Clin. exp. Immunol. (1976) 25, 6–16.

Thyroiditis in T cell-depleted rats: suppression of the autoallergic response by reconstitution with normal lymphoid cells

W. J. PENHALE, W. J. IRVINE, J. R. INGLIS & A. FARMER *Immunology Laboratories, Forrest Road, Edinburgh, and Departments of Endocrinology and Therapeutics, The Royal Infirmary, Edinburgh*

(*Received* 6 *January* 1976)

SUMMARY

Qualitative, quantitative and functional differences were found in lymphoid cells of female thymectomized and irradiated (Tx-X) PVG/c strain rats as compared to normal females of the same strain. Tx-X rats were lymphopenic and had reduced numbers of cells within spleen and cervical lymph nodes, depressed transformation responses of peripheral blood lymphocytes to PHA and a lower percentage killing of their spleen cells by anti-T-cell serum and complement. There was an increased percentage of immunoglobulin-bearing cells in the lymph nodes.

Reconstitution of Tx-X rats by the intravenous route using syngeneic lymph node cells, spleen cells or thymocytes abrogated the autoimmune responses to thyroid components generally observed in this state. Lymph node and spleen cells, but not thymocytes, also prevented thyroid changes when given intraperitoneally. In contrast, bone marrow cells appeared to give enhanced responses.

Quantitative studies showed that the relative proportions of the suppressor or autoregulatory cells in various lymphoid tissues were lymph node > spleen > thymus.

Complete abrogation of the autoimmune responses was possible only when cells were administered within a short time of the final dose of irradiation and moderate thyroid change was again seen if transfer was delayed for 14 days post-irradiation.

At 28 days reconstitution had no influence on the development of the autoimmune responses.

Preliminary characterization studies using an anti-T-cell serum and fractionation of lymph node cells on a linear Ficoll gradient suggested that the autoregulatory cell is a large T cell.

INTRODUCTION

Severe, chronic thyroiditis and autoantibodies to thyroglobulin can be readily induced in rats by thymectomy combined with repeated sublethal irradiation (4 × 200 rad) (Penhale *et al.*, 1973, 1975). These changes are not caused directly by irradiation of the thyroid and it has been postulated that the phenomenon is a consequence of the selective depletion of a subpopulation of T cells which in the normal state is concerned with the suppression of autoallergic reactivity to thyroid components (Penhale *et al.*, 1975). If this hypothesis is correct then it should be possible to inhibit the development of the disease by reconstituting thymectomized and irradiated rats with the appropriate lymphoid cells. In this study we present further evidence for the selectively depleted state of the thymectomized and sublethally irradiated rat and describe experiments in which thyroid changes have been prevented by reconstitution with lymphoid cells from various sources. Some preliminary data on the characterization and distribution of the autoregulatory cell are also given.

Correspondence: Dr W. J. Irvine, Immunology Laboratories, 2 Forrest Road, Edinburgh.

MATERIALS AND METHODS

Rats. Inbred female PVG/c strain rats were used throughout these experiments.

Thymectomy and irradiation. Rats were subjected to the standard thymectomy and irradiation schedule routinely used (Fig. 1) (Penhale *et al.*, 1975). Conditions of irradiation were 54 rad/min at 87 cm from source using filtration through copper only. The total irradiation was 200 rad. Animals were generally bled from the heart and killed by CO_2 60 days following the final irradiation but in one experiment described they were kept for 300 days post-irradiation and bled from the tail at frequent intervals.

Cell transfer. Washed cell suspensions were assessed for viability by trypan blue exclusion and their concentration adjusted before injection either intraperitoneally or intravenously. Cells were administered at various times both during and after irradiation (for precise details see the Results section). Intravenous injections were given into a major tail vein under light methoxyfluorane anaesthesia.

Pathology and serology. Transverse histological sections of both thyroid glands were made following fixation of the entire larynx in formol saline. Sections were stained by haematoxylin and eosin. The severity of thyroiditis was scored arbitrarily from 1 to 4+ according to the criteria previously used (Penhale *et al.*, 1973). Scoring was carried out without prior knowledge of the origin of the specimen.

