Clin. exp. Immunol. (1972) **12,** 455–464.

AGE-DECREASE OF CELLS SENSITIVE TO AN AUTOANTIBODY-SPECIFIC FOR THYMOCYTES AND THYMUS-DEPENDENT LYMPHOCYTES IN NZB MICE

T. SHIRAI,* T. YOSHIKI AND R. C. MELLORS

The Hospital for Special Surgery, affiliated with the New York Hospital, Cornell University Medical College and the Department of Pathology, Cornell University Medical College, New York, N.Y. 10021, *U.S.A.*

(*Received* 24 *April* 1972)

SUMMARY

NZB mice naturally produce an autoantibody which in the presence of complement is specifically cytotoxic for thymocytes and thymus-dependent lymphocytes (T-cells) in the peripheral lymphoid tissues (lymph nodes and spleen) and the circulation of mice. Using a direct cytotoxicity test with a NZB mouse serum pool which contained the high titred autoantibody, a progressive decrease was observed with age in the proportion of the autoantibody-sensitive cells in mesenteric lymph node, spleen, and blood of NZB mice in comparison with mice of other strains (C57BL/6J and NZW). The numerical decrease in the population of autoantibody-sensitive cells was evident at younger age and greater degree in the peripheral blood than in the lymph node and spleen. The age-decrease in the number of autoantibody-sensitive cells in lymph node and spleen contrasted with the numerical increase in nucleated cells in these organs. The age-decrease in the proportion and number of the autoantibody-sensitive cells in the blood exceeded the decrease in the blood lymphocyte count. This finding indicated that T-cells in the blood are selectively depleted with the ageing of NZB mice. A similar observation was made on the blood lymphocytes of (NZB × NZW)F_1 hybrid mice. The depletion of T-cells in the blood in association with the production of natural thymocytotoxic autoantibody is termed autoimmune thymus-dependent lymphocytopenia.

INTRODUCTION

NZB mice are typified by the spontaneous development of autoimmune haemolytic anaemia (Bielschowsky, Helyer & Howie, 1959) and glomerulonephritis (Helyer & Howie, 1963; Holmes & Burnet, 1963) of multifactorial immune-complex type (Mellors *et al.*, 1971). Among the several immunological abnormalities recognized in NZB mice a notable change

* Present address: Department of Pathology, Cancer Institute Hokkaido University, School of Medicine, Sapporo, Japan.

Correspondence: Dr R. C. Mellors, The Hospital for Special Surgery, 535 East 70 Street, New York, N.Y. 10021, U.S.A.

with ageing is the progressive decline in the function of the thymus-dependent immune system. An age-decrease in the capacity of NZB mouse spleen cells to elicit a graft-versus-host reaction has been reported by Stutman, Yunis & Good (1968) and by Cantor, Asofsky & Talal (1970). A question raised was whether this decline was related to an absolute or a proportional decrease in functional cells, in as much as the spleens and to some extent the lymph nodes of NZB mice are known to show an increase in cellular heterogeneity with ageing (Holmes & Burnet, 1963; Mellors, 1966). It has been shown however that a numerical decrease in the pool of long-lived lymphocytes (Denman & Denman, 1970) and of recirculating lymph-node lymphocytes (Zatz, Mellors & Lance, 1971) occurs in NZB mice with ageing. Relevant also is the decreased capacity of ageing NZB mice to reject tumours induced by Moloney sarcoma virus (Gazdar, Beitzel & Talal, 1971).

Besides the main roles both in cellular immunity and in certain humoral antibody responses in cooperation with bone marrow-derived lymphocytes (B-cells) (Claman, Chaperon & Triplett, 1966; Miller & Mitchel, 1968), thymus-dependent lymphocytes (T-cells) may also exert a feedback control over the autoantibody formation by B-cells (Allison, Denman & Barnes, 1971). A relaxation of this control function may help augment the autoantibody formation in NZB mice.

