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Sites of autoantibody production in rats with thyroiditis

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Summary. We have developed a thyroglobulin-specific haemolytic plaque assay and investigated potential sites of autoantibody synthesis in good and poor responder strains of rats immunized with thyroglobulin and in rats subjected to thymectomy and sub-lethal irradiation which subsequently develop thyroiditis spontaneously. The bone marrow appears to be the most important site of thyroglobulin antibody synthesis in all groups, but spleen and cervical lymph nodes are also involved. No thyroglobulin plaque-forming cells could be found in the thyroid. These results imply widespread involvement of the humoral immune system in organ-specific autoimmune processes.

INTRODUCTION

Many observations have been made on the pathogenesis of experimental autoimmune thyroiditis (EAT; Rose, Kong, Okayasu, Giraldo, Biesel & Sundick, 1981) and the importance of antibody-mediated damage has been established (Noble, Yoshida, Rose & Bigazzi, 1976; Jaroszewski, Sundick & Rose, 1978). The site of autoantibody production in these animal models is unknown though we have shown that in human thyroiditis the thyroid is an important site of thyroglobulin antibody synthesis (McLachlan, McGregor, Rees Smith & Hall, 1979; Weetman, McGregor & Lazarus, 1981). The potential therapeutic implication of this observation in man is limited by the inability to study other organs such as spleen. Therefore we have established animal models of EAT to study the sites of autoantibody production to determine whether, as in man, the thyroid is a major site of production, or whether other areas are involved.

We have developed a haemolytic plaque assay to detect cells synthesising thyroglobulin antibody and used this to study three groups of rats with EAT. Thyroiditis was induced by immunization with thyroglobulin in Freund's complete adjuvant in good and poor responder strains (Penhale, Farmer, Urbaniak & Irvine, 1975b) and by thymectomy and irradiation (TX + X) in strain-susceptible rats which subsequently develop spontaneous thyroiditis (Penhale, Farmer & Irvine, 1975a).

In contrast to the human disease, thyroglobulin antibodies did not appear to be synthesized by sufficient numbers of thyroid-derived lymphocytes to be detected by this assay. The predominant site of production in all three groups was bone marrow.

MATERIALS AND METHODS

Animals

August (AUG) and Wistar (WAG) strain rats were obtained from Olac (1976) Bicester, and the Animal

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Unit, Welsh National School of Medicine, respectively. Female animals only were used.

Thyroglobulin preparation

Thyroglobulin was prepared from WAG rat thyroids homogenized in saline on ice. The suspension was centrifuged at 53,000 ***g*** for 70 min, and the supernatant clarified by prefiltration. The thyroglobulin was purified by passage over a Sepharose 6B-CL column and concentrated by ultrafiltration (Amicon UM-10 membrane).

Immunization procedure

An emulsion of 1·3 mg thyroglobulin in an equal volume of Freund's complete adjuvant (Difco, Detroit, U.S.A.) total volume 400 μl was injected subcutaneously into both hind legs of 6 week old AUG or WAG rats and repeated 7 days later. The animals were killed by exsanguination under ether anaesthesia 35 days following the last immunization. This was chosen from preliminary studies as the time of maximum antibody titre and thyroiditis in AUG rats.

Thymectomy and irradiation

Three week old AUG rats were subjected to thymectomy by the technique of Penhale, Farmer, McKenna & Irvine, (1973). Briefly a small incision was made over the sternum under ether anaesthesia and the sternum split to the fourth rib. The fascia covering the thymus was incised and the thymic lobes removed by suction. Absence of thymic remnants was checked visually and the skin closed with stainless steel clips. Two weeks following thymectomy 200 rad whole body irradiation at 50 rad/min was given from a sealed caesium source. This was repeated three times at 2 week intervals to give a total dose of 800 rad. These animals were killed when 18 weeks old.