Antibodies to thyroglobulin were detected by the microhaemagglutination procedure (Penhale *et al.*, 1973).

Lymphoid cell preparation. Young (6–8 weeks old) male PVG/c strain rats were killed in an atmosphere of CO_2 and lymphoid tissues (thymus, cervical lymph nodes, spleen and bone marrow) were removed asceptically and immersed in cold (4–10°C) Hanks's balanced salt solution (HBSS) containing antibiotics and buffered with Hepes buffer (pH 7·2). Bone marrow cells were obtained by flushing the shafts of the tibia and femur with HBSS. Spleen, lymph node and thymus cells were obtained by gently homogenizing the tissue in a glass tube with a hand held homogenizer. All suspensions were made up to a large volume (10× tissue volume approximately) in HBSS and were allowed to stand for 15 min at room temperature to allow particles to sediment. The suspension was centrifuged at 200 *g* for 10 min and the cell pellet adjusted to the required concentration in HBSS.

Velocity sedimentation in linear Ficoll gradients. Velocity sedimentation was carried out as described by Greenberg, Shen & Roitt (1973). Briefly, linear Ficoll gradients of 4–10% were generated by an LKB Ultrograd gradient maker in 50 ml polycarbonate tubes with a gradient height of 9·5 cm. Gradients were established in ten tubes and a 1-ml sample containing 2×10^8 viable lymph node cells was layered onto the surface of each gradient. Tubes were then simultaneously centrifuged at 100 *g* for 15 min. Each gradient was fractionated into twenty aliquots by aspiration. Corresponding fractions from each gradient were pooled, washed three times and the number of viable cells counted. The proportion of Ig bearing cells was then estimated in each fraction. Finally prior to injection, adjacent fractions were pooled to give six bulk fractions and adjusted so that each contained between 1·2 and $2{\cdot}5\times 10^7$ cells/ml.

Preparation of rabbit anti-rat T-cell serum. New Zealand white rabbits were given multiple intravenous injections of 10^8 thoracic duct lymphocytes (TDL) from AO or F1 (AO × HO) rats. They were bled after the seventh dose and after inactivation (56°C for 30 min) the serum was twice precipitated with 40% ammonium sulphate and the precipitate redissolved in the original volume of PBS. After dialysis against PBS at 4°C for 24 hr the reagent was sterilized by filtration and absorbed with bone marrow cells from thymectomized and irradiated PVG × CAM (F1 × F1) rats at 0°C for 60 min (5:1, serum: packed cells). The reagent was tested for specific activity against T cells by microcytotoxicity assay (Greaves *et al.*, 1969), using ^{51}Cr labelled lymphocytes incubated with the reagent and guinea-pig serum absorbed with rat thymocytes as a source of complement. After five absorptions the reagent showed differential killing of lymphoid cells from various sources as follows: thymus, 80%; bone marrow, 10%; spleen, 50%; and lymph node, 70%. Specificity was confirmed by observing that the cells remaining after this treatment were at least 90% immunoglobulin-bearing as determined by immunofluorescence.

Quantitation of surface membrane immunoglobulin (SMIg) bearing B cells. SMIg was detected on washed, gluteraldehyde-fixed leucocytes (Gyöngyössy & Playfair, 1974) after incubation with a polyvalent rabbit anti-rat immunoglobulin serum (Nordic) followed after washing by fluorescein-conjugated goat anti-rabbit immunoglobulin serum (Pentex). Optimal dilutions of antiserum giving plateau levels of positively stained cells were obtained by titration. 5×10^6 Leucocytes were used in a volume of 0·1 ml PBS. The cells were examined under phase contrast to enumerate total number of cells per field and then by ultra-violet epi-illumination to count cells with surface fluorescence. A total of 200 lymphocytes per slide were counted.