NZB mice have been found to produce a natural autoantibody which is cytotoxic, in the presence of complement, for thymocytes of virtually all strains of mice including NZB (Shirai & Mellors, 1971) and for T-cells in the peripheral lymphoid tissues and in the circulation of mice (Shirai & Mellors, 1972; Shirai, Yoshiki and Mellors, 1972). This autoantibody has been termed NTA. NTA appears early in life and is produced virtually throughout the life span of NZB mice. We report here that early in the life of NZB mice a progressive decrease occurs in both the proportion and the number of peripheral blood lymphocytes sensitive to NTA.

MATERIALS AND METHODS

Animals

NZB, NZW and (NZB × NZW)F_1 mice were from our colonies. C57BL/6J mice were obtained from Jackson Memorial Laboratories, Bar Harbor, Maine. These mice have been maintained in a specific pathogen-free environment.

Sera

Blood was collected from the periorbital sinus. An NZB mouse serum pool used for standard serological test in determining the proportion of NTA-sensitive cells was obtained from mice at 17–19 months of age and proved to have extremely high titred NTA (Shirai & Mellors, 1972). Prior to use, this serum pool was once absorbed with an equal volume of AKR/J liver homogenate to exclude G(Gross) natural antibody (Mellors, Aoki & Huebner, 1969). The specificity of the reactions between this standard serum pool and the cells employed in the present experiments was first tested by showing negative reaction with the serum pool absorbed with C57BL/6J thymocytes which had no demonstrable Gross cell surface antigen but positive NTA-reactive antigen.

Cell suspensions

Cells from mesenteric lymph nodes and spleens were obtained by gently dispersing the tissues with a glass tissue grinder (No. 1977, Bellco Glass Inc., Vineland, N.J.) in a

known volume of medium 199 supplemented with 3% foetal calf serum (Microbiological Associates, Bethesda, Maryland) heated to 56°C for 30 min. After large fragments of tissues had settled, free cells were obtained from the supernatant and washed twice at 1000 rev/min for 5 min.

Blood leucocytes were collected by mixing one volume of heparinized blood and two volumes of 6% w/v Dextran in 0·9% NaCl (Grade H, Pharmachem Corp., Bethlehem, Pennsylvania). After 2 hr of incubation at 37°C the cells in the supernatant were collected, washed twice in medium 199 with 3% foetal calf serum and treated with tris buffered ammonium chloride (pH 7·2) (Boyle, 1968) to exclude erythrocyte contamination. The final cell suspensions obtained after two quick washings comprised more than 95% viable cells as determined by trypan blue dye exclusion.

Cytotoxicity test

For the tests in determining the proportion of NTA-sensitive cells in lymph nodes and spleens, a mixture of 0·025 ml of cell suspension (10^7 cells/ml) in medium 199 with 3% foetal calf serum and 0·05 ml standard NZB mouse serum pool at a dilution of 1 : 2 (at this dilution the cytotoxic activity of this standard serum pool for the peripheral lymphocytes was on the plateau) was incubated at 4°C for 60 min, then washed twice and incubated again with 0·05 ml rabbit serum at a dilution of 1 : 20 as a complement source. This rabbit serum was first selected individually for low cytotoxicity for mouse thymocytes and bone marrow cells combined with a high complement level. Complement alone and medium alone (medium 199 with 3% foetal calf serum) were always employed for control. In several tests the standard NZB mouse serum pool previously absorbed with C57BL/6J thymocytes was employed for a negative control to confirm the specificity of the reaction to NTA. For the tests of blood leucocytes $20–30 \times 10^4$ cells were spun down in each test tube. After discarding and wiping off the supernatant 0·05 ml standard NZB mouse serum pool was mixed and the test was carried out in the same manner as mentioned previously. The trypan blue dye exclusion method was employed to determine the dead cells and the live cells. Background level of cell death in control groups was less than 15%. The proportion of the dead cells was estimated by cytotoxicity index calculated as follows:

$$\text{Cytotoxicity index} = \frac{\text{Percentage of dead cells with test serum} - \text{percentage of dead cells with complement alone}}{100 - \text{per cent dead cells with complement alone}} \times 100$$

The total number of NTA-sensitive cells in mesenteric lymph nodes and spleens was estimated by multiplying the total number of cells, obtained by the method employed, by the percentage of dead cells estimated by cytotoxicity index. In the case of blood leucocytes, the number of NTA-sensitive cells in cubic mm of blood was estimated in the same manner.