Serology

The serum thyroglobulin antibody titre at the time of death was assessed by an enzyme-linked immunosorbent assay (ELISA). Briefly rat thyroglobulin (10 μg ml^{-1}) was coated on to micro ELISA plates (MI29A, Dynatech, Billinghurst) with carbonate buffer overnight at 4°. Rat serum diluted 1:100 in phosphate-buffered saline-0·05% Tween 20 (PBS-T) was added for 2 hr at room temperature and then overlaid with anti-rat IgG (Cappel, Cochranville, U.S.A) conjugated to alkaline phosphatase (Sigma, St. Louis, U.S.A) for a further 2 hr. The plates were washed three times with PBS-T between each stage. The optical density following the addition of p-nitrophenyl phosphate substrate was read using a Titertek Multiskanner. The results were expressed as units read from the standard curve produced by a pooled known positive serum, where 1 unit = 1:1000 dilution of standard.

Preparation of lymphoid cell suspensions

The two posterior cervical lymph nodes, the mesenteric lymph nodes and spleen were removed and trimmed of fat. The thyroid was removed with the larynx and the two lobes subsequently dissected free. Bone marrow was obtained from the right femur by washing the marrow cavity out with 5 ml RPMI 1640 medium (Flow, Irvine). Suspensions of cells were prepared by gently and repeatedly squeezing each tissue between blunt forceps in 10 ml RPMI 1640 supplemented with gentamicin 40 mg/litre. A single cell suspension was obtained from the marrow by aspirating the fragments five times through a 19 gauge needle. Each suspension was allowed to stand for 5 min and the supernatant then gently decanted. These cells were washed three times at 200 ***g*** for 5 min at 4°. Cell counts and viability were assessed using a haemocytometer and trypan blue exclusion: the suspensions were always more than 95% viable. The suspensions were maintained at 4° from the time of preparation to assay, which was always less than 60 min. Cell concentrations between $1–10 \times 10^6$ ml^{-1} were used for the plaque assays.

Plaque assay

The protein A plaque-forming cell (PFC) assay of Gronowicz, Continho & Melchers (1976) was adapted for use in the rat to detect cells synthesizing IgG and a thyroglobulin-specific PFC assay similar to that employed in human studies (McLachlan, Bird, Weetman, Rees Smith & Hall, 1981) was devised. Sheep red blood cells (SRBC) in Alsever's solution (Tissue Culture Services, Slough) were washed five times with normal saline. One part of protein A (Pharmacia, Uppsala, Sweden) 0·5 mg/ml^{-1} or rat thyroglobulin 1 mg/ml^{-1} was added to ten parts fresh chromic chloride solution ($2·5 \times 10^{-4}$ M in normal saline, pH 4·6) and the mixture added dropwise to one part SRBC with constant gentle mixing. The cells were incubated at 30° for 30 min, washed three times in Hanks's balanced salt solution (HBSS: Flow, Irvine) and resuspended in five parts HBSS. Tubes containing 800 μl 0·5% agar (Difco, Detroit, U.S.A) in HBSS supplemented with 0·45 mg/ml^{-1} DEAE-dextran (Pharmacia, Uppsala, Sweden) were kept at 46° and to

each tube was added in turn 25 μl developing antiserum (rabbit anti-rat IgG whole molecule diluted 1:10 in HBSS; Miles Yeda, Rehovet, Israel) 50 μl protein A or thyroglobulin-coated SRBC, 100 μl of the cell suspension to be assayed and 25 μl SRBC-absorbed guinea-pig complement (Flow, Irvine; diluted 1:4 in HBSS). The tube was vortexed and three 100 μl drops were placed on a 90 × 15 mm petri dish and covered with 22 × 22 mm coverslips. This formed a thin agar film with a monolayer of SRBC. The dishes were incubated for 4 hr at 37° in a humid atmosphere. Plaques were enumerated under indirect light and the results expressed as the mean (of triplicate) PFC/10^6 live cells added. The presence of a central lymphocyte was verified in random plaques microscopically in all experiments.

