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A DEFECT OF B-LYMPHOCYTE TRANSPORT OF AGGREGATED HGG INTO GERMINAL CENTRES IN NZB AND NZB × NZW F_1 HYBRID MICE

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SUMMARY

Previous experiments have shown (i) that the localization of intravenously injected heat aggregated human γ-globulin (HGG) in the germinal centres of normal mice provides a model for studying the natural uptake of circulating immune complexes in these areas, and (ii) that the aggregated HGG is carried into germinal centres by lymphocytes which have receptors for altered γ-globlin.

Evidence from thymus-cell depletion experiments is now presented which suggests that the lymphocytes concerned are bone-marrow-derived B cells. Defective localization was found in NZB and NZB × NZW F_1 hybrids at different ages and the onset of the defect antedates the onset of autoimmunity and the appearance of histological abnormalities in the spleen. As disease develops it progresses to a complete inability to localize complexes in germinal centres. It is concluded that a functional defect of the bone marrow-derived lymphocyte population exists in these mice.

INTRODUCTION

New Zealand Black mice (NZB) have been extensively studied as a model of autoimmune diseases (Bielschowky, Helyer & Howie, 1959; Howie & Helyer, 1968). During their first year of life, these mice develop a haemolytic anaemia with positive direct Coombs' test, splenomegaly, antinuclear antibodies and renal disease, due to glomerular deposition of antigen–antinuclear antibody complexes (Lambert & Dixon, 1968). Antinuclear antibodies are more pronounced and renal disease is manifested at an earlier stage in the NZB × NZW F_1 hybrid (B/W) mice.

As well as immunologically mediated lesions, these animals show several abnormalities of immune response. Thus an unduly early maturation of their immune system has been

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reported (Playfair, 1968; Evans, Williamson & Irvine, 1968) and a relative inability of young NZB and B/W mice to acquire and maintain tolerance to protein antigens (Weir, McBride, Naysmith, 1968; Staples & Talal, 1969) has been demonstrated. Both hyper-responsiveness (Weir *et al.*, 1968; Morton & Siegel, 1969; Staples & Talal, 1969) and hypo-responsiveness (Stutman, Yunis & Good, 1968; Salomon & Benveniste, 1969) have been observed in these animals, but when their antibody production to a range of different antigens is compared with that of several normal strains it appears simply that they give a higher antibody response to some antigens and a lower antibody response to others (Cerottini, Lambert & Dixon, 1969).

So far as lymphocyte abnormalities are concerned, old NZB mice have a lymphopenia and their lymph nodes also are depleted of lymphocytes (East, de Sousa & Parrott, 1965). Labelling studies with tritiated thymidine (Denman & Denman, 1970) showed that old mice are depleted predominantly of long-lived lymphocytes and there is also evidence from cell-homing studies that in these mice there is a decrease with ageing in the recirculating cell content of lymph nodes and spleen that coincides with the onset and development of autoimmunity (Zatz, Mellors & Lance, 1971). Furthermore, Steinberg, Law & Talal (1970) have shown that the resistance of adult B/W mice to attempts to induce tolerance with ultracentrifuged BGG is overcome by transferring thymus cells from young B/W mice, suggesting that in the adult B/W mouse also the thymus cells are deficient. Thymus deficiency has been related to the development of autoimmunity in NZB by de Vries & Hijmans (1966) and by Holmes & Burnet in B/W (1966).

Previously we studied the uptake of immune complexes or heat-aggregated human gamma globulin (HGG) in germinal centre areas of lymph nodes (Brown, Schwab & Holborow, 1970a) and spleen (Brown *et al.*, 1970b) and found that this uptake is mediated by mobile lymphocytes that have receptors directed to sites exposed on IgG molecules either by complexing with antigen or by mild heat treatment (aggregation) (Brown *et al.*, 1970b). In view of the cellular deficiencies described in NZB and B/W mice, we have therefore now investigated their ability to localize aggregated HGG and immune complexes in the germinal centres of the spleen.

We also present data suggesting that the lymphoid cells responsible for the uptake of aggregated HGG and immune complexes into germinal centre areas in normal mice are of bone marrow origin (B cells) rather than thymus dependent (T cells).

MATERIALS AND METHODS

Mice

NZB, CBA, BALB/c and C_{57} Bl inbred strains from the Taplow colony were used, and F_1 hybrids were bred from NZB female mice crossed with NZW males also maintained at Taplow.

Mice of both sexes and a wide range of ages, from 5 weeks up to 20 months old, were examined.

*Preparation of heat-aggregated HGG labelled with ^{125}I (*Agg HGG)*

Heat aggregated complexes of HGG were prepared and iodinated as described previously (Greenwood *et al.*, 1971a).

