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SUSCEPTIBILITY OF INBRED RAT STRAINS TO EXPERIMENTAL THYROIDITIS: QUANTITATION OF THYROGLOBULIN-BINDING CELLS AND ASSESSMENT OF T-CELL FUNCTION IN SUSCEPTIBLE AND NON-SUSCEPTIBLE STRAINS

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SUMMARY

Ten inbred strains of rats were immunized with crude homologous thyroglobulin emulsified in Freund's complete adjuvant in order to investigate strain susceptibility to the induction of both thyroiditis and antibody to thyroglobulin. Two strains (LH and AUG) were found to be extremely susceptible and had 100% incidence of thyroid lesions which in general varied from moderate to very severe (mean index of pathology ± SE, 2·5 ± 0·2 and 2·1 ± 0·4 respectively). One other strain (HL) also had 100% incidence of lesions but these were consistently mild in character (1·1 ± 0·1). Two strains (DA and SD) were variable, with thyroid change varying from negative to severe. Three strains (LEW, WAG and PVG/c) had occasional lesions and the remaining two strains (AS and CAM) showed no thyroid change.

Four strains (LH, AUG, HL and DA) consistently produced good antibody responses to thyroglobulin (mean titres ± SE 7·3 ± 0·3, 9·5 ± 0·4, 6·9 ± 0·3 and 6·6 ± 0·5 respectively). In contrast, WAG and CAM rats failed to develop autoantibody and the responses of AS, PVG/c and SD strain rats were quite variable. Although the autoantibody response generally correlated well with the presence of thyroiditis in a particular strain, LEW, AS and PVG/c rats often had good antibody levels with minimal thyroid lesions.

Females of the most susceptible strains (LH and AUG) were found to have significantly more severe thyroid lesions and higher antibody titres to thyroglobulin than males.

The most susceptible strains were all found to be of the *Ag-B*^{5} major histocompatibility genotype whilst the least susceptible were of the *Ag-B*^{2} genotype. However, wide interstrain variability was noted within the *Ag-B*^{5} genotype particularly with respect to the induction and extent of thyroid lesions.

It was not found possible to relate the divergence in susceptibility between rat

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strains of *Ag-B*^{5} and *Ag-B*^{2} genotypes to differences in respective numbers of thyroglobulin-binding cells within the circulation of the non-immunized animal. Similarly, there were no differences in response between a susceptible (LH) and non-susceptible (CAM) strain to the phytomitogens PHA and Con A.

INTRODUCTION

Significant variations in the susceptibility of different strains of laboratory animals to the experimental induction of autoimmune disease have been known for many years (Olitsky & Yager, 1949; Boehme, 1965; Hughes & Stedronska, 1973). The genetic basis for these differences has been clearly established in a number of instances by analysis of cross-breeding studies (Lee *et al.*, 1954; Vladutiu & Rose, 1971; Gasser, Newlin & Gonatas, 1973; Williams & Moore, 1973).

Recently, Vladutiu & Rose (1971) demonstrated that in the mouse susceptibility to experimental thyroiditis is determined by an autosomal dominant gene directly linked to the major histocompatibility locus (*H-2*). This finding parallels extensive studies which have shown that in this species the immune response to a wide range of synthetic polypeptide antigens is controlled by a gene designated *Ir-I* situated within this locus (McDevitt & Benacerraf, 1969). In the rat there is also evidence for a close association between one experimental model of autoimmune disease, experimental allergic encephalomyelitis, and the major histocompatibility system (*Ag-B*) of this species (Williams & Moore, 1973; Gasser *et al.*, 1973).

Strain susceptibility to autoimmunity could be determined on a genetic basis at several possible levels:

(1) By the inheritance of autoreactive B cell precursors of antibody-secreting cells. In this context the presence in the circulation and elsewhere of lymphocytes capable of binding thyroglobulin has been described in several species (Bankhurst, Torrigiani & Allison, 1973; Urbaniak, Penhale & Irvine, 1973; Roberts, Whittingham & Mackay, 1973) and there is evidence that these are immunocompetent B cells (Bankhurst *et al.*, 1973; Claggett & Weigle, 1974).

