups of 7-week-old and 16-week-old female and male mice were injected with pooled viable or lysed spleen cells obtained from 36- to 44-week-old syngeneic donors. Two groups of donors were used: (1) animals in which anti-DNP antibody had appeared spontaneously, and (2) animals in which anti-DNP antibody could not be detected. The female and male recipients in the various groups were injected with cells derived from female and male donors, respectively. Animals older than 16 weeks of age were not used as recipients since it had previously been determined that 16-week-old A/Jax mice did not produce anti-DNP antibody after specific immunization (Teague and Friou, 1964; Teague *et al.*, 1968). Controls for each group were injected with medium 199. The results of this experiment are contained in Table 1. It was found that adoptive or passive transfer of autoimmunity to DNP can be accomplished in A/Jax mice, but is dependent upon the age of the recipient. A significant number of the older female and male recipients had developed anti-DNP antibody by 1 week after the cell injection. In none of these recipients had anti-DNP antibody been detectable before the cell injection. In contrast, none of the 7-week-old recipients of this or any other experimental group developed anti-DNP after injection. Controls injected with medium 199 did not develop anti-DNP antibody. Two of five recipient females (16-week-old) injected with cell homogenate from donors with anti-DNP antibody developed detectable anti-DNP antibody within 1 week. These animals remained positive throughout the experiment. Significant numbers of older females injected with viable or disrupted cells from donors which lacked anti-DNP also developed anti-DNP antibody. For example, the number of animals with anti-DNP antibody after the injection of viable spleen cells (donors lacked anti-DNP antibody) increased from three out of eleven to six out of eleven in 6 weeks. In contrast, none of the males injected with viable cells, derived from donors which lacked anti-DNP antibody, and only one out of six injected with similar disrupted cells, developed anti-DNP antibody.

### INJECTION OF SEROLOGICALLY POSITIVE ANIMALS WITH THYMUS CELLS FROM YOUNG DONORS

Groups of eleven 36-week-old female and twelve 72-week-old female retired breeder A/Jax mice which had spontaneously developed anti-DNP antibody (+++ antibody activity) were injected with $7{\cdot}0\times10^6$ viable thymus cells derived from 7-week-old donors. Controls were injected with medium 199. Each animal was bled weekly for 6 weeks, and each individual serum sample was tested for anti-DNP antibody activity (Table 2). Most control animals initially +++ with respect to anti-DNP antibody remained +++ during the 6 weeks of the experiment. A variation of +++ to ++ occurred at least once in four of twelve mice (Nos. 31, 36, 38 and 63). This variation was not considered to be significant and was possibly due to experimental error. Since results in the controls did not vary on a weekly basis in more than 33 per cent of the animals, any variation of ++ or more in the experimental groups was considered to be a significant change. In contrast to the controls, many of the experimental animals apparently had a decrease in the amount of anti-DNP antibody present in their sera. Eight of eleven mice in group II (Nos. 6, 18, 29, 30, 32, 35, 36 and 46) and six of nine mice in Group III (Nos. 2, 9, 12, 19, 35 and 37) appeared to

TABLE 2

ANTI-DNP ANTIBODY ACTIVITY IN FEMALE A/Jax MICE INJECTED WITH $7{\cdot}0 \times 10^6$ VIABLE THYMUS CELLS DERIVED FROM 7-WEEK-OLD FEMALE SYNGENEIC DONORS