*In vivo uptake of *Agg HGG*

*Agg HGG prepared as described above was injected intravenously into mice in various doses. Twenty-four hours after injection mice were anaesthetized with ether, killed by exsanguination and the spleen and a piece of liver removed and weighed. Studies to be reported (Brown, de Jesus & Holborow, unpublished) have shown that at doses up to 100 μg catabolic events are largely completed and maximal uptake in the splenic germinal centres of normal mice has occurred by 24 hr.

The radioactivity of the blood, liver and spleen was measured in a crystal well scintillation detector used in conjunction with a Tracerlab spectrometer. The spleen was then snap-frozen in isopentane at the temperature of liquid nitrogen, and cryostat sections were cut.

Direct Coombs' test

Rabbit antimouse γ-globulin obtained from Wellcome Reagents Ltd, was absorbed with an equal volume of packed washed normal mouse red blood cells (RBC), diluted 1:10 with phosphate buffered saline (pH 8·0), divided into small volumes and frozen.

Agglutination tests were done at room temperature, mixing one drop of diluted anti-globulin serum with one drop of 20% mouse RBC on a glass slide.

Normal mouse RBC were not agglutinated. Large agglutinates appearing rapidly were scored as $+++$, and weaker reactions were scored as $++$; agglutination only visible under the microscope was scored as $+$.

Positive and negative control sera were always included.

Antinuclear factor

Sera from all mice were examined for the presence of antinuclear factor (ANF) by indirect immunofluorescence. Cryostat sections of rat liver were treated with the test serum diluted 1:10, washed and then treated with a 1:16 dilution of a specific rabbit anti-mouse γ-globulin conjugated with FITC (Nordic Pharmaceuticals and Diagnostics).

After washing, sections were mounted in 90% glycerol and examined microscopically with a dark ground condenser, iodine quartz illumination and a FITC-3 interference primary filter with a Wratten 12 secondary filter.

Proteinuria

Proteinuria was detected with 'Albustix' where a $++$ reaction is equivalent to 100 mg albumin per 100 ml urine. Only $++$ or stronger reactions were considered as evidence of renal disease.

Immunofluorescent spleen staining

Cryostat sections of 6-μm thickness were dried under a fan and stained with specific fluorescein conjugated sheep or rabbit antisera raised against HGG (Wellcome Reagents Ltd.) or against mouse γ-globulin (Nordic Pharmaceuticals and Diagnostics).

The anti HGG conjugate was used at a dilution which gave no staining of normal uninjected spleen sections and the antimouse immunoglobulin at dilution that gave no background staining. Staining of both human and mouse immunoglobulin was inhibited by absorbing the conjugates with purified HGG or mouse IgG, respectively.

Anti-mouse lymphocyte serum (ALS)

This serum was obtained from Wellcome Reagents Ltd. According to the manufacturers it had been raised in horses by repeated injections of mouse thymocytes without adjuvant, and had been inactivated at 56°C for 30 min. Activity had been tested by prolongation of graft survival when tail skin from A strain mice was grafted on to CBA strain recipients. Two doses of 0·5 ml of ALS given on days 2 and 5 after operation increased the mean survival time of the grafts from approximately 11 to over 20 days.

Heat inactivated normal horse serum (NHS) was given to the control mice.

Both ALS and NHS were tested for anti-mouse RBC haemagglutinins by titration against a 20% suspension of normal mouse RBC. The ALS agglutinated mouse RBC to a titre of 1/5 and the NHS only when used neat. Since the titres of both were so low, they were administered without absorption by mouse RBC.

Thymectomy

This operation was done in CBA mice 4–6 weeks old under Nembutal anaesthesia, by sucking out the thymus through a midline incision just above the manubrium. At the end of the experiment all thymectomized animals were checked macroscopically for absence of thymus.

Irradiation

Ten to 14 days after thymectomy whole body X-irradiation was done, each mouse receiving 850 rad in about 22 min.

Reconstitution with bone marrow cells

After irradiation mice were reconstituted on the same day with $5–10 \times 10^6$ bone marrow cells, obtained by flushing out femoral marrow from CBA with medium 199 (BDH Chemicals Ltd) containing 10% of foetal calf serum (FCS) and added penicillin and streptomycin. The bone marrow cells were washed once and counted. Viability was tested by the trypan blue method.

RESULTS

1. *Localization of *Agg HGG in splenic germinal centres of thymus-deprived CBA and $C_{57}Bl$ mice.*

(a) *Thymectomized, X-irradiated and bone-marrow reconstituted mice.* Two separate experiments were carried out on different occasions on CBA mice. In each experiment the mice were divided into three groups of three, as follows: group A, thymectomized, X-irradiated and reconstituted with bone marrow cells (see Materials and Methods); group B, sham thymectomized, X-irradiated and reconstituted with bone marrow cells; group C, normal mice of the same age. In experiment I mice were tested 68 days after reconstitution and in experiment II, 45 days after. All mice received intravenously 50 μg of *Agg HGG and were killed 24 hr later.