(2) By the inheritance of potentially autoreactive T cells which could either be directly involved in causing autoimmune damage by the classic cell-mediated mechanism or be required for co-operation with (1). Alternatively, it is possible that a raised threshold to the induction of tolerance in such cells (Playfair, 1971) may be the inherited determinant of autoimmunity rather than specific autoreactivity *per se*.

(3) By the failure to inherit an adequate system of feedback control(s) on (1) and (2) Allison, Denman & Barnes (1971) have postulated that T-cell regulation of autoimmune responses may constitute an important mechanism in the suppression of autoimmunity. A number of studies (East, Sousa & Jaquet, 1967; Teague & Friou, 1969; Penhale *et al.*, 1973; Welch, Rose & Kite, 1973; Nishizuka *et al.*, 1973) provide evidence in support of this concept. T-cell regulation could conceivably operate in both a central and peripheral manner in this situation.

In view of these non-exclusive possibilities it is likely that susceptibility is determined by several genes and that it is quantitative rather than 'all or none' in nature.

In this study the susceptibility of a number of inbred rat strains to the induction of thyroiditis has been related to the presence and numbers of thyroglobulin-binding cells in the

circulation, to the major histocompatibility genotype and to general T-cell function as assessed by the measurement of their response to non-specific phytomitogens.

MATERIALS AND METHODS

Rats.

The following strains of inbred rats were used in the present studies: Lewis (LEW), Campbell (CAM), AS, Dark Agouti (DA), Sprague–Dawley (SD), August (AUG), Hooded Lister (HL), Wistar (WAG), Liverpool Hooded (LH) and PVG/c.

The strains, either breeding pairs or young stock, were obtained as follows: LEW by courtesy of Dr J. F. Mowbray, Experimental Pathology Department, St Mary's Hospital Medical School, London, W2; CAM by courtesy of Professor A. Buxton, Department of Veterinary Pathology, Royal (Dick) School, of Veterinary Studies, Edinburgh; AS by courtesy of Professor J. R. Batchelor, McIndoe Memorial Research Unit, Queen Victoria Hospital, East Grinstead, Sussex; DA by courtesy of Mr D. Drewitt, Department of Clinical Surgery, Medical School, Edinburgh; SD by courtesy of Dr W. L. Ford, Department of Pathology, Medical School, Edinburgh; AUG and HL, Animal Suppliers Ltd, London; WAG and LH, MRC Centre for Laboratory Animals, Carshalton, Surrey; PVG/c OLAC Ltd, Bicester, Oxford.

Only rats between 8 and 10 weeks old were used in these studies and, where possible, equal numbers of males and females.

Injections

Lyophilized rat thyroglobulin was made up to a concentration of 10 mg/ml in sterile physiological saline and emulsified with an equal volume of Freund's complete adjuvant, containing sonicated *Mycobacterium tuberculosis* H37Rv at 6 mg/ml. 0·05–0·1 ml was injected into each footpad (total 0·2–0·4 ml per rat). A suspension of *Bordetella pertussis* of 20×10^{10} organisms/ml was prepared by concentrating 'Wellcome' *pertussis* vaccine five-fold. Rats were injected with 0·05 ml intradermally into the dorsum of each foot at the same time as the thyroglobulin injection was given. At 17 days following immunization all rats were bled by cardiac puncture and killed.

Histology

Rat larynxes with thyroids *in situ* were fixed in 10% formal saline and stained with haematoxylin and eosin. All slides were examined without knowledge of their origin. The severity of thyroiditis was scored as follows.

1 = occasional foci of thyroiditis.
2 = multiple discrete foci.
3 = diffuse thyroiditis with some loss of follicular structure.
4 = diffuse thyroiditis with complete obliteration of follicular structure.

Preparation of rat thyroglobulin (rat Tg)

Thyroid glands, largely obtained from random-bred Wistar rats, were collected and stored at −20°C until preparation of the extract. The tissue was gently homogenized at 4°C in sterile physiological saline (6·0 ml saline/1·0 g tissue) for 5 min, and the homogenate allowed

to stand overnight at 4°C. The preparation was then centrifuged at 60,000 *g* for 1 hr at 4°C, and the supernatant mixed with saturated ammonium sulphate (pH 7·0) to give a final saturation of the mixture of 40%. The resulting precipitate was spun down at 2000 *g* for 15 min and the thyroglobulin pellet dissolved in three times its volume of distilled water and dialysed overnight against distilled water and lyophilized.

