Reticuloendothelial Activity of New Zealand Black Mice[1] (34624)

JANE I. MORTON AND B. V. SIEGEL

Division of Immunology and Department of Pathology, University of Oregon Medical School, Portland, Oregon 97201

New Zealand Black (NZB) mice develop with age, autoimmune disease characterized by Coombs positive hemolytic anemia (1, 2). This is accompanied by depression of the primary (3–5), but not the secondary (6), antibody response following immunization with sheep erythrocytes. Because alterations in reticuloendothelial system (RES) activity may influence antibody formation (7), a study was undertaken to assess possible changes in RES activity in NZB strain mice which might contribute to immunologic abnormality.

Materials and Methods. Fifth generation NZB mice of both sexes raised in this laboratory from an original stock received as generations 57 and 58 from W. H. Hall, Otago University Medical School, Dunedin, were used in the present study. The female BALB/c mice employed were obtained from Jackson Laboratory, Bar Harbor, Maine. Direct Coombs tests were carried out as described by Norins and Holmes (8).

The phagocytic activity of the reticuloendothelial system was assayed by determining the rate of clearance of carbon from the blood following intravenous injection. Mice were injected with 0.1 ml (6 mg) of a suspension of colloidal carbon (Pelikan, Gunther Wagner, Hannover, Germany, No. C11/1431a) prepared after a modification of the procedure of Parker and Finney (9). Ten-lambda blood samples, collected by orbital bleeding at 5-min intervals over a period of 30 min, were diluted in 3 ml of 0.1% $NaHCO_3$ and optical densities were read at 620 mμ in a Beckman Model B spectrophotometer (10). Samples were read against a blank of similarly prepared blood obtained from the same animal immediately prior to carbon injection. Results are expressed as half-times ($t_{1/2}$) of clearance, in minutes, of colloidal carbon determined from the semilogarithmic plot of optical density versus time.

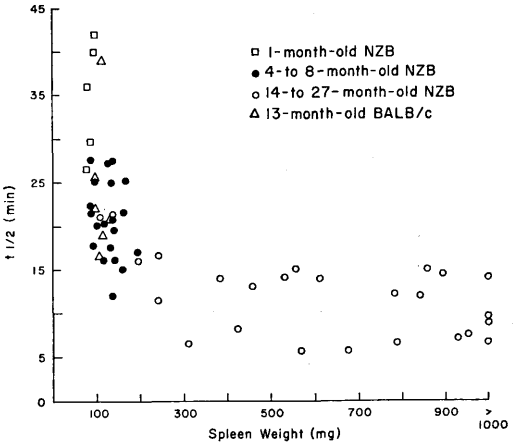

FIG. 1. Relationship of half-time carbon clearance values to spleen weights of 1- to 27-month-old mice of the NZB strain and of 13-months-old BALB/c mice.

Technetium-99m sulfur colloid (Tesuloid TM, E. R. Squibb & Sons, New Brunswick, N.J.) was inoculated intravenously in a 0.1-ml volume containing 0.1 mg of thiosulfate colloid with an activity which ranged from 30 to 80 μCi in different preparations. Counts of radioactivity of excised spleens and livers were carried out 30 min following injection employing a Nuclear Chicago Model 132A Spectroscaler connected to a 2-in. sodium iodide crystal scintillation probe.

Results. Carbon clearance rates for NZB mice of various ages. Half-time clearance values as a function of spleen weights for NZB mice from 1 to 27 months of age are plotted in Fig. 1. As shown, when spleen weights exceeded 200 mg, clearance was sig-

[1] This work was supported by U.S. Atomic Energy Commission Contract RLO-1927-43.

TABLE I. Liver Weights of NZB and BALB/c Mice of Different Ages Studied for Carbon Clearance.

Strain	Age (months)	No. of mice	Av liver wt (g) (range)
NZB	1	5	0.91 (0.80–0.97)
	4–5	15	1.57 (1.24–1.95)
	8	6	1.61 (1.47–1.68)
	14–27	26	2.52 (1.81–4.64)
BALB/c	13	7	1.39 (1.24–1.50)

nificantly accelerated. However, the rate of clearance did not manifest a consistent increase with augmentation of spleen size beyond 200 mg. Three NZB mice over 14 months of age which were Coombs positive did not show increased spleen weights or notable liver enlargement. These animals had individual clearance half-times of 16, 21, and 21 min, values comparable to those exhibited by younger mice showing normal-sized spleens. This suggested that organ size rather than age or the presence of autoantibody may have been responsible for accelerated clearance. In this regard, 1-month-old NZB mice, having spleens weighing in the range of 77–95 mg, demonstrated slower clearance rates than NZB mice, 4 months and older, whose spleen weights generally exceeded 100 mg (Fig. 1). Thirteen-month-old BALB/cJ mice having spleen weights of 96–145 mg evidenced clearance rates comparable to those observed for NZB mice with similar spleen sizes (Fig. 1).