RESULTS

Immunological status of Tx-X PVG/c strain female rats

Table 1 summarizes the quantitative and functional changes found in leucocytes of the peripheral blood and lymphoid organs of female Tx-X rats at 60 days post-irradiation. These indicate that the immunological status of these rats differs in several respects from normal females of the same strain. Both the numbers of circulating lymphocytes and the mitogenic response to PHA were depressed and this finding was similar to that described previously in random bred Wistar rats after Tx-X (Penhale *et al.*, 1973). Fewer lymphoid cells were recovered following homogenization of the lymph nodes and

TABLE 1. Immunological status of female Tx-X PVG/c strain rats 60 days after final irradiation

Parameter	Tx-X	Normal
Total WBC count in peripheral blood ($\times 10^3$/mm^3)	2·5±0·2 (9)	4·9±0·8 (6)
Differential WBC ($\times 10^3$/mm^3)		
Lymphocytes	1·1±0·1	3·4±0·2
Monocytes	0·6±0·1	0·8±0·2
Polymorphs	0·7±0·1	0·6±0·1
Others	0·1±0·01	0·1±0·02
Spleen/body wt. ratio ($\times 10^4$)	2·7±0·4 (15)	2·5±0·2 (9)
Total spleen cell recovery ($\times 10^6$)	76±19 (10)	175±19 (10)
Mitogenic index* of peripheral blood lymphocytes	3·7±1·8 (31)	23·5±17·5 (13)
Immunoglobulin-bearing lymph node cells (%)	58·5±5·7 (11)	38·1±4·8 (10)
Killing of spleen cells by anti-T-cell serum and complement (%)	29·0±7·0 (4)	47·5±5·7 (4)

Figures in parentheses indicate number of animals investigated.

* For details see Penhale *et al.* (1973).

spleen and there was a reduction in lymph node mass which was particularly noticeable in the lymph nodes of the cervical region. However, a corresponding decrease was not observed in the weight of the spleen as there was no alteration in the spleen/body weight ratios. The lower recovery of cells obtained from this organ presumably indicates an increase in connective tissue, reticular or other elements following thymectomy and irradiation. Histologically, there was evidence of depletion of the paracortical areas of the lymph nodes and also the thymus-dependent regions of the spleen. Lymph node cells of Tx-X rats had a higher proportion of immunoglobulin bearing cells than normal, showing that the main effect of Tx-X was upon the T-cell compartment. Nevertheless, cytotoxic studies using anti-T-cell serum and complement revealed that there was a substantial residual T-cell population at least in the spleen.

Reconstitution of Tx-X rats by repeated intravenous administration of lymphoid cells

It was initially considered that it might be necessary to give cell replacement after each irradiation. Accordingly, lymphoid cells were injected intravenously within 24 h of each dose (Fig. 1). The effect of

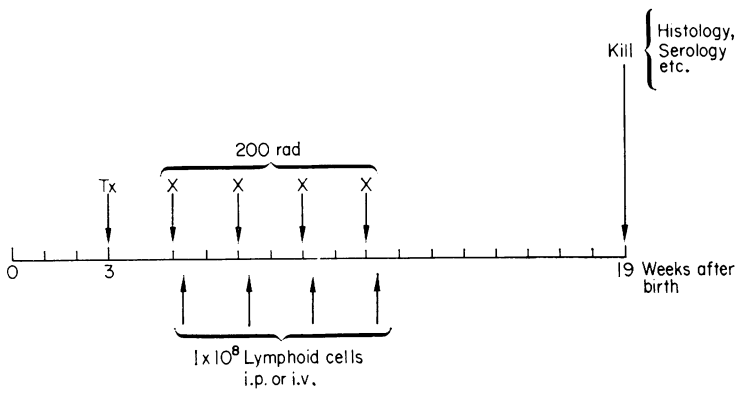

FIG. 1. Standard thymectomy and irradiation schedule and time of reconstitution using four repeated doses lymphoid cells. Tx = thymectomy; X = irradiation, four doses of 200 rads each.

TABLE 2. Reconstitution of thymectomized and irradiated rats with four repeated injections of lymphoid cells intravenously*

Cells transferred (10^8 per injection)	No. of rats	Thyroid pathology: Mean score ± s.e.m.	Thyroid pathology: Incidence (%)	Antibody to rat thyroglobulin: Mean titre† ± s.e.m.	Antibody to rat thyroglobulin: Incidence (%)
Thymocytes	12	0·8±0·4	25	1·6±0·9	25
Spleen cells	6	0·2±0·2	17	2·3±1·5	33
Lymph node cells	7	0	0	0	0
Control (Tx-X only)	17	2·2±0·4	77	6·4±1·4	65

* For details, see Fig. 1.
† log 2.