For the antigen-binding studies, 10 mg of crude thyroglobulin was further purified by gel-filtration on Sephadex G-200 with Tris–HCl buffer (pH 8·0). The thyroglobulin fraction was dialysed against water and lyophilized.

Iodination procedure

The chloramine-T method as modified by Hunter (1967) was used with minor modifications as previously reported (Urbaniak *et al.*, 1973). A thousand microcuries of carrier-free ^{125}I (Amersham, IMS 30) were reacted with 5 μg rat Tg and 10 μl chloramine-T (5 mg/ml) at room temperature for 60 sec at pH 7·5. The reaction was terminated by the addition of 0·75 ml sodium metabisulphite (160 μg/ml) followed by 0·2 ml carrier KI (10 mg/ml). The labelled protein was then separated from unreacted ^{125}I by Sephadex G-200 column fractionation. The specific activity of the ^{125}I rat Tg varied from 75 to 91 μCi/ug. The labelled thyroglobulin was used within 24 hr of preparation.

Labelling of lymphocytes with ^{125}I-labelled thyroglobulin

The conditions for antigen binding were as described by Byrt & Ada (1969) and Bankhurst *et al.* (1973). 5×10^6 lymphocytes were incubated with 100–125 ng ^{125}I rat Tg for 30 min at 0–4°C over ice in a final volume of 0·5 ml Eagle's Basal medium (EBM) containing 0·01% sodium azide. The cells were then washed three times with ice-cold EBM, resuspended in 0·1 ml foetal bovine serum and smeared onto gelatin-coated slides.

Autoradiography

The method of autoradiography was as previously described (Urbaniak *et al.*, 1973). The lymphocyte smears were air dried, fixed in methanol:water (90:10 v/v), washed in distilled water and subsequently dipped into Ilford K2 nuclear emulsion. Slides were then dried in the darkroom and stored in plastic light-proof boxes and exposed at 4°C for 19–20 days. Slides were developed with Kodak D19 developer, fixed in 30% sodium thiosulphate, washed and subsequently stained with Leishman's stain.

Antigen-binding lymphocytes

Slides were examined under oil immersion. 10^3 lymphocytes were counted per slide and only single intact cells without obvious damage were counted (Ada, 1970; Bankhurst *et al.*, 1973). Background was never more than three grains per cell and only lymphocytes with five or more grains per cell were considered positive. Slides were coded and examined at random without prior knowledge of their origin.

Lymphocyte transformation

Leucocytes were obtained from peripheral blood using a modification of the method pescribed by Wilson (1967). Four millilitres of blood was obtained by cardiac puncture and withdrawn into a 5 ml disposable syringe containing 0·5 ml tri-sodium citrate (final concentration 1% w/v), and 0·5 ml 10% dextran (mol. wt. 250,000; final concentration 1% w/v). The blood/dextran was mixed thoroughly and then allowed to sediment in the syringe for

1 hr at room temperature. The leucocyte-rich layer was removed and made up to 10 ml with Hanks's balanced salt solution (HBSS) pH 7·2, buffered with 20 mM Hepes buffer and containing 10 i.u./ml preservative-free heparin (Evans Medical) and spun at 1500 *g* for 10 min. The cell pellet was resuspended in a further 10 ml heparinized HBSS and spun at 200 *g* for 10 min. The cells were finally resuspended in 5·0 ml culture medium (RPMI 1640 supplemented with 20% horse serum, buffered with 20 mM Hepes buffer and containing 200 u/ml penicillin, 100 μg/ml streptomycin; all obtained from Biocult Labs Ltd, Scotland). Differential counts were carried out and the concentration of cells adjusted to give 1×10^6 lymphocytes/ml.