Liver weights of these different groups of mice are presented in Table I. It can be seen that liver weights increased with age, normal growth accounting for the differences between 1-month-old and 4- to 8-month-old NZB mice, while in the case of old, overtly autoimmune mice part of the size increase probably reflected organ enlargement compensatory to hemolytic stress. Examination of individual spleen and liver weights of old mice indicated that these two organs did not necessarily enlarge in parallel; that is, one animal might have a relatively small spleen and a markedly enlarged liver, while in the case of another mouse the situation might be reversed. This could provide one explanation for the failure to observe continuous increases in clearance rates with progressive spleen enlargement (Fig. 1). Liver weights of 13-month-old BALB/c mice were noted to be somewhat less than liver weights of 4- to 8-month-old NZB mice.

Effect of phenylhydrazine-induced hemolytic anemia on clearance rates of young NZB mice. It seemed of interest to explore further the relationship between the autoimmune disease syndrome and increased reticuloendothelial activity. In this connection, phenylhydrazine (phenylhydrazine HCl, Matheson, Coleman & Bell, Norwood, Ohio) was employed to experimentally induce hemolytic anemia in young preautoimmune NZB mice.

The individual carbon clearance rates for 4-month-old NZB mice, both untreated and injected intraperitoneally with 3 mg of phenylhydrazine (ϕH) dissolved in 0.3 ml of 0.85% NaCl, are shown in Table II. When ϕH was administered 14–20 hr before assay, responses fell into two distinct categories: in one (group 1) there was delayed uptake of colloid suggestive of RES blockade, while in the second (group 2) clearance rates were largely within the range of rates observed for control animals. These ϕH-treated mice demonstrated an approximately 30% increase in spleen weights (av, = 162 mg; range, 131–200), slightly increased liver sizes (av, 1.63 g; range, 1.44–1.80) and markedly

TABLE II. Carbon Clearance Rates [$t_{½}$ (min)] for Individual 4-Month-Old NZB Mice, Control and Treated with 3 mg of Phenylhydrazine (ϕH).

Untreated controls		Phenylhydrazine-treated mice; time (hr) of ϕH injection prior to carbon clearance assay (hr): 14–20	120
27.5	Group 1:	60	16.5
21.5		43	11.5
20.0		37	7.5
17.5			6.5
17.0	Group 2:	20	6.0
17.0		18	4.0
16.0		18	
		16	
		14	

TABLE III. Effect of Splenectomy (Splx) on Carbon Clearance Rates in 1-Year-Old NZB Mice.

After splenectomy (days)	Treatment	No. of mice	Av $t_{½}$ (min) (range)
1	Splx	4	25.2 (21.5–31.0)
	Sham	5	19.8 (15.0–25.0)
6	Splx	6	16.3 (10.5–24.0)
	Sham	4	8.2 (7.0–10.0)

depressed hematocrit values (av, 33%; range, 31–35). There were virtually no differences in either spleen or liver sizes or in hematocrit values between those animals showing RES blockade (group 1) and those showing normal rates of carbon clearance (group 2).

Injection of phenylhydrazine, 120 hr prior to assay, resulted in accelerated carbon clearance (Table II); these clearance rates were comparable to those observed for autoimmune animals showing splenomegaly (Fig. 1). The phenylhydrazine-treated mice manifested markedly enlarged spleens (av, 466 mg; range, 369–590) and depressed hematocrits (av, 30%; range, 21–37). Liver weights, however, were normal (av, = 1.45 g; range, 1.20–1.83). Spleen weights of the untreated control mice averaged 123 mg (range, 91–194), livers averaged 1.43 g (range, 1.24–1.65) and hematocrits averaged 47% (range, 44–51). All mice showed negative direct Coombs tests on the day of clearance assay. The results obtained with ϕH-treatment would suggest that hemolysis and resultant spleen enlargement alone are sufficient to produce increased RES activity and that changes in liver size and the presence of autoantibody may not be necessary in this regard.

Effect of splenectomy on carbon clearance rates in old NZB mice. Carbon clearance studies following splenectomy were next undertaken to evaluate the extent of participation of the spleen in the observed acceleration of clearance in aged NZB mice. One-year-old NZB mice were splenectomized or sham operated under Nembutal anesthesia either 6 days or 1 day prior to assay. As shown in Table III, mice splenectomized 6 days previously showed slower rates of clearance than sham-operated controls. Clearance rates for most of the splenectomized mice were similar to the rates observed for preautoimmune NZB mice (Fig. 1), while the sham-operated controls displayed accelerated rates characteristic of old animals with enlarged spleens (Fig. 1).