Student's t-tests	*Pathology*	*Antibody*
Control *vs* thymocyte-treated	$P<0·01$	$P<0·01$
Control *vs* spleen-treated	$P<0·01$	$P<0·1$
Control *vs* lymph node-treated	$P<0·002$	$P<0·005$

four injections each of 10^8 spleen, lymph node or thymic lymphoid cells in Tx-X recipients is shown in Table 2. Cells derived from all three sources significantly inhibited both the development of thyroiditis and autoantibodies to thyroglobulin under these conditions. Lymph node cells had the most striking effect, abrogating completely the usual autoimmune responses seen after Tx-X (Fig. 2). Moderate to severe thyroid change was seen in two of twelve rats treated with thymocytes but only minimal thyroid change was observed in one of the six rats given splenic cells.

Reconstitution of Tx-X rats by repeated intraperitoneal administration of lymphoid cells

In view of the effectiveness of multiple intravenous administration of lymphoid cells in abrogating thyroiditis in Tx-X recipients the effect of four repeated doses of cells (Fig. 1) when given by the intraperitoneal route was investigated. These results are summarized in Table 3. In contrast to the previous experiments, thymocytes administered under these conditions failed to inhibit the autoimmune response and the mean thyroid pathology and autoantibody titres were similar to the control untreated Tx-X group. Spleen cells, however, proved as effective by this route as when given intravenously and lymph node cells again completely inhibited all autoallergic thyroid responses. On the other hand, both severe infiltrative change and high titres of autoantibody were a regular feature in the Tx-X recipients of bone marrow cells. In this instance there appeared to be the possibility of enhancement of the autoimmune effect as compared to that of the untreated Tx-X controls, although this did not reach statistical significance ($P>0·1$).

Quantitation of the suppressor effect of lymphoid cells on the development of thyroiditis

The preceding studies indicated that both qualitative and quantitative differences exist in the suppressor effect of lymphoid cell populations obtained from different lymphoid organs. This effect was therefore titrated in the various lymphoid cell preparations by giving graded doses of cells intraperitoneally. A single dose regimen was used in these experiments and this was given within 24 hr of the final dose of irradiation (Fig. 3). Table 4 summarizes the results obtained. Spleen cells were found to be inhibitory only at the highest dose of 10^8 cells. In contrast, lymph node cells had an observable effect down to the level of 10^6 cells. As expected from the previous studies thymocytes were not inhibitory at any dose level under these conditions. It was therefore concluded that a higher proportion (approximately 100-fold) of suppressor or autoregulatory cells reside in lymph nodes than in the spleen and that the spleen contains a greater proportion of active or mature cells than the thymus (when intravenous and intraperitoneal studies are considered together).

The effect of time of reconstitution on the development of thyroiditis in Tx-X PVG/c strain rats

Since reconstitution with lymphoid cells given immediately after irradiation proved effective in abrogating the autoallergic response, it was of interest to investigate the influence of the timing of reconstitution.

Previous studies (Penhale *et al.*, 1975) have shown that both thyroid lesions and autoantibodies begin