Control experiments

Several experiments were performed to validate the two plaque assays. Complement or antiserum was omitted and plaques against uncoated SRBC were sought. Cells were incubated with cycloheximide 100 $\mu g/ml^{-1}$ (Sigma, St. Louis, U.S.A) for 45 min at 37°, washed three times and then tested in the assays to determine any contribution by adherent or secreted antibody. To assess the specificity of the thyroglobulin PFC assay, cells were tested against bovine serum albumin (BSA)-coated SRBC (prepared in an identical fashion) and also inhibition of the PFC response was sought using free thyroglobulin 25 $\mu g/ml^{-1}$ added to the cells. To assess the protein A assay further, three female AUG rats were given 10^9 SRBC intraperitoneally, killed after 5 days and the spleens removed. The protein A PFC response for IgG and IgM (the IgM PFC assay used rabbit anti-rat IgM whole molecule 1:10 in HBSS, Miles-Yeda, Rehovet, Israel, as developing antiserum) was compared with SRBC-specific PFC response. This was performed by mixing cells with uncoated SRBC and complement in identical amounts to identify IgM plaques and anti-IgG was added to identify the PFC response. Although the peak obtained from Sepharose separation of the thyroid extract was taken to give maximum purity of thyroglobulin, the possiblity existed of contamination by other molecules such as IgG. To exclude contamination by raised anti-IgG antibodies in our immunized animals giving plaques (and also reacting in the ELISA) sera were tested for anti-rat IgG antibodies by haemagglutination using rat IgG-coated SRBC. This assay was known to give positive reaction (titre > 1:10240) in rabbits immunized with rat IgG.

Culture conditions

Various attempts were made to culture lymphocytes and then examine the PFC response. Other groups have observed the difficulties encountered in the culture of rat lymphocytes but their techniques for overcoming these using indomethacin, carbonyl iron or nylon wool extraction proved unhelpful (Corvalan & Howard 1978; Mattingly & Kemp 1979). However, we have already observed that by using small doses of thyroglobulin a marked increase in IgG and thyroglobulin antibody synthesis can be demonstrated by lymphocytes from animals with EAT. We therefore cultured lymphocytes for 4 days in 12 × 75 mm plastic tubes (Falcon plastics) containing 1 ml of culture medium comprised of RPMI 1640 supplemented with 10% foetal bovine serum (Flow, Irvine) and gentamicin 40 mg/l^{-1} with or without thyroglobulin 10^{-2} $\mu g/ml^{-1}$, this giving the optimal response in our system. The cells were incubated in duplicate at 37° in 5% CO_2 in air and pooled before assay, performed after three washes in RPMI 1640.

RESULTS

PFC assay control experiments

PFC were not detected by either assay when complement or antiserum were omitted or when uncoated SRBC were used. In five experiments using cycloheximide, the PFC response in both assays was inhibited by 75% or more, indicating that the PFC response depended on *de novo* synthesis of antibody. PFC were never formed against BSA-coated SRBC (four experiments). In the presence of free thyroglobulin, the thyroglobulin PFC response was always abolished ($P < 0.001$), but there was no effect ($P > 0.05$) on total IgG PFC (thirty experiments, Wilcoxon-Rank Test).

Table 1. Comparison of the number of splenic PFC/10^6 cells detected by uncoated SRBC and protein A-coated SRBC in three rats immunized with SRBC

	IgM PFC/10^6		IgG PFC/10^6	
Animal no.	SRBC	Protein A	SRBC	Protein A
1	6200	6250	3750	6560
2	3550	3200	4050	7300
3	1000	2400	400	1750

Table 2. Thyroglobulin antibody titres and splenic weights (mean ± SD) in the groups studied

Strain	Treatment	Thyroglobulin antibody titre (u.)	Spleen Weight (g)	Number of animals
AUG	Nil	80 ± 179*	0·44 ± 0·03	5
AUG	Injected Tg/CFA	20,333 ± 1751	0·63 ± 0·15	6
AUG	Thymectomy + irradiation	36,000 ± 13740	1·86 ± 0·07	6
WAG	Nil	0	0·57 ± 0·14	6
WAG	Injected Tg/CFA	5800 ± 5763	0·58 ± 0·04	6

* A single animal positive, titre 400 u.; the remainder negative.