White blood cell and differential counts

The white blood cell count was made by the customary method using Turk's solution and haemocytometer. White blood cell differential counts were performed with slides of blood smears stained with Wright's solution.

RESULTS

Lymph nodes

The age-changes in the number of nucleated cells and the proportion (%) and number of NTA-sensitive cells obtained from the mesenteric lymph nodes were compared in mice of strains NZB, NZW and C57BL/6J (Table 1). The total number of nucleated cells in the lymph nodes of NZB mice increased with age particularly beyond 14 months. The number of nucleated cells in the lymph nodes of NZW and C57BL/6J mice decreased with age beyond 9 months.

The proportion (%) of NTA-sensitive cells in the lymph nodes of NZB mice declined progressively with age. The proportion of NTA-sensitive cells in the lymph nodes showed an upward trend with the ageing of NZW mice and a slight increase followed by a small decline after 14 months in C57BL/6J mice.

TABLE 1. The proportion and total number of NTA-sensitive cells in mesenteric lymph nodes of mice at various ages

Strain of mice	Age (months)	No. tested	Total No. cells obtained ($\times 10^6$)	NTA-sensitive cells %	Total No. NTA-sensitive cells ($\times 10^6$)
			Mean ± SD	Mean ± SD	Mean ± SD
NZB	1–4	10	34 ± 14·4	58 ± 5·4	20 ± 3·1
	5–9	13	41 ± 12·4	46 ± 9·4	19 ± 7·5
	10–14	7	50 ± 30·6	32 ± 8·5	15 ± 6·4
	15–20	3	92 ± 93·6	19 ± 14·4	9 ± 2·4
NZW	1–4	5	49 ± 16·1	56 ± 7·2	32 ± 15·3
	5–9	8	48 ± 16·3	69 ± 3·9	38 ± 18·3
	10–14	5	23 ± 6·9	74 ± 4·7	17 ± 5·1
	15–20	8	25 ± 8·2	71 ± 4·9	18 ± 6·1
C57BL/6J	1–4	16	22 ± 7·6	58 ± 4·0	12 ± 4·1
	5–9	11	24 ± 7·6	61 ± 7·8	14 ± 4·0
	10–14	10	16 ± 5·3	56 ± 5·9	9 ± 2·8
	15–20	13	16 ± 5·7	40 ± 8·4	7 ± 2·7

The total number of NTA-sensitive cells in the mesenteric lymph nodes of NZB mice decreased with age and was clearly evident beyond 14 months. This decrease contrasted with the increase in the total number of nucleated cells obtained from the mesenteric lymph nodes. The total number of NTA-sensitive cells in the lymph nodes of NZW and C57BL/6J mice also decreased after 9 months of age. This decrease was in parallel with that of the total number of nucleated cells obtained from the lymph nodes.

Spleens

The age-changes in the total number of nucleated cells and the proportion and number of NTA-sensitive cells obtained from the spleens of three strains of mice are shown in Table 2. The total number of nucleated cells obtained from the spleens of NZB mice increased very substantially and progressively with age. The number of nucleated cells

TABLE 2. The proportion and total number of NTA-sensitive cells in spleens of mice at various ages