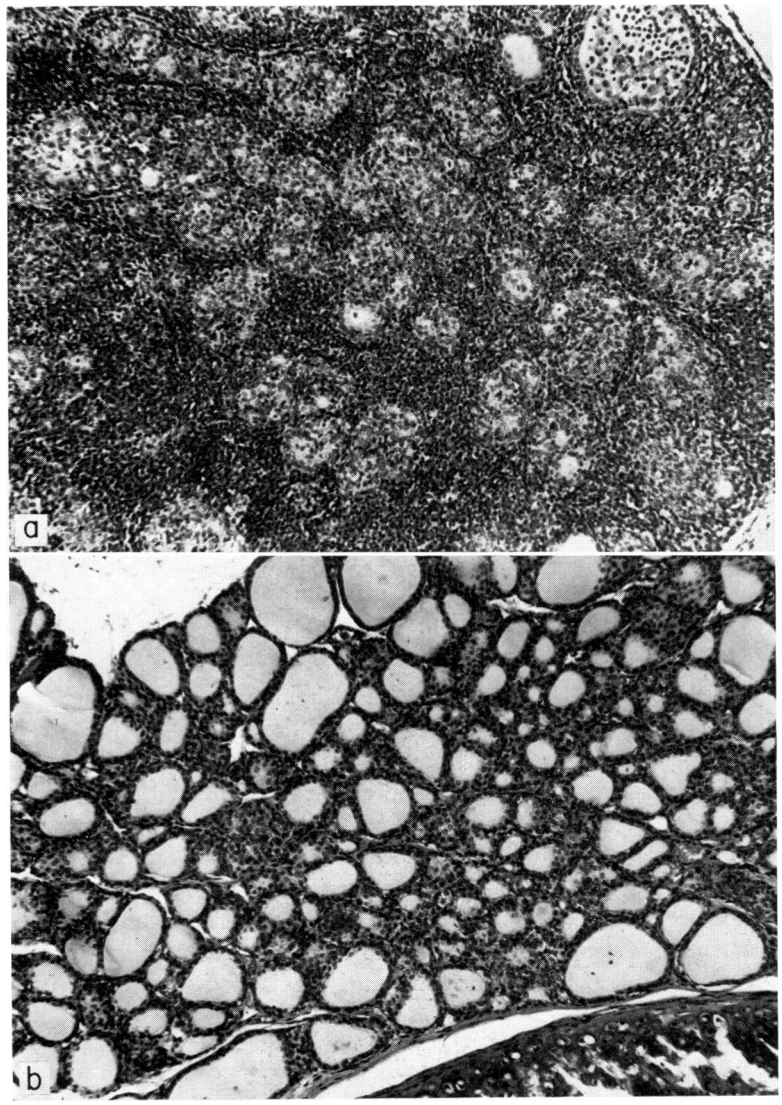

FIG. 2. (a) Thyroid of a rat after thymectomy and irradiation. Note the heavy mononuclear infiltration and absence of normal follicular architecture. H & E; magnification × 100. (b) Thyroid of a rat after thymectomy, irradiation and reconstitution with lymph node cells (4×10^8 i.v., as indicated in Fig. 1). Note the absence of cellular infiltration and the normal appearance of the follicles. H & E; magnification × 100.

to appear at approximately 8 weeks following thymectomy and are well established by 10 weeks. Since this appeared to be a critical period in the development of the disease, lymphoid cells were accordingly given both before and after the 10 week stage. Table 5 presents the results found after transferring lymph node cells or thymocytes in single or multiple doses. Although lymph node cells completely inhibited the autoimmune response when given i.p. 1 day after Tx-X, substantial autoantibody levels and thyroid change were seen if cell transfer was delayed until 14 days post-irradiation. Nevertheless

TABLE 3. Reconstitution of thymectomized and irradiated rats with four repeated injections of lymphoid cells intraperitoneally*

Cells transferred, four doses 10^8 per injection	No. of rats	Thyroid pathology: Mean score ± s.e.m.	Thyroid pathology: Incidence (%)	Antibody to rat thyroglobulin: Mean titre† ± s.e.m.	Antibody to rat thyroglobulin: Incidence (%)
Thymocytes	8	2·9±0·6	75	4·4±1·4	71
Spleen cells	8	0·1±0·1	12	0·5±0·5	12
Bone marrow cells	6	3·5±0·5	100	7·2±1·6	83
Lymph node cells	6	0	0	0	0
Control (Tx-X only)	9	2·3±0·6	78	6·6±1·8	66

* For details, see Fig. 1.
† log 2.