The results of the protein A assay in animals immunized with SRBC is shown in Table 1. Cells from the immunized animals did not produce plaques against protein-A-coated SRBC in the presence of complement alone, that is, the process of cell coating prevented the lysis which occurs in a conventional anti-SRBC assay. It can be seen that the protein A assay detected as many cells synthesizing IgM of all specificities as there were of anti-SRBC specificity. However roughly twice as many PFC were found for IgG by protein A than by the indirect anti-SRBC assay, consistent with our findings of spontaneous IgG PFC, presumably of wide specificities, in the spleens of normal animals (see below). These results suggest that the protein A PFC assay as adapted for use in the rat is a useful means of detecting cells secreting immunoglobulin, although it is unlikely to detect all the cells secreting a given class. We subsequently sought only IgG PFC since in these preliminary experiments we were never able to detect anti-thyroglobulin PFC of IgM class, despite strong IgG class thyroglobulin PFC responses. No anti-IgG antibodies were detected by haemagglutination in eighteen animals with EAT of either kind or in three pooled serum samples, each from more than ten AUG rats with EAT induced by immunization.

Serology

The serum antithyroglobulin antibody titres for the groups studied are shown in Table 2. All animals in the injected and TX + X groups had antibodies but as expected titres were much higher in AUG rats. One AUG control rat had detectable antibodies presumably reflecting the development of spontaneous thyroiditis in this susceptible strain (2% incidence in our colony).

Also shown are the splenic weights. The difference between AUG immunized animals and controls was significant (Student's t test $P < 0{\cdot}05$) but there was no difference in the WAG groups. The splenic weight of the TX + X animals must at least in part reflect their age.

Tissue distribution of PFC

The tissue distributions of PFC after the procedures aimed at inducing thyroiditis were determined and the results are summarized in Tables 3 and 4. In both groups of EAT AUG rats, all animals had thyroglobulin PFC in the bone marrow and the majority of TX + X animals also had PFC in the spleen. By contrast, only one of the AUG controls had PFC and this animal was found to have serum thyroglobulin antibodies (detailed above). Only half of the WAG animals that received thyroglobulin had thyroglobulin PFC and none were detected in the WAG controls.

The numbers of IgG PFC in the marrow were the same for both groups of animals that received thyroglobulin, but were increased in the TX + X animals compared with AUG controls ($P < 0{\cdot}05$, Student's t test). Also, in the TX + X group splenic IgG PFC were increased ($P < 0{\cdot}01$) and lymph node PFC were decreased ($P < 0{\cdot}001$), cervical lymph node IgG PFC were decreased in AUG and WAG immunized animals ($P < 0{\cdot}001$ and 0·01, respectively) and mesenteric

Table 3. Mean spontaneous thyroglobulin PFC/10^6 cells (±SD) in EAT and control animals

Strain	Treatment	Thyroglobulin PFC/10^6: mean±SD Marrow	Spleen	Cervical node	Mesenteric node	Thyroid	Number of animals
AUG	Nil	5±12 (1)	0	0	0	0	5
AUG	Injected Tg/CFA	47±37 (6)	0	0	0	0	6
AUG	Thymectomy+ irradiation	149±171 (6)	407±328 (5)	0	0	0	6
WAG	Nil	0	0	0	0	0	6
WAG	Injected Tg/CFA	0	6±10 (3)	0	0	0	6

Numbers in parentheses indicate the number of animals in each group with thyroglobulin PFC.

node PFC decreased in AUG immunized rats ($P<0{\cdot}001$). The number of splenic PFC was decreased in the WAG immunized rats ($P<0{\cdot}01$) but not in the AUG group.