Although the normal mice (group C) had higher counts for radioactivity in their spleens than mice of groups A and B, no significant difference was seen between groups in the intensity of staining of the germinal centres for HGG (Table 1). The lower counts in the treated mice were probably due to incomplete recovery after irradiation and reconstitution.

TABLE 1. Failure of depletion of thymus-derived lymphocytes to decrease splenic uptake of 50 μg *Agg HGG injected i.v. into CBA mice

Experiment	Group	Interval in days between reconstitution and injection of *Agg HGG	Spleen		
			Cps	Mean cps	G.C. staining for HGG
I	A	68	115·91	95·43	+++
			80·09		++
			90·30		++
	B	68	84·73	84·39	++
			82·80		++
			85·65		++
	C	68	93·36	111·65	++
			122·74		+++
			118·87		+++
II	A	45	181·5	118·1	+++
			117·9		+++
			54·8		+
	B	45	106·1	109·0	+++
			133·5		+++
			87·4		++
	C	45	238·5	195·8	++++
			179·1		+++
			170·0		+++

A = Thymectomized mice, lethally X-irradiated, bone marrow reconstituted.
B = Sham-thymectomized mice, lethally X-irradiated, bone marrow reconstituted.
C = Normal mice of same age.
Cps = counts/second in the whole spleen.
G.C. = germinal centres.

(b) *Thymectomized mice chronically treated with ALS.* Four weeks after thymectomy twenty-four C_{57}Bl mice, divided into two groups of twelve, received intraperitoneally 0·5 ml of ALS or NHS daily on 5 consecutive days in each week, for a period of 2 months. Five of the twelve ALS treated and nine of the twelve NHS treated mice survived this treatment. Ten days after the last injection of either ALS or NHS, the mice were given 50 μg of *Agg HGG intravenously and killed after 24 hours. The radioactivity in spleen, liver and blood was measured, and cryostat sections of the spleen were examined by immunofluorescence for HGG, horse γ-globulin and mouse IgG. As controls, three C_{57}Bl mice thymectomized at the same time but receiving no further treatment, and four normal C_{57}Bl mice of the same age as the thymectomized mice were included.

Isotope uptake (Table 2). No difference in the mean splenic uptake of *Agg HGG was found between the thymectomized ALS treated mice and the thymectomized controls, but the mean splenic count in the non-thymectomized mice was lower than in any of the thymectomized groups. This difference may not have been significant, but it may be noted that in the previous experiment (IA) also a similar difference was observed between the thymectomized and the sham-thymectomized groups (Table 1).

TABLE 2. Failure of thymectomy and ALS to prevent uptake of *Agg HGG in splenic germinal centres of C_{57}Bl mice

Thymectomy	Treatment	Spleen					Antibody response†	
		HGG retention (cps)	Mean (cps)	G.C. staining for HGG	G.C. staining for mouse γ	G.C. staining for horse γ	Anti-whole horse serum	Anti-horse γ
Yes	ALS	70·0	100·5	++	+	+	Neg	Neg
		133·1		++	Neg	+	Neg	Neg
		100·1		++	Neg	Neg	Neg	Neg
		108·8		++	Neg	Neg	Neg	Neg
		90·4		++	+	+	Neg	Neg
Yes	NHS	99·8	88·9	+++	++++	+++	+	+
		102·5		+++	++++	+++	+	+
		110·8		++	+++	++	Neg	Neg
		78·4		++	++	+++	+	+
		88·2		+++	++	++	Neg	Neg
		96·9		++	+++	+++	+	+
		60·7		++	+++	+++	+	+
		84·2		+++	++	+++	+	+
		78·3		++	+++	+++	+	+
Yes	None	111·5	100·0	++	+	Neg	N.T.	N.T.
		109·2		+++	+	Neg	N.T.	N.T.
		79·4		++	+	Neg	N.T.	N.T.
No	None	62·4	68·65	++	+	Neg	Neg	Neg
		68·3		++	+	Neg	Neg	Neg
		80·7		+++	+	Neg	N.T.	N.T.
		63·2		++	+	Neg	N.T.	N.T.

Cps = counts/second in the whole spleen.
G.C. = germinal centre.
N.T. = not tested.
* Agg HGG = Heat-aggregated HGG labelled with ^{125}I.
† Detected by immunodiffusion.