Cells from each strain of rat were tested at the same time with both phytohaemagglutinin (PHA-P, Difco Labs, Surrey) and concanavalin A (Con A, Grade IV, Sigma Chemical Co., London). PHA-P was reconstituted as recommended by the manufacturers and then diluted with culture medium to give a range of doubling concentrations from 12·5 to 100·0 μl/ml of reconstituted material. Twenty microlitres of each dilution were then dispensed in triplicate into the wells of a microtitre tray (Linbro IS-FB-96-TC), followed by 200 μl of cell suspension (2×10^5 lymphocytes). This gave final PHA concentrations of 1·25–10·0 μl/ml. Triplicate unstimulated cultures were also set up. The cultures were incubated at 37°C in a humidified atmosphere for 72 hr. Tritiated thymidine of specific activity 2·0 Ci/mmole and total thymidine concentration 1×10^{-3} mM (prepared by diluting [^{3}H]thymidine of specific activity 5·0 Ci/mmole, from Radiochemical Centre, Amersham, in cold thymidine) was added in 20-μl volumes for the final 16 hr of culture. Con A was diluted in medium to give doubling concentrations of 25–200 μg/ml, and the cultures set up exactly as described for PHA. The cultures were terminated and extracted by the communal extraction and rinse procedure previously described (Penhale *et al.*, 1974). Briefly, 200-μl aliquots from each well were dispensed on to glass fibre filters and air dried. The filters were then bulk treated successively in 5% trichloracetic acid, phosphate-buffered saline, pH 7·0, and absolute methanol. The filters were dried at 60°C, placed in scintillation vials containing 5·0 ml of scintillator (NE 233, Nuclear Enterprises, Edinburgh) and counted for 1 min in a Packard Tricarb 2425 scintillation counter.

Tanned cell haemagglutination procedure for the detection of antibody to thyroglobulin

A micro-haemagglutination test using coated tanned sheep red cells (SRBC) was used. Heparinized erythrocytes were tanned and coated, as described by Herbert (1967), with 100 μg/ml rat Tg. Sera were diluted 1/5 in physiological saline and heat-activated at 56°C for 1 hr. A double absorption was carried out by adding two 0·1-ml volumes of packed SRBC at intervals of 30 min to 0·2 ml of diluted serum and incubating at 37°C for a total of 1 hr. After centrifugation to remove SRBC the absorbed sera were subsequently dispensed in 25-μl volumes in microtrays by the Takatsi procedure, equal volumes of sensitized SRBC were added and the test incubated at room temperature for 2 hr before reading.

RESULTS

Strain susceptibility to the induction of thyroiditis

The extent of thyroid lesions induced in individual rats by immunization with crude homologous thyroglobulin and Freund's complete adjuvant is shown in Fig. 1. Marked strain differences were observed. For example, no lesions were found in any individuals of the AS or CAM strains, whereas thyroid change was regularly seen in both LH and AUG

strains with the most severe lesions consistently occurring in LH rats. Wide variations in the response to immunization were noted in both DA and SD rats suggesting that considerable heterogeneity exists within the two strains, at least with respect to this particular characteristic. When strains were grouped according to their major histocompatibility type it was apparent that the poorest responders were found in the *Ag-B*^{1} and *Ag-B*^{2} groups and the best within the *Ag-B*^{5} group. However, considerable interstrain variation was seen within the *Ag-B*^{5} group and whilst LH and AUG rats had severe lesions, HL rats consistently produced mild lesions and PVG/c rats were generally found to have normal thyroid histology.

Strain variation in autoantibody response

Strain dependency of autoantibody production was also noted (Fig. 2). Antibody was not detected in any individuals of the WAG strain and at low titre in only one animal of the

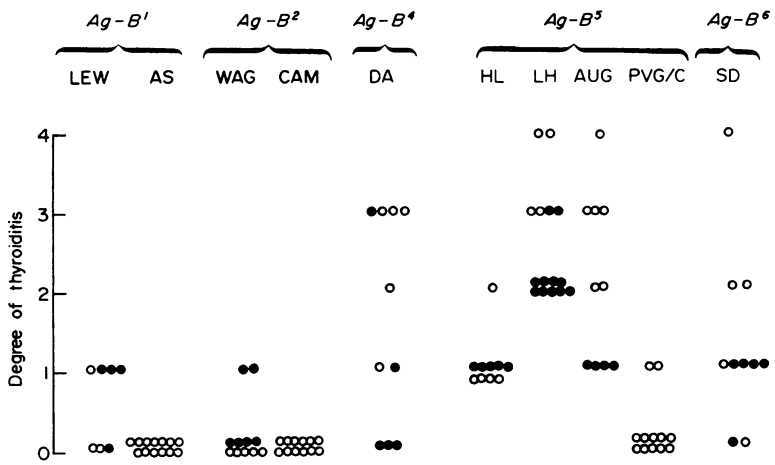

FIG. 1. Degree of thyroiditis in inbred rat strains. Open circles, females; closed circles, males. Gradation of thyroid lesions as described under Materials and Methods section.