Lymphocyte recovery from the thyroid was poor. No cells were recovered from control animals: in the TX+X group $0{\cdot}5$–$1{\cdot}8\times10^6$, in AUG+TG $0{\cdot}1$–$0{\cdot}5\times10^6$, and WAG+TG $0{\cdot}1$–$0{\cdot}4\times10^6$ cells were obtained. An IgG PFC response was only demonstrated in three of the TX+X animals and no thyroglobulin PFC were found.

PFC responses following culture

The addition of thyroglobulin to cultures usually enhanced IgG and thyroglobulin PFC compared to similar cultures without thyroglobulin. In preliminary studies such an effect was never found when bovine serum albumin was substituted for thyroglobulin. Figure 1 demonstrates the magnitude of the increase which occurred in IgG PFC (all cultures) and in thyroglobulin PFC (four of six cultures) using cervical lymph node lymphocytes from immunized AUG rats.

Following culture in the presence of thyroglobulin the bone marrow of all animals of each group with EAT yielded thyroglobulin PFC (Table 5). PFC could also be demonstrated in the spleen and cervical lymph node cultures of the majority of animals. No thyroglobulin PFC were found in any of the control animal cultures with or without thyroglobulin.

Table 4. Mean spontaneous IgG PFC/10^6 cells (±SD) in EAT and control rats

Strain	Treatment	IgG PFC/10^6: mean±SD Marrow	Spleen	Cervical node	Mesenteric node	Thyroid	Number of animals
AUG	Nil	1503±112	998±507	2229±1035	4038±1827	ND	5
AUG	Injected Tg/CFA	1548±452	946±444	11±12	117±116	0	6
AUG	Thymectomy+ irradiation	3507±1708	3163±1383	20±17	13±15	6±9	6
WAG	Nil	1609±204	1783±521	1757±2516	1516±1014	ND	6
WAG	Injected Tg/CFA	1114±791	909±322	850±1076	18±32	0	6

ND, not done due to insufficient numbers of cells.

Table 5. Mean thyroglobulin PFC/10^6 cells (±SD) in 4 day cultures with thyroglobulin 10^{-2} μg ml^{-1}

Strain	Treatment	Thyroglobulin PFC/10^6: mean ± SD				Number of animals
		Marrow	Spleen	Cervical node	Mesenteric node	
AUG	Nil	0	0	0	0	5
AUG	Injected Tg/CFA	467±234 (6)	133±82 (5)	683±531 (5)	17±41 (1)	6
AUG	Thymectomy+ Irradiation	279±260 (6)	528±598 (6)	293±218 (6)	238±550 (3)	6
WAG	Nil	0	0	0	0	6
WAG	Injected Tg/CFA	340±270 (6)	68±121 (3)	500±282 (6)	0	6

Numbers in parentheses indicate the number of animals in each group with thyroglobulin PFC.

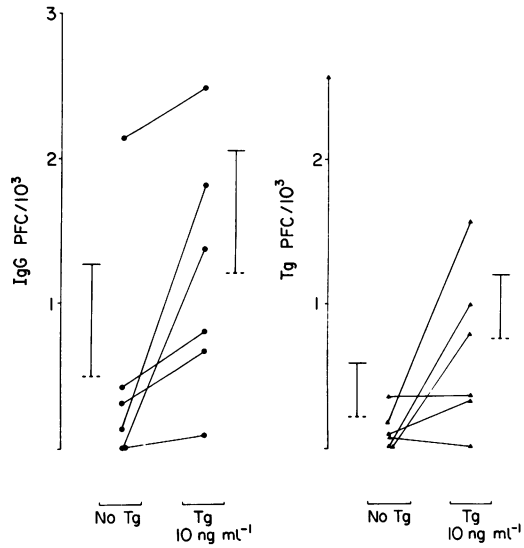

Figure 1. IgG and thyroglobulin PFC/10^6 cells in cultures of cervical lymph node lymphocytes obtained from immunized AUG rats, in the presence or absence of thyroglobulin. The dotted line and bar indicate mean + 1 SD for each response.