Immunofluorescence study (Table 2). There was no significant difference between the four groups in intensity of staining of splenic germinal centres for HGG. However, with anti-mouse IgG conjugate the ALS treated group showed no or only faint staining, whereas in the NHS treated group the staining of germinal centres for autologous IgG was bright.

Staining for horse γ-globulin in the splenic germinal centres was very bright in the NHS treated group, but negative or only faint in the ALS group. All controls were negative.

Antibody to horse serum and horse γ-globulin. Tested by immunodiffusion, the ALS treated mice had no detectable antibody to either horse serum or horse γ-globuin but seven out of nine of the NHS treated had.

2. *Localization of aggregated HGG in splenic germinal centres of NZB and B/W mice*

(a) *Immunofluorescence in young NZB and B/W* (Table 3). 50-μg doses of aggregated HGG were injected intravenously into fifty-five normal mice—BALB/c, C_{57}Bl or CBA—

of various ages (2–10 months), seventeen B/W females (1·5–4 months) and thirteen NZB of both sexes (1·5–3 months).

All animals were tested for a direct Coombs' reaction, ANF and proteinuria, before injection of aggregated HGG. All had normal tests with the exception of seven older B/W mice which had ANF scored as +.

In spleen sections from mice of all the strains at this young age group killed 24 hr after injection, HGG was present in the germinal centres, as shown by immunofluorescence. The staining was bright, specific and well-defined, being distributed in the characteristic dendritic intercellular pattern already described (Brown *et al.*, 1970a; 1970b; Greenwood *et al.*, 1971a) and there was no apparent difference in intensity of staining between any of the strains examined. Staining for mouse γ-globulin revealed a similar but usually less bright staining pattern in the splenic germinal centres of all the mice. In four NZB mice this staining was as bright as that of the HGG.

TABLE 3. Relation between the ability of mice to localize intravenously injected aggregated HGG in splenic germinal centres and the presence of autoimmune disease

Strain	Number of mice tested	Age in months	Dose of Agg HGG (μg)	Coombs' test	ANF	Renal disease*	Splenic germinal centres stained for	
							HGG	mouse IgG
BALB/c	30	2–10	50	Neg	Neg	Neg	++	+
$C_{57}Bl$	15	2–10	50	Neg	Neg	Neg	++	+
CBA	10	3–10	50	Neg	Neg	Neg	++	+
B/W	17	1·5–4	50	Neg	Neg/+	Neg	++/+	+
NZB	13	1·5–3	50	Neg	Neg	Neg	++	+/++
NZB	9	10–20	50–500	+++	+/+++	+	Neg	Neg/+
B/W	15	6–8	50–500	+/++	+++	+	Neg	Neg

* Renal disease was considered to be positive when proteinuria tested by "Albustix" was ++ or stronger (correspondent to 100 mg/100 ml urine or more).

Anti-mouse IgG conjugate also gave a fainter but definite staining of the whole white pulp with the exception of the periarteriolar areas. This appeared to be due to the presence of immunoglobulins on and/or between the small lymphocytes which constitute the bulk of the white pulp.

(b) *Immunofluorescence in old NZB and B/W* (Table 3). Fifteen B/W females from 6 to 8-months old and nine from NZB 10 to 20-months old, all with features of well developed autoimmune disease—i.e. positive Coombs' and ANF tests and marked proteinuria—lacked the ability to localize aggregated HGG in the spleen at a detectable level.

Even when the dose of HGG was increased from 50 to 500 μg no localization was found in any area of the splenic white pulp of the old mice.

A similar lack of staining was apparent with anti-mouse IgG. The dendritic intercellular pattern of mouse IgG found in germinal centres of young animals had disappeared completely in the old B/W mice and in five of the oldest NZB mice. In four NZB aged 10 months faint germinal centre staining for mouse IgG was still present.

(c) *Splenic uptake of isotope-labelled aggregated HGG in old NZB and B/W mice.* When aggregated HGG labelled with ^{125}I (*Agg HGG) was used, the amount of isotope detected in the spleen correlated with the presence or absence of a fluorescent dendritic staining pattern for HGG in the germinal centres (Table 4). Thus, three old NZB, and five old female B/W mice with autoimmune disease, and three healthy adult BALB/c mice, were injected intravenously with a standard dose of 75 μg of *Agg HGG and killed 24 hr after this injection.

Splenic uptake in all but one of the diseased NZB and B/W mice was much lower than in the healthy control mice, coinciding with a lack of localization in the germinal centres by immunofluorescence. In two of the old NZB mice there was marked splenomegaly, and in one of these the whole spleen radioactivity count was normal and in the other 50% of normal. When the results were expressed as cps/100 mg of tissue however, the splenic uptake was shown to be depleted to the same extent in these as in the other diseased mice in this group (Table 4).