| Group No.* | Age of recipient (weeks) | Mouse No. | Anti-DNP antibody activity in recipients | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Before transfer | Weeks after transfer | | | | | |
| | | | | 1 | 2 | 3 | 4 | 5 | 6 |
| I | 36 | 4 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | | 22 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | | 25 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | | 29 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | | 31 | +++ | +++ | ++ | +++ | +++ | +++ | +++ |
| | | 36 | +++ | +++ | +++ | ++ | ++ | +++ | +++ |
| | | 38 | +++ | +++ | +++ | ++ | +++ | +++ | +++ |
| | | 49 | +++ | +++ | ++ | +++ | +++ | +++ | +++ |
| | | 61 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | | 63 | +++ | +++ | +++ | +++ | +++ | ++ | +++ |
| | | 67 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | | 68 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| II | 36 | 3 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | | 6 | +++ | − | − | − | − | − | − |
| | | 18 | +++ | − | − | − | − | − | − |
| | | 28 | +++ | ++ | + | + | ++ | +++ | +++ |
| | | 29 | +++ | + | − | − | − | − | − |
| | | 30 | +++ | ++ | ++ | + | − | − | − |
| | | 32 | +++ | + | + | − | − | − | − |
| | | 35 | +++ | + | + | + | − | − | − |
| | | 36 | +++ | + | − | − | − | − | − |
| | | 38 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| | | 46 | +++ | − | − | − | − | − | − |
| III | 72 | 2 | +++ | + | − | − | − | − | − |
| | | 7 | +++ | + | ++ | D† | D | D | D |
| | | 9 | +++ | + | − | − | − | − | − |
| | | 11 | +++ | + | − | D | D | D | D |
| | | 12 | +++ | − | − | − | − | − | − |
| | | 17 | +++ | +++ | ++ | + | D | D | D |
| | | 19 | +++ | + | − | − | − | − | − |
| | | 21 | +++ | ++ | ++ | + | +++ | +++ | +++ |
| | | 30 | +++ | ++ | ++ | + | +++ | +++ | +++ |
| | | 35 | +++ | + | − | − | − | − | − |
| | | 37 | +++ | +++ | + | + | − | − | − |
| | | 38 | +++ | +++ | ++ | ++ | +++ | +++ | +++ |

* Members of Group I were injected with medium 199: members of Groups II and III were injected with thymus cells.

† D, Animal dead.

have decreased anti-DNP activity by 1–3 weeks after the injection of thymus cells. Three recipients in Group III died and are not considered. The decrease in anti-DNP antibody in most of the animals was not a transient phenomenon. Animals that eventually became negative remained so throughout the remainder of the experiment.

## IMMUNIZATION OF OLDER ANIMALS WITH DNP AFTER INJECTION OF THYMUS OR SPLEEN CELLS FROM YOUNG DONORS

Groups of nine to ten female and male 36-week-old A/Jax mice which had not developed anti-DNP antibody spontaneously were injected with viable syngeneic thymus or spleen cells derived from 4-week-old donors (Table 3). Controls were injected with medium 199.

TABLE 3

ANTI-DNP ANTIBODY ACTIVITY IN 36-WEEK-OLD A/Jax MICE AFTER INJECTION OF VIABLE SYNGENEIC THYMUS OR SPLEEN CELLS (4-WEEK-OLD DONORS) AND IMMUNIZATION WITH DNP

| Material injected | Donor recipient sex | Anti-DNP antibody activity in recipients | | | | | |
|---|---|---|---|---|---|---|---|
| | | Before transfer | Weeks after injection | | | Weeks after DNP immunization* | |
| | | | 1 | 2 | 3 | 1 | 2 |
| $4{\cdot}7 \times 10^6$ thymus cells | Female | 0/9† | 0/9 | 0/9 | 0/9 | 0/9 | 0/9 |
| $4{\cdot}4 \times 10^6$ thymus cells | Male | 0/9 | 0/9 | 0/9 | 0/9 | 0/9 | 0/9 |
| $7{\cdot}4 \times 10^6$ spleen cells | Female | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| $5{\cdot}3 \times 10^6$ spleen cells | Male | 0/9 | 0/9 | 0/9 | 0/9 | 0/9 | 0/9 |
| Medium 199 | Female | 0/10 | 0/10 | 0/10 | 0/10 | 6/10 | 9/10 |
| Medium 199 | Male | 0/10 | 0/10 | 0/10 | 0/10 | 6/10 | 7/10 |

* DNP immunization was given immediately after week 3 sera collections.
† Number of mice with anti-DNP antibody/total number of mice injected.

One, 2 and 3 weeks later all mice were bled and their sera examined for anti-DNP antibody activity. None of these sera contained detectable anti-DNP antibody. None of the animals which had been injected with viable thymus or spleen cells were induced to produce antibody to DNP following immunization with DNP. However, most control mice injected with medium 199 were induced to produce anti-DNP following immunization.