Rate differences between splenectomized and sham-operated mice were also observed when splenectomy was performed 1 day before carbon clearance assay (Table III). Here, however, both splenectomized and sham-operated animals showed delayed clearance compared to unoperated NZB mice of similar age and with enlarged spleens (Fig. 1). This might suggest residual effects attributable to operative procedures, possibly Nembutal anesthesia, which would account for the relatively impaired clearance rates of both the control and experimental mice.

Effect of serum transfer from autoimmune to preautoimmune NZB mice. The following experiment was performed to test for the possibility that enhanced levels of serum opsonin may contribute to increased clearance rates in the case of older NZB mice. A serum pool was first prepared from 33 strongly Coombs positive NZB mice, 13- to 19-months of age. Eight 4-month-old Coombs negative NZB mice were injected intraperitoneally with 0.5 ml of this serum: of these, 4 animals were injected 22 hr prior to assay and 4 mice 2–4 hr before assay. The average half-time clearance for the first group of mice was 19.5 min, and for the second 16 min. Since these clearance times were more comparable to those for young NZB mice than those noted for Coombs positive animals (Fig. 1), there was apparently little or no accelerative effect on reticuloendothelial system activity by such serum transfer.

Distribution of technetium-99m sulfur colloid in spleens and livers of NZB mice. The partition of radioactive colloid between spleen and liver of NZB mice of various ages was investigated in an attempt to assess the relative efficiencies of different-sized spleens in taking up particulate material. The data presented in Table IV indicate that there was

TABLE IV. Relative Distribution of Technetium-99m Sulfur Colloid in Spleens and Livers of NZB Mice.

Group	Av spleen wt (g) (range)	Age (months)	No. of mice	Ratio of (radioactivity/mg of spleen) : (radioactivity/mg of liver)
1	0.090 (0.080–0.110)	<3	6	0.401 (0.280–0.544)
2	0.104 (0.090–0.120)	4–7	5	0.405 (0.291–0.832)
3	0.205 (0.150–0.280)	11–18	6	0.302 (0.203–1.750)
4	0.566 (0.310–0.810)	11–18	10	0.242 (0.137–0.666)
5	1.480 (1.140–2.000)	11–18	4	0.241 (0.114–0.532)

a decrease in the ratio of radioactivity/mg spleen to radioactivity/mg liver with spleen enlargement. In the case of a few of the animals showing markedly enlarged spleens (groups 4 and 5) there was little or no increase in total colloid uptake with spleen enlargement. In general, however, accelerated clearances with increased organ weights appeared to more than offset the decreased clearance efficiency obtaining on a per unit weight basis. The conclusion that spleen clearance efficiency was altered is based on the premise that the clearance capacity per unit liver weight remained constant. However, because the determined ratios pertain to relative radioactivity, the present results could be attributed alternatively to changes in liver phagocytosis.

Discussion. Increased rates of carbon clearance were noted in old, autoimmune NZB mice with associated hepatosplenomegaly. That the presence of autoantibody was not necessary to bring about such changes in phagocytic activity was indicated by the results of two different experiments. In one, injection of serum from old Coombs positive NZB mice into young Coombs negative animals did not bring about a significant alteration in clearance rates. In a second experiment, phenylhydrazine injection into young NZB mice was observed to result in a nonautoimmune hemolytic anemia with subsequent splenomegaly and accelerated carbon clearance. A relationship between organ size and clearance rate was further suggested by the observation that splenectomy of old splenomegalic NZB mice resulted in a delay in carbon clearance. These findings would suggest that compensatory increases in spleen and liver sizes following hemolytic stress could eventuate in an augmentation of phagocytic activity. In this connection, the studies of Jandl, *et al.* (11) have indicated that the sequestration of particulate matter *per se,* of damaged autologous erythrocytes, for example, may stimulate reticuloendothelial hyperplasia.

The question still remains as to the relationship, if any, between these changes in RES activity and the immunodepression observed in old, overtly autoimmune NZB mice. Antigen-processing by phagocytic cells may be necessary for the preservation of antigen in an available immunogenic form, for the formation of informational RNA, or for the complexing of antigen with RNA to form a highly immunogenic "super-antigen" (7). Only a very small amount of antigenic material ingested by such cells, however, appears to be actually passed on to antibody-forming cells (12). This may be explained by the observation that cells classed as phagocytic are heterogeneous in their activity. For example, although splenic macrophages may process and retain immunogenic material, the Kupffer cells of the liver apparently destroy antigen (13, 14). Hence, measurements of capacity to remove injected colloid do not provide the necessary information to distinguish possible alterations in the balance between these two types of phagocytic function; nor do they elucidate the extent to which such alterations influence the immune process.