TABLE 4. Splenic uptake of 75 μg of *Agg HGG given intravenously to old healthy and diseased mice

Strain	Age in months	Autoimmune disease†	Splenic			
			Weight (mg)	Uptake in		Germinal centres staining for HGG
				Cps	Cps/100 mg	
BALB/c	6	Absent	160	148·7	92·94	+++
		Absent	155	164·7	106·25	+++
		Absent	95	146·3	154·00	+++
NZB	10–14	Present	1095	163·2	14·90	Neg
		Present	1200	63·1	5·26	Neg
		Present	265	28·5	10·75	Neg
B/W	6–8	Present	240	16·6	6·91	Neg
		Present	295	44·6	15·12	Neg
		Present	295	23·2	7·86	Neg
		Present	165	20·2	12·24	Neg
		Present	155	14·3	9·22	Neg

Cps = Isotope incorporation expressed as counts/second/spleen.
Cps/100 mg = Isotope incorporation expressed as counts/second/100 mg of spleen.
* Agg HGG: Heat-aggregated HGG labelled with ^{125}I.
† Autoimmune disease was considered to be present when there was a positive direct Coombs' and ANF tests together with a proteinuria of ++ or stronger.

3. *Effect of age and disease on the splenic uptake of *Agg HGG*

(a) *NZB mice.* NZB mice were grouped according to age and tested for Coombs' and ANF positivity and presence of proteinuria. Eight mice aged between 1·5 and 2 months, twelve aged 3–7 months, two aged 8 months, nine aged 10–11 months, four aged 14 and six aged 20 months were examined. Each received intravenously 50 μg of *Agg HGG in PBS (pH 8·0) and were killed 24 hours later.

Equal numbers of mice of normal strains—BALB/c, $C_{57}Bl$ or CBA—were included as controls for each age group and tested in the same way. The mean (cps) splenic uptake of *Agg HGG in the normal control mice in each age group was taken as 100% for that particular age, and individual splenic uptakes in the NZB mice in each age group were expressed as percentages of the normal. It was found that NZB mice 1·5–2-months old, although still apparently localizing HGG normally in their splenic germinal centres as judged by immunofluorescent staining, and still normal in other respects as judged by negative Coombs' and ANF tests, and the absence of proteinuria (Fig. 1), nevertheless were already by this age defective (mean uptake 66·5% of normal) in retaining *Agg HGG in their spleens (Fig. 2).

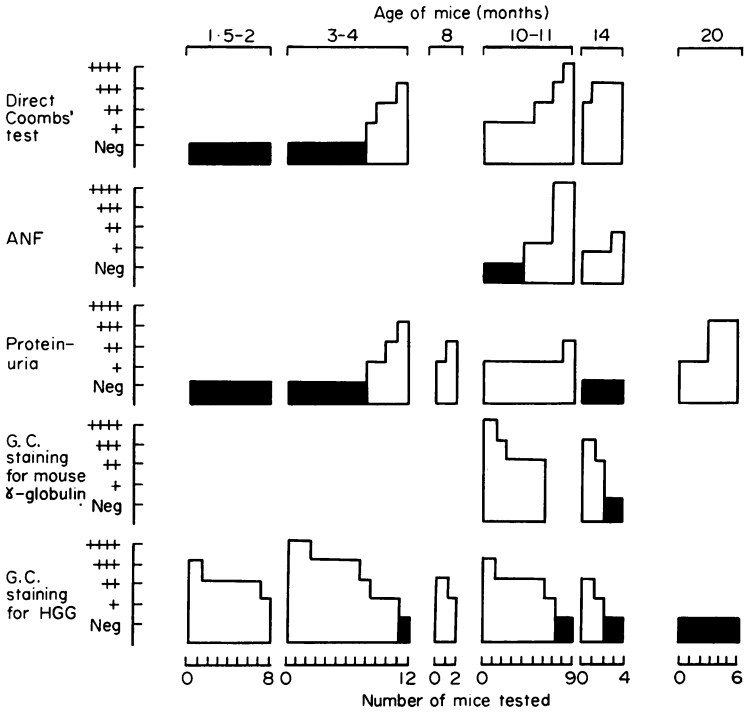

FIG. 1. The relation between direct Coombs' test, ANF, proteinuria and splenic (G.C.) germinal centre staining for HGG and mouse γ-globulin in NZB mice of different ages, injected intravenously with 50 μg Agg HGG labelled with ^{125}I. In control mice (BALB/c, $C_{57}Bl$ or CBA) germinal centre staining for mouse IgG was not more than + or + + at any age; for HGG it was + + + or + + + + at all ages. Solid bars, negative results. Open bars, positive results.