## DISCUSSION

The transfer of lymphoid cells to syngeneic and allogeneic recipient animals has been used extensively to demonstrate that such tissues contain cells with immunological memory and/or potential for both immediate and delayed immunological responses to various antigens. The discovery of the existence of spontaneous autoimmunity in inbred NZB/BL (Bielschowsky, Helyer and Howie, 1959) and A/Jax (Friou and Teague, 1963, 1964) mice provided the opportunity to evaluate the effect of adoptive transfer to autoimmune disease and/or autoimmunity with lymphoid cells in these strains. It has been demonstrated that young adult NZB/BL mice developed Coombs positive erythrocytes and haemolytic anaemia after being injected with viable spleen cells obtained from Coombs positive animals (Holmes, Gorrie and Burnet, 1961). The results of these studies indicated that the antibody was derived from active synthesis rather than from passive transfer. This conclusion was reached mainly on the observations of the persistence of the Coombs positive state after transfer, evidence of a progressively fatal disease, and failure to transfer similar reactions with homogenates of the viable cell suspension which was used to transmit the disease. The inability to transmit the disease with cell free material was interpreted as indicating that infectious agents probably were not involved in the aetiology of the haemolytic disease (Holmes *et al.*, 1961). However, the ability to transmit the haemolytic disease with viable spleen cells has been questioned. In another study, no weanling or newborn NZB/BL mice injected with syngeneic spleen cells obtained from Coombs positive donors became Coombs positive during a 35-day observation period (East, de Sousa and Parrott, 1965). In spite of these findings, the role of a microbial agent as a possible participant in the production of these phenomena must be considered. Recent

reports have shown most convincingly that cell-free filtrates obtained from a lymphoma which appeared spontaneously in NZB/BL mice would induce the early development of anaemia and nephritis when injected into pre-weanling NZB/BL (Mellors and Huang, 1966) and Swiss mice (Mellors and Huang, 1967). Electron microscopic examinations of the lymphoma cells and the kidneys revealed the presence of structures which were thought to be viral particles (Mellors and Huang, 1966). It was speculated that once the virus is established in the host, the agent or its products might either directly injure cells of the renal glomeruli, stimulate the lymphoid cells, cause disease by hypersensitivity mechanisms, or expand the population of lymphoid cells and cell types susceptible to viral action and transform some of these into cells with autoimmune and neoplastic behaviour (Mellors and Huang, 1966). However, the possibility that agents such as this may cause selective damage to homeostasis mechanisms concerned with preventing the development of autoimmunity was not considered.

The present experiments were performed to determine if anti-DNP antibody could be detected in the sera of 7-week-old A/Jax mice following the injection of viable spleen cells or homogenates of these preparations which were obtained from older syngeneic animals. Previously, it had been shown that anti-DNP antibody does not appear in A/Jax mice until after 22 weeks of age and that 16-week-old animals could not be induced to produce anti-DNP antibody after immunization (Teague *et al.*, 1968). The results of the present studies revealed that neither the presence of nor the absence of anti-DNP antibody in the donor's sera, nor the viability of the cells was a critical factor determining whether or not anti-DNP antibody would appear in the recipients. The most critical factor appeared to be the age of the recipient since none of the 7-week-old recipients developed anti-DNP antibody. In contrast, most animals which were 16 weeks old when injected with viable cells or homogenates produced anti-DNP. The persistence of this antibody in most recipients suggests that the majority of the antibody was derived from active antibody synthesis rather than from antibody passively transferred in the innoculum. The production of this antibody was probably not due to stimulation with syngeneic nuclear material since older animals which lacked anti-DNP antibody did not produce anti-DNP after being injected with thymus or spleen cells derived from young donors. Although these findings indicate that an unknown factor in the cell free material of A/Jax mice may have been responsible for the production of anti-DNP antibody by the 16-week-old animals, the nature of this factor is unknown. Quite possibly, it may have been an infectious agent. If so, its exact role in the process is unknown. However, if such an agent were the only requirement for the genesis of autoimmunity to DNP in A/Jax mice, one could expect that most of the older donors would have had such an agent in their spleens and that animals of any age would have been demonstrably affected by the injection. Since an age dependent susceptibility to the passive transfer of autoimmunity to DNP was seen in the present investigation, and since previous work has shown that the capacity to respond to DNP following immunization is influenced by the age of the animal (Teague *et al.*, 1968) it seems that either microbial agents are not involved, or that the development of this type of autoimmunity may require more than one variable.

The observation that anti-DNP antibody decreased or disappeared from most animal's sera after treatment with cells derived from young syngeneic animals emphasizes that the functional state of the thymus or its dependent lymphoid tissues may be an important component of the mechanism which is responsible for controlling autoimmunity to DNP in A/Jax mice. The results indicate that either the thymus cells or the resulting thy-

mocyte–host interaction was capable of preventing further development of cells with autoimmune potential for DNP as expressed by the disappearance of anti-DNP antibody from the serum. Other experiments revealed that when 36-week-old A/Jax mice which lacked anti-DNP antibody were treated with viable thymus or spleen cells from young syngeneic mice and later immunized with DNP, none of the experimental animals were induced to produce anti-DNP. In contrast, untreated controls began to produce specific antibody as the result of immunization. These findings demonstrate that the spleen as well as the thymus of young animals can influence autoimmunity to DNP. These data also suggest that neither the spleen nor the thymus of the young animals contained enough cells with autoimmune potential for DNP to escape the homeostatic influence which may have been present in either these organs or those of the host.