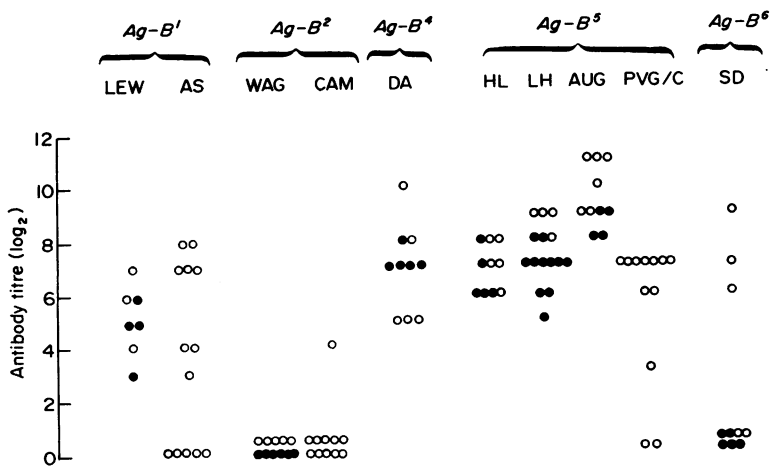

FIG. 2. Antibody titre to thyroglobulin in inbred rat strains. Open circles, females; closed circles, males.

CAM strain. Substantial titres of antibody were found in all other strains although in some cases (AS, SD and PVG/c strains) within-strain responses were quite variable. The highest titres were observed in the AUG strain and consistently high titres were also observed in the HL and LH rats. When strains were grouped according to *Ag-B* type there was again an indication of inter-group differences. For example, the lowest responses were observed within the $Ag\text{-}B^2$ genotype and the highest within the $Ag\text{-}B^5$. In general, autoantibody response paralleled the severity of thyroiditis in the same animal and overall there was a good correlation between these parameters. However, some exceptions were observed where although substantial titres of antibody were present only low-grade thyroiditis was found. This was seen particularly in the two strains examined belonging to the $Ag\text{-}B^1$ genotype and also PVG/c strain rats.

Overall responses both in respect of thyroiditis and autoantibody are summarized in Table 1. The $Ag\text{-}B^5$ genotype as a whole was found to differ significantly both in incidence and extent of thyroid injury and antibody titre from both $Ag\text{-}B^1$ and $Ag\text{-}B^2$ strains. Similarly $Ag\text{-}B^1$ rats differed significantly from $Ag\text{-}B^2$ strain rats in all respects except in severity of the thyroid lesions (Table 2).

TABLE 1. Thyroid pathology and autoantibody response in the different rat strains following immunization with homologous thyroglobulin in Freund's complete adjuvant

Genotype	Strain	Number	Thyroid pathology		Autoantibody	
			% Incidence	Mean severity	% Incidence	Mean log titre ± SEM
$Ag\text{-}B^1$	LEW	7	57	0·6±0·2	100	5·1±0·5
	AS	13	0	0	63	3·7±0·9
$Ag\text{-}B^2$	WAG	11	18	0·2±0·1	0	0
	CAM	12	0	0	9	0·3±0·3
$Ag\text{-}B^4$	DA	10	70	1·6±0·4	100	6·6±0·5
$Ag\text{-}B^5$	HL	10	100	1·1±0·1	100	6·9±0·3
	LH	15	100	2·5±0·2	100	7·3±0·3
	AUG	10	100	2·4±0·4	100	9·5±0·4
	PVG/c	12	17	0·2±0·1	84	5·3±0·8
$Ag\text{-}B^6$	SD	10	80	1·3±0·4	30	2·2±1·1

Relationship between sex and autoimmune response

The severity of thyroiditis and titre of autoantibody were compared in males and females in strains where there were adequate numbers of each sex and where good responses were observed overall. The results are summarized in Table 3. This shows that with one exception (antibody levels in male and female DA strain rats) there was a divergence between males and females both in mean pathology and autoantibody titre. In all cases means were greater in females than males and in LH and AUG strains these differences were highly significant. These findings therefore indicate that females are generally more liable to develop severe lesions and high autoantibody titres than males following immunization with homologous thyroglobulin.