This quantitative defect in splenic retention became steadily more marked with age and development of disease, falling to a mean of 20% of normal uptake when the mice reached 20 months of age (Fig. 2). Because old NZB mice develop marked splenomegaly this quantitative defect in their splenic retention of aggregated HGG with ageing and disease becomes more evident when the uptake for each 100 mg of splenic tissue is calculated. This falls

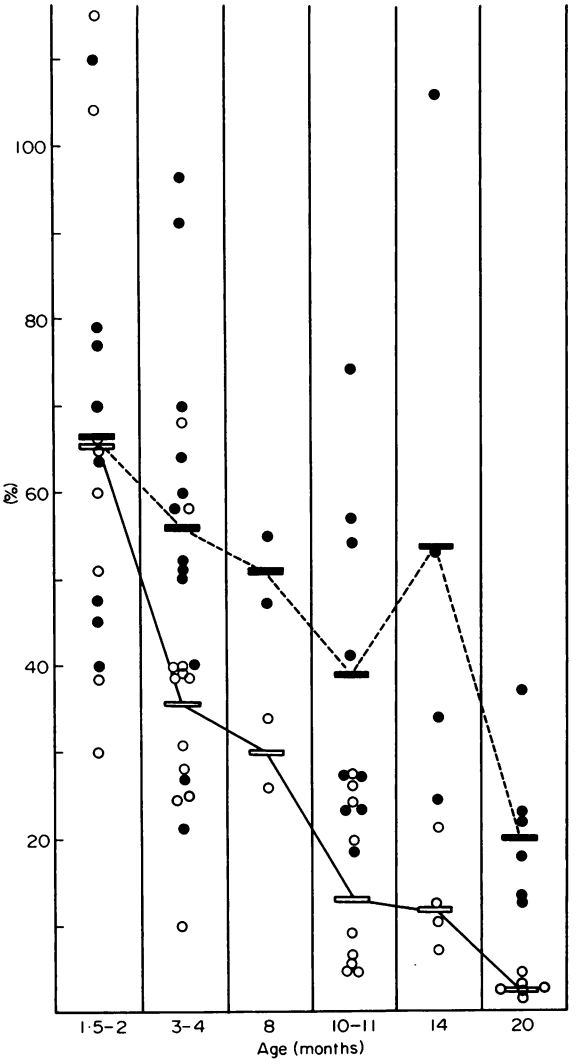

FIG. 2. 24-hr splenic uptake of aggregated HGG in NZB mice of different ages, expressed as percentages of the mean uptake of the same i.v. dose (50 μg Agg HGG) by the spleens of normal mice of the same ages. ●, Individual percentage uptake/whole spleen; solid bars, mean percentage uptake/whole spleen; ○, individual percentage uptake/100 mg of splenic tissue; open bars, mean percentage uptake/100 mg splenic tissue. Note that when uptake in the whole spleen (●) is corrected for spleen weight (○) the defective uptake is more clearly shown, i.e. the uptake curve falls off sharply with age. (—).

from 65·5% of the normal uptake at 1·5–2 months to 3·5% at 20 months (Fig. 2). The fall in uptake was accompanied by a decrease in the brightness of staining of the splenic germinal centres for HGG and mouse γ, and eventually neither was detectable (Fig. 1).

(b) *B/W female mice.* Three B/W females 1·5 months old and three groups of five females,

3–3·5, 5–6 and 7–8 months old respectively were tested and injected as above, and compared with normal control mice in the same way.

As before, the mean splenic uptake in the control mice at each age group was taken as 100% for that age, and the uptake in B/W mice was calculated as a percentage of this normal.