DISCUSSION

The thyroglobulin PFC assay described here appears to be specific for the detection of cells synthesizing thyroglobulin antibody. Artefacts were excluded by verifying the presence of a central lymphocyte and the inhibition of the PFC response by cycloheximide indicates that cytophilic antibody is not involved in plaque production. Since plaques were not produced in the absence of complement, killer cells cannot be implicated. The specificity of the response was demonstrated not only by inhibition with free thyroglobulin but also by detection of PFC in rats with EAT which had not been immunized with the antigen used to coat the SRBC. Although the majority of our thyroglobulin preparation seemed likely to be thyroglobulin, the possibility existed that contaminants could have produced false plaques. Several lines of evidence argue against this. Free thyroglobulin only inhibited thyroglobulin-specific plaques and not total IgG plaques. Anti-thyroglobulin antibodies occurred spontaneously in TX+X animals and in a control animal and it seems improbable that all could have developed PFC against a contaminant of our preparation. Finally anti-IgG antibodies were not found in the sera of EAT animals suggesting that significant IgG contamination at least does not exist in our preparation. The protein A assay has also been modified for use in the rat although the data on SRBC immunized rats indicate that not all antibody-secreting cells are detected, which accords with Gronowicz *et al.* (1976). The possible reasons for this include the different antibody affinities involved in the assays, different susceptibilities of the SRBC to lysis (Protein A plaques are smaller than SRBC only plaques) and perhaps different maturities of the PFC. There is also a marked prozone phenomenon involved in Protein A responses (Gronowicz *et al.*, 1976 and unpublished data).

The results suggest that in AUG strain rats with EAT the bone marrow is the most important site of autoantibody synthesis: in all animals, marrow-

derived PFC were demonstrated both spontaneously and following culture. Of particular interest was the single AUG control animal in which thyroglobulin PFC were found in the bone marrow, since this rat proved to have spontaneous thyroiditis on the basis of positive serum antibodies. These observations are in accord with the extensive data confirming the importance of the marrow as a site of antibody production (Benner, Hijmans & Haajiman, 1981) and it is noteworthy that in an animal model of non-organ specific autoimmunity, the NZB mouse, the bone marrow is the major site of autoantibody synthesis against erythrocytes (Cohen 1980).

There was a much lower titre of serum antibody in the immunized WAG rats as expected. These animals also differed from the AUG rats in that no PFC could be found in the marrow; moreover, spontaneous PFC could only be found in the spleens of half the animals. Besides suggesting that sites of antibody synthesis differ between the strains, this observation indicates that some animals could produce serum antibodies without PFC being detected. Either alternative sites not examined were also involved, or the PFC assay was not sensitive enough to detect such cells.

The results following culture permitted the identification of PFC in other tissues. All of the WAG rats with EAT could be shown to have marrow PFC, and the numbers of PFC was increased in each group with EAT after culture. In the majority of animals PFC could be detected in the spleen and cervical lymph node cultures and in four animals in mesenteric lymph node cultures.

The size of the PFC response following culture was greater than we had expected, although for reasons stated above it is impossible to estimate the percentage of thyroglobulin PFC in each tissue following culture since the thyroglobulin and Protein A PFC assays are not directly comparable and the latter at least seems to underestimate the number of immunoglobulin synthesizing cells (see above). Since the same thyroglobulin PFC assay was employed for spontaneous and cultured plaques, the rise in PFC following culture is likely to be a real phenomenon. One possibility could be that adherent cells, which are known to have a striking effect on rat lymphocyte cultures (Mattingly & Kemp, 1979), may be specifically activating these cells in our culture system. Another explanation would be that precursor cells, not detected in the spontaneous assays, become activated in culture. These possiblities are currently being investigated.