TABLE 2. Statistical analysis of mean differences in thyroid pathology and autoantibody in the different *Ag-B* genotypes

(i) Differences in incidence (χ^2 analysis)

	Pathology	Autoantibody
Ag-B^5 vs *Ag-B*^{1}	$P<0{\cdot}001$	$P<0{\cdot}01$
vs *Ag-B*^{2}	$P<0{\cdot}001$	$P<0{\cdot}001$
Ag-B^1 vs *Ag-B*^{2}	$P<0{\cdot}05$	$P<0{\cdot}001$

(ii) Differences in severity of thyroiditis and mean titre of autoantibodies (Student's *t*-test)

	Pathology	Autoantibody
Ag-B^5 vs *Ag-B*^{1}	$P<0{\cdot}001$	$P<0{\cdot}001$
vs *Ag-B*^{2}	$P<0{\cdot}001$	$P<0{\cdot}001$
Ag-B^1 vs *Ag-B*^{2}	N.S.	$P<0{\cdot}001$

TABLE 3. Relationship between sex and autoimmune response

			Pathology		Autoantibody	
Strain	Sex	Number	Mean ± SEM	*P* value	Mean ± SEM	*P* value
AUG	♂	4	1·0 ± 0	<0·01	8·5 ± 0·3	<0·05
	♀	6	2·8 ± 0·3		10·4 ± 0·4	
LH	♂	11	2·2 ± 0·1	<0·001	6·8 ± 0·3	<0·01
	♀	4	3·5 ± 0·3		8·8 ± 0·3	
DA	♂	5	1·4 ± 0·6	N.S.	6·6 ± 0·2	N.S.
	♀	5	2·4 ± 0·4		6·6 ± 1·0	
SD	♂	5	0·8 ± 0·2	N.S.	0	N.S.
	♀	5	1·8 ± 0·7		4·4 ± 1·9	
All strains	♂	25	1·4 ± 0·9	<0·001	5·7 ± 3·0	>0·05
	♀	20	2·6 ± 1·1		7·6 ± 3·2	

Quantitation of thyroglobulin-binding cells in the peripheral blood of non-immunized rats of susceptible and non-susceptible strains

It is possible that the difference between susceptible and non-susceptible strains is due to quantitative or qualitive differences in lymphocytes capable of ultimately producing thyroglobulin antibodies following stimulation. Since antigen-binding lymphocytes are generally considered to be potentially immunocompetent B cells the presence and number of thyroglobulin-binding cells were accordingly investigated in non-immunized susceptible (*Ag-B*^{5}) and non-susceptible (*Ag-B*^{2}) strains. Table 4 shows that such cells were found in all rats examined, irrespective of their susceptibility. Furthermore, no significant differences in relative numbers of labelled cells or in the numbers of grains per lymphocyte were noted between any of these strains, nor between the two genotypes as a whole.

TABLE 4. Quantitation of thyroglobulin-binding lymphocytes in the peripheral blood of *Ag-B²* (non susceptible) compared with *Ag-B⁵* (susceptible) strain rats

Genotype	Strain	Number of rats	Thyroglobulin-binding cells per 10^3 lymphs (mean ± SEM)
Ag-B²	WAG	5	4·6 ± 1·2
	CAM	5	1·8 ± 0·2
Ag-B⁵	LH	5	4·2 ± 0·7
	HL	5	4·0 ± 0·6
	PVG/c	5	3·0 ± 1·0
	AUG	4	2·2 ± 0·4

Student's *t*-test: *Ag-B²* vs *Ag-B⁵*, not significant.