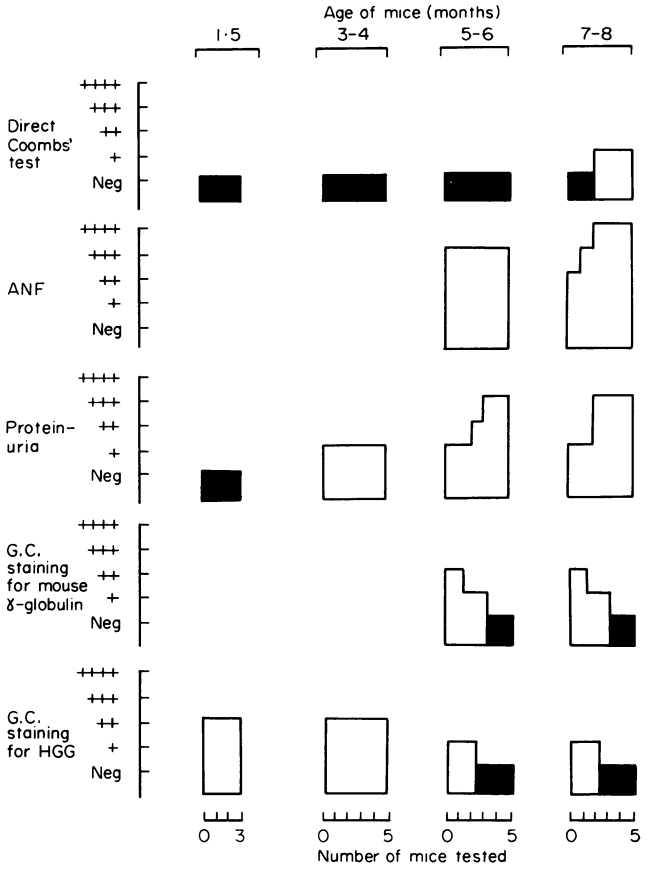

FIG. 3. The relation between direct Coombs' test, ANF, proteinuria and splenic (G.C) germinal centre staining for HGG and mouse γ-globulin in B/W female mice of different ages, injected intravenously with 50 μg Agg HGG labelled with ^{125}I. In control mice (BALB/c, $C_{57}Bl$ or CBA) germinal centre staining for IgG was not more than + or ++ at any age; for HGG it was +++ or ++++ at all ages. Solid bars, negative results; open bars, positive results.

Again we found that at an early age—1·5 month—and before any signs of disease were detected and while germinal centres staining for HGG was still apparently normal (Fig. 3), the ability to transport *Agg HGG to the spleen and to retain it there in germinal centres was diminished to 64·5% of the normals (Fig. 4). As before the defect became more marked with age and disease, falling to 17% of the normal uptake when the mice were

7–8 months old. Because of the absence of gross splenomegaly in the B/W mice the curves for whole spleen uptake and uptake/100 mg of tissue were similar (Fig. 4). This decrease

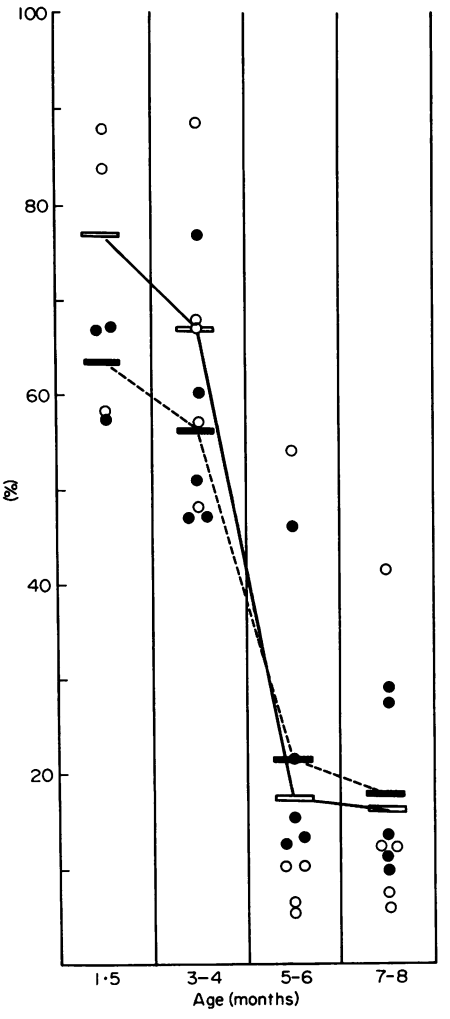

FIG. 4. 24-hr splenic uptake of aggregated HGG in B/W female mice of different ages, expressed as percentage of the mean uptake of the same i.v. dose (50 μg *Agg HGG) by the spleens of normal mice of the same ages. ●, Individual percentage uptake/whole spleen; solid bars, mean percentage uptake/whole spleen; ○, individual percentage uptake/100 mg of splenic tissue; open bars, mean percentage uptake/100 mg splenic tissue. Note that because of the absence of gross splenomegaly in the B/W mice the curves for whole spleen uptake (- - -) and uptake/100 mg tissue (—) are similar.

in splenic retention was accompanied by a decrease in the intensity of immunofluorescent staining for HGG and mouse γ in the splenic germinal centres and by the development of the signs of disease (Fig. 3).