The phenomenon of increased clearance rates in overtly autoimmune NZB mice

manifesting splenomegaly and impairment of primary immune capacity may be similar to the situation of accelerated carbon clearance in runted, neonatally thymectomized mice showing enlarged spleens and diminished capacity to respond to immunologic stimuli (15). In this connection, the syndrome produced by neonatal thymectomy has been described by De Vries *et al.* (16) as autoimmune in character. Although a causal relationship between increased RES activity and diminished immune competence in such animals could exist, conceivably as a consequence of an altered balance between antigen-destruction and antigen-processing, other studies have suggested that the immunodepression observed under conditions of NZB autoimmunity (3–5) and of neonatal thymectomy (17) may be related to a paucity of antigen-sensitive cells. Thus, while defective antigen-handling as a factor in immune depression in overtly autoimmune NZB mice has not been excluded by the present study, it also seems possible that concurrent changes in immune competence and RES activity in these animals may be independent consequences of the disease process and do not bear a direct cause and effect relationship to one another.

Summary. Carbon clearance rates were observed to be significantly increased in autoimmune NZB mice with enlarged spleens. Similar acceleration could be induced in young NZB mice by intraperitoneal injection of phenylhydrazine 5 days prior to assay. Marked anemia and splenomegaly were also noted to develop following such phenylhydrazine treatment. Splenectomy of 1-year-old NZB mice was observed to result in delayed clearance of carbon particles. There was little effect on the rate of carbon clearance in young NZB mice injected intraperitoneally with a serum pool obtained from old Coombs positive animals. Assessment of spleen and liver radioactivity following intravenous injection of technetium-99m sulfur colloid indicated a somewhat reduced efficiency of colloid uptake by the enlarged spleen on a per milligram weight basis; however, this reduced efficiency was not sufficient, generally, to counteract the increased clearance capacity of the whole organ. The present findings would suggest that the increased rate of carbon clearance observed in overtly autoimmune NZB mice may be a result of the hepatosplenomegaly associated with the disease state.

We are grateful to Dr. E. L. Dobson, Donner Laboratory, University of California, Berkeley, for supplying the Pelikan carbon and for his helpful discussions regarding these studies.

1. Bielchowski, M., Helyer, B. J., and Howie, J. B., Proc. Univ. Otago Med. Sch. **37**, 9 (1959).

2. Holmes, M. C., and Burnet, F. M., Ann. Intern. Med. **59**, 265 (1963).

3. Diener, E., Int. Arch. Allergy, Appl. Immunol. **30**, 120 (1966).

4. Morton, J. I., and Siegel, B. V., J. Reticuloendothel. Soc. **5**, 567 (1968).

5. Morton, J. I., and Siegel, B. V., J. Reticuloendothel. Soc. **6**, 78 (1969).

6. Morton, J. I., and Siegel, B. V., Immunology **19**, 481 (1969).

7. Shands, J. W., *in* "Modern Trends in Immunology" (R. Cruickshank and D. M. Weir, eds.) Vol. 2, p. 86. Appleton-Century-Crofts, New York (1967).

8. Norins, L. C., and Holmes, M. C., J. Immunol. **93**, 897 (1964).

9. Parker, H. G., and Finney, C. R., Amer. J. Physiol. **198**, 916 (1960).

10. Bailey, J. M., Clough, J., and Stearman, M., Proc. Soc. Exp. Biol. Med. **115**, 642 (1964).

11. Jandl, J. H., Files, N. M., Barnett, S. B., and MacDonald, R. A., J. Exp. Med. **122**, 299 (1965).

12. Franzl, R. E., Nature (London) **195**, 457 (1962).

13. Cohn, Z. A., J. Exp. Med. **120**, 869 (1964).

14. Inchley, C. J., Clin. Exp. Immunol. **5**, 173 (1969).

15. Schooley, J. C., Kelley, L. S., Dobson, E. L., Finney, C. R., Havens, V. W., and Cantor, L. N., J. Reticuloendothel. Soc. **2**, 396 (1965).

16. De Vries, M. J., Van Putten, L. M., Balner, H., and Van Bekkum, D. W., Rev. Fr. Etud. Clin. Biol. **9**, 381 (1964).

17. Miller, J. F. A. P., *in* "The Thymus: Experimental and Clinical Studies" (G. E. W. Wolstenholme and R. Porter, eds.), p. 53. Little, Brown, Boston (1966).

Received Oct. 22, 1969. P.S.E.B.M., 1970, Vol. 133.