T-cell function in non-immunized rats of a susceptible and non-susceptible strain as assessed by their response to phytomitogens

Strain divergence in susceptibility may be attributable to qualitative or quantitative differences in certain aspects of T-cell function, such as their ability to co-operate in the formation of autoantibodies to thyroglobulin to invoke strong cell-mediated immune

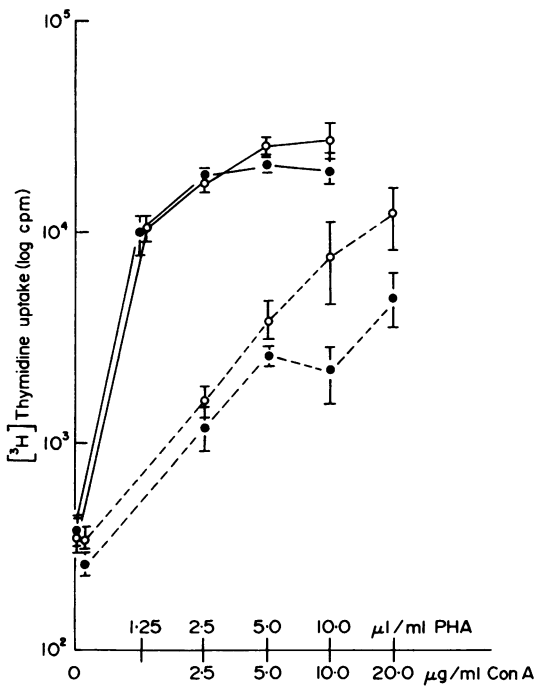

FIG. 3. Response of peripheral blood leucocytes to PHA and Con A in susceptible and non-susceptible strains of rats. Open circles, CAM rats; closed circles, LH rats. Solid line, PHA responses; interrupted line, Con A responses. Each point represents mean response ± SE of eight rats.

reactivity against thyroglobulin determinants or to act in a regulatory role thereby inhibiting autoantibody induction. It is possible that differences in some of these aspects might be reflected in the capacity of their T cells to undergo cell division following stimulation. This capacity may be approximately assessed in a non-specific manner by stimulation with appropriate T-cell mitogens such as PHA and Con A. In this investigation the degree of stimulation by these mitogens was used as an index of T-cell function in a susceptible (LH) and a non-susceptible (CAM) strain. Fig. 3 shows that the dose–response curves to these mitogens followed similar patterns in both strains. The lower mean incorporations observed with Con A at higher dose levels in CAM rats were not significantly different from those of the LH strain.

DISCUSSION

This study has shown that a wide divergence in response to homologous thyroglobulin is found in different inbred rat strains both in the extent of lesions induced within the thyroid gland and also in the level of autoantibody to thyroglobulin. This strain variation is in conformity with similar experience with actively induced thyroiditis in the mouse (Vladutiu & Rose, 1971) and with other experimentally induced autoimmune disease in the rat (Hughes & Stedronska, 1973). Such differences may be either genetically determined or may be the result of non-genetic influences. These possibilities are not mutually exclusive and it is likely that both effects may have contributed to the present observations. The involvement of genetic influences is suggested by the association found here between *Ag-B* type and susceptibility, notably in the divergence between $Ag\text{-}B^2$ and $Ag\text{-}B^5$ genotypes. This association suggests a close link between susceptibility to thyroiditis, the magnitude of the thyroglobulin autoantibody response and the major histocompatibility allele. Such an association can only be confirmed and more precisely defined by cross-breeding studies between susceptible and non-susceptible strains and also by the investigation of susceptibility in congenic strains.

However, it is difficult to explain the wide interstrain variability seen within the $Ag\text{-}B^5$ group, particularly the poor response of PVG/c strain rats on this basis. Since typing with *Ag-B* specific sera was not carried out to confirm the histocompatibility type of each strain it is possible that some rats did not fall within the genotypes expected although in view of the well-documented backgrounds of all the strains used this would appear unlikely. Other possibilities may also exist for the poor responsiveness of the PVG/c strain, such as the occurrence of mutation or recombination resulting in the association of a new low response gene with the $Ag\text{-}B^5$ allele in this particular strain, or alternatively, PVG/c strain rats may possess an effective genetically determined, but *Ag-B*-independent, regulatory mechanism which suppresses the usual level of response to thyroglobulin seen within the group as a whole. It is also possible that non-genetic influences of an environmental or other nature may have been involved, since although every attempt was made to keep conditions constant for all strains, it was not possible to immunize all the rats simultaneously. Such environmental factors may be of importance if the response gene involved is incompletely penetrant and this phenomenon may also account for the variability observed within the DA and SD strains.