In contrast to the situation in Hashimoto's thyroiditis and Graves' disease (Weetman *et al.*, 1981) no PFC could be found in the thyroid. Although cell recovery was always small, similar numbers were employed in the human experiments using fine needle aspirate specimens. Insufficient numbers of cells were obtained for culture to be performed in addition to the spontaneous PFC assay and it may be that PFC would then have been found (as was the case for example in cultures of WAG marrow). In the obese strain chicken, which develops spontaneous thyroiditis, thyroglobulin antibodies are produced by germinal centres in the thyroid gland (Boyd & Wick, 1980) but there are marked differences between avian and mammalian lymphatic systems, not least of which is the absence of lymph nodes in the former. Rabbit thyroglobulin PFC have been detected in the spleen and thyroids of rabbits given xenogeneic thyroglobulin but although thyroiditis was induced in this model, the presence of antibodies against thyroglobulin derived from two species makes interpretation difficult (Clinton & Weigle, 1972). It is clear however that the stage of the disease at the time of assay is critical since these workers were able to show that splenic PFC predated those in the thyroid. One piece of evidence which corroborates this major difference between EAT induced by immunization and human thyroiditis is the demonstration that the thyroid infiltrate in guinea-pigs is predominantly composed of T cells (Paget, McMaster & van Boxel, 1976) whereas in Hashimoto's thyroiditis 40% of the lymphocytes are B cells (Totterman, 1978). The results in the TX+X group in which thyroiditis develops spontaneously would argue further against the thyroid being a major site of antibody synthesis in EAT since IgG PFC could be demonstrated, yet no thyroglobulin-specific PFC were found , despite high serum antibody titres and readily detectable PFC responses elsewhere.

The results of the protein A assay for IgG are less readily interpretable. Despite bigger spleens the immunized AUG animals had the same number of IgG PFC, whereas in the TX+X AUG rats spleen size and PFC number were increased. This may have been due to age and the extent of disease in these animals. However the diminution in IgG PFC in the spleens of WAG EAT rats and in the lymph nodes of the EAT animals as a whole is difficult to explain. The number of IgG PFC increased following culture in the EAT rats but decreased in the controls. That IgG PFC as well as thyroglobulin PFC were often increased by culture with thyroglobulin may be due to the non-specific polyclonal stimulation known to occur in

antigen-induced cultures (Volkman, Lane & Fauci, 1981). It is known that T cells and macrophages can modulate plaque production (Strelkauskas, Wilson, Callery, Chess & Schlossman, 1977; Weiss & Fitch 1978) and it may be that at the time of assay some such regulatory cells may have been responsible for inhibition of plaque production. This possibility is currently under investigation, since it has implications for the absence of plaques produced by the thyroid-derived lymphocytes.

There is increasing evidence for the importance of humoral mechanisms as effectors of EAT, especially in TX + X animals (Ahmed & Penhale, 1981), and we are at present studying the effect of various agents on antibody production in these models (Keast, Rennie, Weetman, Foord & Hall, 1981). The demonstration of diffuse autoantibody production in EAT has particular significance in the interpretation of such experiments and suggests that organ-specific autoimmunity may still involve widespread immune involvement.

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REFERENCES

Ahmed A.A. & Penhale W.J. (1981) Thyroid transplantation in rats developing autoimmune thyroiditis following thymectomy and irradiation. *Clin. exp. Immunol.* **45,** 480.

Benner R., Hijmans W. & Haajiman J.J. (1981) The bone marrow: the major source of serum immunoglobulins, but still a neglected site of antibody formation. *Clin. exp. Immunol.* **46,** 1.

Boyd R. & Wick G. (1980) Effector mechanisms in spontaneous autoimmune thyroiditis of obese strain chickens. In: *Autoimmune Aspects of Endocrine Disease* (Ed. by A. Pinchera, D. Doniach, G. F. Fenzi and L. Baschieri). Academic Press, London.

Clinton B.A. & Weigle W.O. (1972) Cellular events during the induction of experimental thyroiditis in the rabbit. *J. exp. Med.* **136,** 1605.