Strain of mice	Age (months)	No. tested	Total No. cells obtained ($\times 10^6$)	NTA-sensitive cells (%)	Total No. NTA-sensitive cells ($\times 10^6$)
			Mean ± SD	Mean ± SD	Mean ± SD
NZB	1–4	10	203 ± 61·2	30 ± 5·5	61 ± 24·5
	5–9	13	364 ± 174·9	21 ± 8·0	86 ± 46·5
	10–14	6	980 ± 695·4	12 ± 8·1	57 ± 9·1
	15–20	3	1433 ± 873·7	3 ± 2·1	32 ± 18·2
NZW	1–4	5	225 ± 43·7	33 ± 4·7	74 ± 18·1
	5–9	8	203 ± 70·1	37 ± 6·2	73 ± 23·7
	10–14	5	333 ± 162·0	36 ± 9·2	128 ± 90·2
	15–20	8	316 ± 130·4	32 ± 7·3	98 ± 59·8
C57BL/6J	1–4	14	179 ± 50·8	32 ± 4·7	57 ± 14·2
	5–9	9	240 ± 72·1	33 ± 5·3	79 ± 26·2

in the spleens of NZW and C57BL/6J mice showed a significant but smaller increase with age.

The proportion of NTA-sensitive cells in the spleen of NZB mice decreased very greatly and progressively, almost linearly, with age. The proportion of NTA-sensitive cells in the spleens of NZW and C57BL/6J mice was virtually constant over the age-range studied.

The total number of NTA-sensitive cells in the spleens of NZB mice increased at 5–9

TABLE 3. The proportion and total number of NTA-sensitive cells in the blood of mice at various ages

Strain of mice	Age (months)	No. tested	Total No.* cells obtained ($\times 10^3$)	Lymphocytes (%)	NTA-sensitive cells (%)	Total No.* NTA-sensitive cells ($\times 10^3$)
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
NZB	1–3	18	2·4 ± 0·61	61 ± 8·5	43 ± 7·9	1·0 ± 0·31
	4–6	10	1·6 ± 0·42	60 ± 4·9	30 ± 10·5	0·5 ± 0·15
	7–11	10	2·1 ± 1·09	53 ± 7·4	13 ± 5·1	0·3 ± 0·19
	12–20	8	2·0 ± 1·21	48 ± 14·9	15 ± 7·3	0·3 ± 0·10
NZW	1–3	7	5·5 ± 0·83	69 ± 9·6	30 ± 4·6	1·6 ± 0·19
	4–6	9	3·9 ± 0·69	81 ± 6·4	52 ± 5·0	2·0 ± 0·35
	7–11	8	2·8 ± 0·86	78 ± 6·0	56 ± 6·9	1·5 ± 0·37
	12–20	12	2·2 ± 0·52	76 ± 9·7	51 ± 13·0	1·1 ± 0·29
(NZB × NZW)F_1	4–6	8	2·1 ± 0·51	62 ± 15·5	47 ± 8·4	1·0 ± 0·24
	7–11	10	2·6 ± 1·21	48 ± 10·2	22 ± 7·1	0·5 ± 0·22
C57BL/6J	1–3	10	2·3 ± 0·41	78 ± 8·8	54 ± 6·8	1·2 ± 0·24
	4–6	8	1·9 ± 0·40	87 ± 7·6	56 ± 3·5	1·0 ± 0·22
	7–11	12	2·1 ± 0·68	80 ± 11·0	51 ± 7·2	1·1 ± 0·28

* Number of nucleated cells per cubic mm.

months of age and declined progressively thereafter with age. The total number of NTA-sensitive cells in the spleens of NZW mice increased after 9 months of age reaching a maximum at 10–14 months. The total number of NTA-sensitive cells in the spleens of C57BL/6J mice also increased over the age-range studied.

Blood

The age-changes in the number of nucleated cells (per cubic mm), lymphocyte differential count (%), and the proportion and number of NTA-sensitive cells obtained from the blood of NZB, NZW, (NZB × NZW)F_1 and C57BL/6J mice are given in Table 3.

The number of nucleated cells obtained from the blood of NZB mice showed, on the average, small age-changes although great variations were observed in individuals after 6 months of age. The number of nucleated cells obtained from NZW mice at 1–3 months of age was much greater than that found in the other strains of mice tested, and decreased progressively with age to the level of the other strains. Small age-changes were observed in the number of nucleated cells obtained from the blood of (NZB × NZW)F_1 and C57BL/6J mice over the age-range studied.