Numerous investigators have suggested that the thymus influences the development of autoimmunity, but the precise mechanism by which it operates, if any, is unknown. It has been speculated that autoimmunity results from somatic mutation in immunopoietic stem cells which permits subsequent antibody producing cells ('forbidden clones') to proliferate and express their autoimmune activity (Burnet, 1958). The essential genetic lesion was thought to result in increased lability of immunologically competent stem cells to undergo somatic mutation toward resistance to immunological homeostasis (Holmes *et al.*, 1961). According to this interpretation, these clones of cells would be eliminated in the normal animal by the thymus (Burnet and Holmes, 1964). Thymic abnormalities have been described in NZB/BL mice in which there is extensive germinal centre formation. Attempts were made to relate these changes to the autoimmune disease which occurs in members of this strain (Burnet and Holmes, 1964). Other studies of NZB/BL mice have revealed severe depletion of thymic epithelial cells (deVries and Hijmans, 1967). However, the finding that germinal centres are present in the thymus may reflect some secondary process rather than a primary factor. More direct evidence of the relationship of the thymus to the development of autoimmune processes has been obtained from numerous experiments in which the thymus was surgically removed at birth in various experimental animals. Members of certain strains of mice subjected to thymectomy at birth develop, as young adults, Coombs positive erythrocytes (Yunis, Hong, Grewe, Martinez, Cornelius and Good, 1967), anaemia (deVries, Van Putten, Balner and Van Bekkum, 1964) and antinuclear antibodies (Teague, 1967; Thivolet, Monier, Ruel and Richard, 1967). Neonatal thymectomy of NZB/BL mice results in an earlier appearance of haemolytic anaemia (Howie and Helyer, 1966). Similar treatment of A/Bi mice enhances the early development of both anti-DNP and -DNA antibodies and moderate to severe glomerulonephritis with both $\gamma$-globulin and $\beta_{1C}$ present in mesangial distribution (Teague, 1967). Although other lymphoid tissues have not been frequently suggested as possible participants in immunological homeostasis, studies in rabbits have shown that removal of the appendix and spleen results in the spontaneous development of autoimmune phenomena (Sutherland, Archer, Peterson, Ecker and Good, 1965).

If the thymus or the thymus dependent lymphoid tissues were the only factor involved in the development of autoimmunity in experimental animals, it could be expected that any neonatally thymectomized animal would develop autoimmunity. However, since results to the contrary have been reported (East and Parrott, 1965; Howie and Helyer, 1966), it appears that at least one other factor is necessary for the development of autoimmunity. It has been speculated that cells with autoimmune potential result from somatic mutation (Burnet, 1958; Burch and Rowell, 1965) and are recognized and eliminated by

the thymus of the normal animal (Burnet and Holmes, 1964). If somatic mutation is responsible for the emergence of autoimmune cells, the process may be influenced by the genetic constitution of the animal (Holmes and Burnet, 1964; Teague *et al.*, 1968). It is possible, therefore, that the rates of somatic mutation toward autoimmunity as well as the functional status of the homeostasis mechanism are both governing factors which ultimately determine whether or not autoimmunity will develop. The results of the present investigation have demonstrated that both the thymus and the spleen can participate in prevention or elimination of autoimmunity to DNP. Thus, it may be that this activity represents an inherent capacity of the thymus dependent lymphoid tissues in general, rather than the thymus alone (Burnet and Holmes, 1964) to discriminate, perhaps by the presence of some recognition factor, between autoimmune and non-autoimmune developing or mature cells.

It is tempting, therefore, to suggest that any treatment, agent or genetic lesion which can selectively damage the thymus or the thymus dependent lymphoid tissues, directly by interfering with thymic function or indirectly by interfering with either the normal activities of thymus dependent lymphocytes or the development of these cells from their precursors, will result in a deficiency of a homeostasis system which, in turn, would provide an environment for the successful maturation of autoimmune populations of cells. The frequency by which cells with autoimmune potential appear, may be governed by the rate of somatic mutation. The rate or potential for somatic mutation may vary among different strains of animals according to their genetic constitution. This may also influence the increase in rate during ageing in certain strains.

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