DISCUSSION

In NZB mice histological abnormalities in the thymus at an early age have been demonstrated (DeVries & Hijmans, 1966; Holmes & Burnet, 1966) and in both NZB and B/W mice cell mediated immune responses are depressed. The data presented by various authors point to a deficiency of thymus-dependent cells increasing with age in these animals, e.g. increased survival of skin or tumour allografts (Teague *et al.*, 1970), hypo-responsiveness of spleen cells to mitogenic agents (Leventhal & Talal, 1970; Rodey, Good & Yunis, 1971), inability of the spleen cells of aged as compared with young NZB mice to induce a graft-versus-host reaction (Cantor, Asofsky & Talal, 1970). A depletion of long-lived lymphocytes in old NZB mice was demonstrated by autoradiographic analysis by Denman & Denman (1970) and a similar conclusion were reached by Zatz, Mellors & Lance (1971) who in a study of the migration patterns of injected ^{51}Cr-labelled lymphocytes into lymphoid organs found a decreased recirculating cell content in lymph nodes and spleens from ageing NZB as compared with CBA mice. Using the regression time of virus-induced murine sarcomas as an indication of immunological competence, Gazdar, Beitzel & Talal (1971) confirmed that cell mediated immune responses are depressed in aged NZB and B/W mice, and found moreover that this depression precedes the onset of detectable autoimmune disease. All these findings indicate that NZB and B/W mice become relatively deficient in thymus-dependent lymphocytes.

There is good evidence from animal experiments that thymectomy or depletion of thymus-derived lymphocytes may lead to the development of autoantibodies. Thus neonatally thymectomized rabbits (Sutherland *et al.*, 1965) and mice (Teague, 1967; Thivolet *et al.*, 1967) often develop positive Coombs' test and antinuclear antibodies, and the development of antinuclear antibodies that occurs naturally in ageing A/J mice (Teague & Friou, 1964, 1969) is reversed by transfer of thymus cells from young syngeneic mice. Furthermore, with chronic administration of ALG to C_{57}Bl mice antinuclear antibody appears (Denman, Denman & Holborow, 1966), and the transient induction of a positive Coombs' test in young NZB recipients of old NZB spleen cells is rendered permanent if the young recipient NZB have received ALG treatment (Holborow & Denman, 1968).

It has already been suggested that the bone marrow derived-lymphocyte population also is abnormal in these mice. Zatz *et al.* (1971) reported that after the age of 3 months and coinciding with the onset of autoimmunity, the number of non-recirculating cells in the spleens of NZB mice sharply increases as compared with normal mice, while the recirculating population falls. Staples, Steinberg & Talal (1970) however have produced evidence that by the same age the bone marrow-derived lymphocytes of NZB mice are already less easily made tolerant to BGG than those of normal mice, even in the presence of competent thymus-derived cells. Furthermore, Cerottini *et al.* (1969) have shown that B/W mice are poor responders to keyhole limpet haemocyanin, an antigen thought to be thymus independent (Taylor, 1969).

We have reported previously that the uptake of Agg HGG and immune complexes into germinal centres of lymphoid tissue is probably mediated by lymphocytes that have receptors for IgG altered either by mild heat aggregation or by complexing with antigen (Brown *et al.*, 1970a; 1970b). We also found that mice infected with malaria were unable to localize either Agg HGG or their own immune complexes into their germinal centres during the period of humoral immunosuppression that coincides with the peak of para-

sitaemia (Greenwood *et al.*, 1971a) although their cell mediated immune response remained unaffected (Greenwood, Playfair & Torrigiani, 1971b). This finding suggested to us that the cells responsible for the uptake of Agg HGG or immune complexes in germinal centres are more likely to be cells of the bone marrow-derived than of the thymus-dependent population of lymphocytes.

The results presented here support this idea. Thus, thymus-deprived mice (thymectomized and chronically ALS injected, or thymectomized, lethally X-irradiated and bone marrow reconstituted) are able to localize Agg HGG and immune complexes in their splenic germinal centres at least as well as normal mice. Indeed, the isotope experiments suggest (Tables 1 and 2) that thymectomy may even increase the uptake in germinal centres. Thymectomized lethally X-irradiated and bone marrow reconstituted mice have been shown by Davies (1969) to have only the thymus-independent zones of their lymphoid tissues repopulated, and by chromosome marker studies the repopulating cells were proved to be descendants of the bone marrow donor cells. Our experimental results thus point to the conclusion that the localization of Agg HGG and of autologous immune complexes in germinal centres depends upon uptake by bone marrow-derived lymphocytes.

In the work reported here we have found that in NZB and B/W mice localization is defective from an early age, even before the onset of autoimmunity. Since in both types of mice germinal centres are present (East, 1970) and apparently normal, it must be concluded that there is a fault in the transport mechanism and therefore that a functional abnormality of the bone marrow cell population exists in these animals which antedates the onset of autoimmune disease.

This conclusion is in accordance with the suggestion of Staples *et al.* (1970) that during the process of immunological maturation in the NZB mice not only the thymus-dependent but also the bone marrow lymphocytes are affected, and our results in both NZB and B/W mice indicate that these defects combine to contribute to the ultimate emergence of the autoimmune disease.

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