The lymphocyte differential count decreased with the age of NZB mice and decreased similarly in (NZB × NZW)F_1 mice. The lymphocyte differential count in NZW and C57BL/6J mice reached a maximum at 4–6 months of age and then showed a slight decrease over the age-range studied.

The proportion of NTA-sensitive cells in the blood of NZB mice decreased progressively with age and was already clearly evident at 4–6 months. The level reached a minimum at 7–11 months of age and was constant thereafter. A similar decrease was also observed in (NZB × NZW)F_1 mice over the age-range studied (4–11 months). In both cases the age-decrease in the proportion of NTA-sensitive cells exceeded the decrease in the blood lymphocyte differential count. The proportion of NTA-sensitive cells in the blood of NZW mice at 1–3 months of age was lower than that found in other strains of mice at comparable ages. However, this proportion reached a normal level (comparable with C57BL/6J mice) at 4–6 months and showed, on the average, small age-changes thereafter. The changes in the proportion of NTA-sensitive cells in the blood of C57BL/6J mice were small over the age-range studied (1–11 months).

The total number of NTA-sensitive cells in the blood of NZB mice decreased progressively with age as was clearly evident at 4–6 months. The number reached a minimum at 7–11 months and was constant thereafter. A similar rapid numerical decrease was observed in (NZB × NZW)F_1 mice. NZW mice at younger ages (1–6 months) had a greater number of NTA-sensitive cells in the blood than found in the other strains of mice at comparable ages. The total number of NTA-sensitive cells declined beyond 6 months. However, the numerical level in NZW mice at 12–20 months of age was comparable to the number of NTA-sensitive cells in the blood of NZB mice at 1–3 months. Virtually no age-changes were observed in the total number of NTA-sensitive cells in C57BL/6J mice over the age-range studied.

Although not shown in Table 3, the decline in the proportion of NTA-sensitive cells in male and female NZB mice was virtually the same. Female NZB mice had a slightly lower total number of nucleated cells in the blood and similarly a slightly lower total number of NTA-sensitive cells than found in male mice.

It was noted that the nucleated cells in NZB mice, particularly after 7 months of age,

showed a rather high proportion of dead cells following incubation with complement alone. When the proportion of dead cells exceeded 15% by incubation with complement alone, these data were not included in Table 3. Similar findings were observed in some NZW mice after 12 months of age. This observation was not made on the cells collected from the blood of NZB mice at 2 months of age or C57BL/6J and NZW mice before 11 months. In addition, this type of reaction was not common in tests performed in cells in lymph nodes and spleens.