Cohen P.L. (1980) Bone marrow as the major site of antierythrocyte autoantibody production in NZB mice. *Arthr. Rheum.* **23,** 1045.

Corvalan J.R.F. & Howard J.C. (1978) Primary *in vitro* antibody formation in the rat: partial characterization and properties of an inhibitor cell present in normal spleen. *J. Immunol.* **8,** 331.

Gronowicz E., Coutinho A. & Melchers F. (1976) A plaque assay for all cells secreting Ig of a given type or class. *Europ. J. Immunol.* **6,** 588.

Jaroszewski J., Sundick R.S. & Rose N.R. (1978) Effects of antiserum containing thyroglobulin antibody on the chicken thyroid gland. *Clin. Immunol. Immunopath.* **10,** 95.

Keast D., Rennie D., Weetman A.P., Foord S. & Hall R. (1981) The influence of methimazole on autoimmune thyroid disease in the rat. *Ann. Endocrinol.* **42,** 66A.

McLachlan S.M., Bird A.G., Weetman A.P., Rees Smith B. & Hall R. (1981) Use of plaque assays to study thyroglobulin autoantibody synthesis by human peripheral blood lymphocytes. *Scand. J. Immunol.* **14,** 233.

McLachlan S.M., McGregor A.M., Rees Smith B. & Hall R. (1979) Thyroid-autoantibody synthesis by Hashimoto thyroid lymphocytes. *Lancet* **i,** 162.

Mattingly J.A. & Kemp J.D. (1979) Suppression of secondary plaque-forming cell responses by rat splenic adherent cells: evidence for dependance on prostaglandin production. *Cell Immunol.* **48,** 195.

Noble B., Yoshida T., Rose N.R. & Bigazzi P.E. (1976) Thyroid antibodies in spontaneous autoimmune thyroiditis in the Buffalo rat. *J. Immunol.* **117,** 1447.

Paget S.A., McMaster P.R.B. & van Boxel J.A. (1976) Experimental autoimmune thyroiditis in the guinea pig characterization of the infiltrating lymphocyte populations. *J. Immunol.* **117,** 2267.

Penhale W.J., Farmer A. & Irvine W.J. (1975a) Thyroiditis in T cell-depleted rats. Influence of strain, radiation dose adjuvants and antilymphocyte serum. *Clin exp. Immunol.* **21,** 362.

Penhale W.J., Farmer A., McKenna R.P. & Irvine W.J. (1973) Spontaneous thyroiditis in thymectomized and irradiated Wistar rats. *Clin exp. Immunol.* **15,** 225.

Penhale W.J., Farmer A., Urbaniak S.J. & Irvine W.J. (1975b) 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. *Clin exp. Immunol.* **19,** 179.

Rose N.R., Kong Y.C.M., Okayasu I., Giraldo A.A., Biesel K. & Sundick R.S. (1981) T-cell regulation in autoimmune thyroiditis. *Immunol. Rev.* **55,** 299.

Strelkauskas A.J., Wilson B.S., Callery R.T., Chess L. & Schlossman S.F. (1977) T-cell regulation of human peripheral blood B-cell responsiveness. *J. exp. Med.* **146,** 1765.

Totterman T.H. (1978) Distribution of T-, B- and thyroglobulin-binding lymphocytes infiltrating the gland in Graves' disease, Hashimoto's thyroiditis and de Quervain's thyroiditis. *Clin. Immunol. Immunopathol.* **10,** 270.

Volkman D.J., Lane H.C. & Fauci A.S. (1981) Antigen-induced *in vitro* antibody production in humans: a model for B cell activation and immunorgulation *Proc. natn. Acad. Sci.* **78,** 2528.

Weetman A.P., McGregor A.M. & Lazarus J.H. (1981) Thyroid derived lymphocytes synthesise thyroglobulin antibodies. *Clin. Sci.* **61,** 17P.

Weiss A. & Fitch F.W. (1978) Suppression of the plaque-forming cell response by macrophages present in normal rat spleen. *J. Immunol.* **120,** 357.