In general, there was a good correlation between the thyroglobulin antibody titre in particular strains and the severity of thyroiditis. Vladutiu & Rose (1971) found a similar relationship in experimental thyroiditis in mice. These observations are in accord with recent

studies which have tended to reinstate the humoral factor in the pathogenesis of organ-specific autoimmune disease (Wick *et al.*, 1970; Clinton & Weigle, 1972; Penhale *et al.*, 1973; Nishizuka *et al.*, 1973). In this context antibody is likely to be involved either directly, or combined in the form of complex with the autoantigenic determinants of thyroglobulin in arming lymphoid cells and thereby endowing them with directional cytotoxicity for thyroid cells (Calder *et al.*, 1973a, b). Although in the majority of animals thyroid lesions were accompanied by substantial antibody titres, in certain cases, particularly rats of the LEW and AS strains, moderate titres of antibody were seen without concomitant thyroid damage. Recently Rose *et al.* (1973) have similarly shown that BRVR and BSVS strain mice respond closely with respect to thyroid antibody titres but differ sharply in their cellular response to thyroid antigen. Rose *et al.* (1973) have suggested as one possible explanation of this lack of correlation between autoantibody and thyroid pathology that a non-histocompatibility associated gene in the non-susceptible BRVR strain may regulate the autoimmune response at the level of thyroid tissue damage. A peripheral control mechanism of this nature operating locally within the thyroid gland could also account for the present findings in the AS and LEW rats. Furthermore, it is possible that such a mechanism is operating within all strains but at different, and possibly genetically determined, levels of efficiency. It may be envisaged that a certain threshold level of autoantibody would be required to overwhelm the regulatory mechanism and initiate thyroid damage. The expression of overt thyroid autoimmunity within a particular strain would then be dependent on the loss of equilibrium between autoantibody production and local suppressor activity.

In addition to strain dependency of autoimmunity, a clear-cut difference in susceptibility between the sexes was also noted. This is in agreement with other studies on experimentally induced thyroiditis and with the long-established observation that there is a higher incidence of clinical autoimmune thyroid disease in females than in males. This is therefore evidence for a further genetic influence on susceptibility although whether this is the consequence of a direct sex-linked genetic determinant of autoimmune responsiveness or an indirect effect of endocrine function remains to be elucidated.

The presence of lymphocytes capable of specifically binding thyroglobulin has now been described in a number of species in the normal, non-autoimmune state (Bankhurst *et al.*, 1973; Urbaniak *et al.*, 1973; Roberts *et al.*, 1973; Claggett & Weigle, 1974). Such an observation must clearly have great relevance to the pathogenesis of autoimmune disease. However, it was not found possible in this investigation to relate either the degree of susceptibility or the humoral response to thyroglobulin in the various strains to the number of these cells within the circulation although circulating numbers may not reflect either similar distributions within other sites or total numbers on a strain to strain basis. A similar finding in relation to the response to the synthetic polypeptide (T, G)-A--L has recently been observed. Hammerling & McDevitt (1973) have found that the same frequency of antigen-binding cells was seen in both high and low non-immunized responders. Since there is now evidence from a number of sources (Bankhurst *et al.*, 1973; Claggett & Weigle, 1974) that thyroglobulin-binding cells are immunocompetent B cells, it would appear that the degree of immune responsiveness to thyroglobulin is not determined by specifically reactive B cells but possibly by some aspect of T cell function.

No evidence was obtained from the T-cell mitogen studies to suggest that differences existed between susceptible and non-susceptible strains in terms of general function as assessed by their capacity for cell division following non-specific stimulation. It would

appear therefore that an explanation for genetic differences in susceptibility to the development of thyroiditis should be sought at a more subtle level such as the specifically autoreactive thymus-derived lymphocyte. This possibility would fit with basic data which has shown that histocompatibility associated immune response genes appear to be expressed through thymus-derived lymphocytes (Green, Paul & Benacerraf, 1972).

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