DISCUSSION

NZB mice were found to produce a natural thymocytoxic autoantibody called NTA which is cytotoxic for the thymocytes of virtually all strains of mice tested (Shirai & Mellors, 1971, 1972). When determined by the cytotoxicity test at 4°C, a 50% incidence of NTA occurred within 1 month after birth. The test at 37°C revealed a 50% incidence of NTA at 2–3 months of age. NTA was found to be produced virtually thoughout the life span, and the autoantibody titre increased with age (Shirai & Mellors, 1972). A similarity to θ-alloantigen system was shown by the presence of NTA-reactive antigen in the brain tissue of mice. Since a marked decrease of θ-alloantigen-bearing cells occurs in the lymph nodes or spleens of thymectomized mice (Raff & Wortis, 1970; Schlesinger & Yron, 1970) and of congenitally athymic nude mice (Raff & Wortis, 1970), the cells in peripheral lymphoid tissues sensitive to θ-alloantibody have been considered to be thymus-dependent lymphocytes (T-cells). A NZB mouse serum pool which contained high titred NTA was also cytotoxic for some cells in the peripheral lymphoid tissues and the circulation of mice (Shirai & Mellors, 1972). An extreme reduction or a total depletion of these cells was observed in thymectomized C57BL/6J mice and congenitally athymic nude mice (Shirai, Yoshiki & Mellors, 1972). This finding suggested that NTA-sensitive cells in the peripheral lymphoid tissues and the circulation were also T-cells. The question asked was whether a depletion of these NTA-sensitive T-cells occurred in NZB mice with ageing; an affirmative answer was obtained in the present experiments. The earliest change was a progressive decrease in both the proportion and the number of NTA-sensitive cells in the peripheral blood. The decrease was clearly evident in NZB mice at 4–6 months of age as compared with 1–3 months and was fully manifest at 7–11 months of age. As compared with the changes in the blood, the decrease of NTA-sensitive cells in lymph node and spleen was less marked and more delayed, particularly in respect of the decline in the total number. Whether NTA is a cause or an effect of the depletion of NTA-sensitive T-cells in NZB mice with ageing is not yet known. However, a causal relation is suggested by the following considerations. The age-increase in the prevalence and titre of NTA in NZB mice (Shirai & Mellors, 1972) is inversely related to the linear decrease of NTA-sensitive cells in the blood. In analogy to the major site and mode of action of antilymphocyte serum (Lance, 1969; Martin & Miller, 1968), NTA may act on T-cells mainly in the circulation. A suggested mechanism of T-cell depletion in the blood is that of sensitization phagocytosis (Shirai & Mellors, 1971). It is to be noted that the administration of θ-alloantiserum has failed to elicit *in vivo* activity (Raff, 1970). However, it is noteworthy that NZB mice produce NTA virtually throughout their life span and that the autoantibody is always in excess in the circulation. Since mice generally have a low complement level, the activity of NTA may be mild but prolonged. In as much as T-cells in the peripheral lymphoid tissues and the circulation have a lower con-

centration of NTA-reactive antigen on the cell surface than do thymocytes as shown by lower sensitivity to NTA (Shirai & Mellors, 1972), a considerable accumulation of NTA on the antigenic site of T-cells may be necessary in order to initiate biological activity. Zatz *et al.* (1971) showed that a progressive decrease in the recirculating (thymus-dependent) pool of lymph-node lymphocytes occurred in NZB mice with age. The decrease was clearly evident in the mice before 10 months of age. The observation by Zatz *et al.* (1971) is somewhat parallel to the present finding of a decrease in the number of NTA-sensitive T-cells in the peripheral blood (Table 3). These studies indicate that recirculating T-cells are selectively depleted in NZB mice with ageing. The depletion of T-cells in the blood in association with the production of NTA may be termed *autoimmune thymus-dependent lymphocytopenia.* Raff (1971) presented the hypothesis, along with supporting experimental evidence, that there were two types of T-cells in mice, namely T1 and T2, both sensitive to θ-alloantibody. Cantor *et al.* (1970) demonstrated the synergy in graft-versus-host reaction between the spleen cells of young and old NZB mice; both types of T-cells may be involved (Cantor & Asofsky, 1972) although a conclusion has not been obtained. It is possible that recirculating T-cells (T2) are selectively deficient in the spleen and lymph node of old NZB mice.

It is to be noted that the blood leucocytes of NZB mice with ageing showed an increasing population of cells which were killed by *in vitro* incubation with complement alone. A possible interpretation is prior *in vivo* sensitization of T-cells with NTA, although further clarification of this point is necessary. Those lymphocytes which were killed only when incubated with NTA followed by complement may also have small amounts of NTA on the cell surface but not in sufficient quantity to cause cytolysis upon the addition of complement alone. The blood leucocytes of some old NZW mice were similarly killed by incubation with complement alone. Care must be taken in studies on the membrane immunoglobulins of lymphocytes, particularly when using broadly reactive anti-immunoglobulin serum. Since NTA is mostly IgM immunoglobulin (Shirai & Mellors, 1972) and the light chain of mouse IgM immunoglobulin is mainly λ (Potter and Lieberman, 1967), the use of anti-κ serum may be preferred in studies of the membrane immunoglobulin-bearing cells in mice (Takahashi *et al.*, 1971) particularly of the strain NZB as well as some others at old age. Bhoopalam *et al.* (1971) recently reported that a diminution in the proportion of membrane immunoglobulin-bearing cells, particularly the IgG-receptor carrying lymphocytes, in the blood leucocytes occurred in NZB mice with ageing. The cells bearing membrane immunoglobulins are considered to be for the most part thymus-independent lymphocytes (B-cells) (Takahashi *et al.*, 1971; Unanue *et al.*, 1971), although certain T-cells may also have them (Bankhurst, Warner & Sprent, 1971; Greaves, 1970). In the present experiment, the proportion and the number of blood lymphocytes not sensitive to NTA did not decrease but rather increased with ageing of NZB mice.

The further clarification of the relation between the recirculating T-cell depletion and autoantibody formation in NZB mice is of considerable importance. A hypothesis is that certain T-cells may exert an active controlling function over autoantibody-forming B-cells; relaxation of this control function may be of importance in the formation of certain autoantibodies and natural antibodies in NZB mice (Allison, Denman & Barnes, 1971). In addition, a depletion of normal T-cell function may also allow the development of abnormal clones of T-cells or B-cells. Although the genesis of NTA, whether under genetic or viral influence, is unknown, it is reasonable to suggest that the continuous production of NTA in

NZB mice is linked to the formation of several other autoantibodies or natural antibodies and the disease syndromes of these mice.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institute for Arthritis and Metabolic Diseases, N.I.H., and the Susan Greenwall Foundation, Inc.

The excellent technical assistance of Mr G. Rodney, Mrs P. Marinaccio, Miss M. Hendricks and Mr L. Dienes is gratefully acknowledged.

This work is the Research Department Publication No. 439.

REFERENCES

ALLISON, A.C., DENMAN, A.M. & BARNES, R.D. (1971) Cooperating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity. *Lancet*, **ii**, 135.

BANKHURST, A.D., WARNER, N.L. & SPRENT, J. (1971) Surface immunoglobins on thymus and thymus-derived lymphoid cells. *J. exp. Med.* **134**, 1005.

BHOOPALAM, N., YAKULIS, V.J., COSTEA, N. & HELLER, P. (1971) Membrane immunoglobulins of lymphocytes in NZB mice. *J. Immunol.* **107**, 1501.

BIELSCHOWSKY, M., HELYER, B.J. & HOWIE, J.B. (1959) Spontaneous hemolytic anemia in the mice of the NZB/BL strain. *Proc. Univ. Otago Med. School*, **37**, 9.

BOYLE, W. (1968) An extension of the ^{51}Cr-release assay for the estimation of mouse cytotoxins. *Transplantation*, **6**, 761.

CANTOR, H., ASOFSKY, R. & TALAL, N. (1970) Synergy among lymphoid cells mediating the graft-versus-host response. I. Synergy in graft-versus-host reactions produced by cells from NZB/BL mice. *J. exp. Med.* **131**, 22.

CANTOR, H. & ASOFSKY, R. (1972) Synergy among lymphoid cells mediating the graft-versus-host response. III. Evidence for interaction between two types of thymus-derived cells. *J. exp. Med.* **135**, 764.

CLAMAN, H.N., CHAPERON, E.A. & TRIPLETT, R.F. (1966) Thymus-marrow cell combinations. Synergism in antibody production. *Proc. Soc. exp. Biol. (N.Y.)*, **122**, 1167.

DENMAN, A.M. & DENMAN, E.L. (1970) Depletion of long-lived lymphocytes in old New Zealand Black mice. *Clin. exp. Immunol.* **6**, 457.

GAZDAR, A.F., BEITZEL, W. & TALAL, N. (1971) The age related response of New Zealand mice to a murine sarcoma virus. *Clin. exp. Immunol.* **8**, 501.

GREAVES, M.F. (1970) Biological effects of anti-immunoglobulins: evidence for immunoglobulin receptors on 'T' and 'B' lymphocytes. *Transplant Rev.* **5**, 45.

HELYER, B.J. & HOWIE, J.B. (1963) Spontaneous autoimmune disease in NZB/Bl mice. *Brit. J. Haematol.* **9**, 119.

HOLMES, M.C. & BURNET, F.M. (1963) The natural history of autoimmune disease in NZB mice. A comparison with the pattern of human autoimmune manifestations. *Ann. Intern. Med.* **59**, 265.

LANCE, E.M. (1969) The mechanism of action of anti-lymphocyte serum. Studies of antibody eluate. *J. exp. Med.* **130**, 49.

MARTIN, W.J. & MILLER, J.F.A.P. (1968) Cell to cell interaction in the immune response. IV. Site of action of antilymphocyte globulin. *J. exp. Med.* **128**, 855.

MELLORS, R.C. (1966) Autoimmune and immunoproliferative diseases of NZB mice and hybrids. *Int. Rev. exp. Pathol.* **5**, 217.

MELLORS, R.C., AOKI, T. & HUEBNER, R.J. (1969) Further implication of murine leukemia-like virus in the disorders of NZB mice. *J. exp. Med.* **129**, 1045.

MELLORS, R.C., SHIRAI, T., AOKI, T., HUEBNER, R.J. & KRAWCZYNSKI, K. (1971) Wild-type Gross leukemia virus and the pathogenesis of the glomerulonephritis of New Zealand mice. *J. exp. Med.* **133**, 113.

MILLER, J.F.A.P. & MITCHELL, G.F. (1968) Cell to cell interaction in the immune response. I. Hemolysin-forming cells in neonatally thymectomized mice reconstituted with thymus or thoracic duct lymphocytes. *J. exp. Med.* **128**, 801.

POTTER, M. & LIEBERMAN, R. (1967) Genetics of immunoglobulins in the mouse. *Advanc. Immunol.* **7,** 91.

RAFF, M.C. (1970) Role of thymus-derived lymphocytes in the secondary humoral immune response in mice. *Nature (Lond.),* **226,** 1257.

RAFF, M.C. (1971) Surface antigenic markers for distinguishing T and B lymphocytes in mice. *Transplant. Rev.* **6,** 52.

RAFF, M.C. & WORTIS, H.H. (1970) Thymus dependence of θ-bearing cells in the peripheral lymphoid tissues of mice. *Immunology,* **18,** 931.

SCHLESINGER, M. & YRON, I. (1970) Serologic demonstration of a thymus-dependent population of lymph node cells. *J. Immunol.* **104,** 798.

SHIRAI, T. & MELLORS R.C. (1971) Natural thymocytotoxic autoantibody and reactive antigen in New Zealand Black and other mice. *Proc. nat. Acad. Sci. (Wash.),* **68,** 1412.

SHIRAI, T. & MELLORS, R.C. (1972) Natural cytotoxic autoantibody against thymocytes in NZB mice. *Clin. exp. Immunol.* (In press).

SHIRAI, T., YOSHIKI, T., & MELLORS, R.C. (1972) Thymus dependence of cells in peripheral lymphoid tissues and in the circulation sensitive to natural thymocytotoxic autoantibody in NZB mice. *J. Immunol.* (In Press).

STUTMAN, O., YUNIS, E.J. & GOOD, R.A. (1968) Deficient immunologic functions of NZB mice. *Proc. Soc. exp. Biol. (N.Y.).* **127,** 1204.

TAKAHASHI, T., OLD, L.J., MCINTIRE, K.R. & BOYSE, E.A. (1971) Immunoglobulin and other surface antigens of cells of the immune system. *J. exp. Med.* **134,** 815.

UNANUE, E.R., GREY, H.M., RABELLINO, E., CAMPBELL, P. & SCHMIDTKE, J. (1971). Immunoglobulins on the surface of lymphocytes. II. The bone marrow as the main source of lymphocytes with detectable surface-bound immunoglobulin. *J. exp. Med.* **133,** 1188.

ZATZ, M.M., MELLORS, R.C. & LANCE, E.M. (1971) Changes in lymphoid populations of ageing CBA and NZB mice. *Clin. exp. Immunol.* **8,** 491.