Student's t-tests	*Pathology*	*Antibody*
Control *vs* thymocyte-treated	$P>0·25$	$P>0·2$
Control *vs* spleen-treated	$P<0·005$	$P<0·01$
Control *vs* lymph node-treated	$P<0·005$	$P<0·01$
Control *vs* bone marrow-treated	$P>0·1$	$P>0·45$

there was a reduction in the severity of thyroid change and mean titre of antibody to homologous thyroglobulin as compared to control Tx-X animals although these differences were not significant ($P<0·1$ and $P<0·45$ respectively). When given at 28 days these cells completely failed to modify the autoimmune response. A similar pattern was also observed in the thymocyte transfer study. In this instance cells were given intravenously instead of intraperitoneally. Furthermore, as it appeared likely from the preceding experiment that a single dose of cells would prove to be ineffective, repeated doses were also given. From Table 5 it will be seen that when delayed until 14 days post-irradiation neither a single dose nor multiple doses eliminated the autoimmune responses although their severity was reduced, and in the case of the single dose both thyroid changes and autoantibody responses were significantly depressed when compared to the Tx-X control group ($P<0·00025$). Tx-X rats given thymocytes at 42 days post-irradiation showed no modification of autoimmune changes as compared to the control group.

These studies therefore indicated that reconstitution with lymphoid cells could not reverse already established autoantibody responses and thyroid lesions at least within the period of this particular experiment (approximately 40 days). To further substantiate this finding, lymph node cells were given in repeated doses to Tx-X rats with long-standing autoantibody levels to thyroglobulin. These animals were not killed at the standard time but were allowed to continue for up to 300 days post-irradiation and bled at intervals during this period. 10^8 Lymph node cells were given at the times indicated in Fig. 4

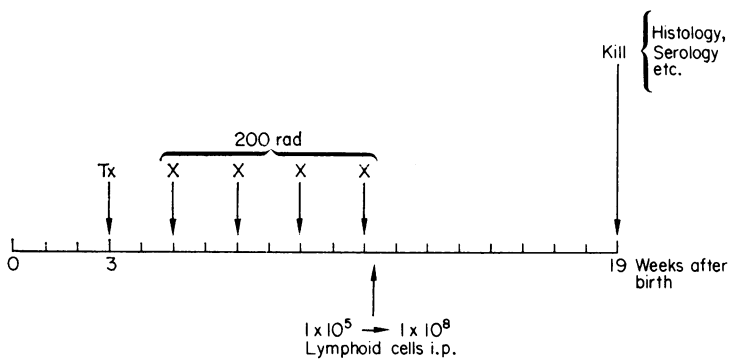

FIG. 3. Standard thymectomy and irradiation schedule and time of single reconstitution with varying numbers of lymphoid cells. Tx = thymectomy; X = irradiation.

TABLE 4. Quantitation of the suppressor effect of lymphoid cells on the development of thyroiditis in thymectomized and irradiated rats*

Cells transferred	No. of rats	Thyroid pathology: Mean score ±s.e.m.	Thyroid pathology: Incidence (%)	Antibody to rat thyroglobulin: Mean titre† ±s.e.m.	Antibody to rat thyroglobulin: Incidence (%)
Spleen cells					
10^5	4	2·0±0·7	75	6·8±2·5	75
10^6	5	2·8±0·7	80	9·2±2·1	100
10^7	2	3·5±0·5	100	13·5±0·5	100
10^8	8	0·1±0·1	17	0·5±0·5	33
Lymph node cells					
10^5	3	3·7±0·3	100	5·3±0·3	100
10^6	5	1·8±0·6	83	0·8±0·8	17
10^7	4	0·5±0·5	25	1·0±1·0	25
10^8	6	0	0	0	0
Thymus cells					
10^6	4	2·0±0·9	75	6·6±2·9	75
10^7	5	2·4±0·7	80	6·2±1·9	80
10^8	8	2·9±0·6	75	4·8±1·5	72
Control (Tx-X only)	10	2·5±0·4	90	7·3±1·0	100

* For details of reconstitution see Fig. 3.
† log 2.

and the animals were maintained for an extended period following the primary reconstitution (100 days). Again, the administration of cells had no effect upon the immune response profile as compared to similarly maintained control animals (Fig. 4). Furthermore, all animals in this experiment, both LN cell recipients and controls, were found to have severe thyroid lesions when eventually killed.

Characterization of the autoregulatory cell

(a) *Treatment of lymph node cells with anti-T-cell serum and complement.* Considerable evidence implicates the T cell as the mediator of immunological suppressor functions in other experimental systems (Basten, 1974) and the mode of induction of autoimmune change in the present model suggests that the

TABLE 5. The effect of the timing of reconstitution on the development of thyroiditis in thymectomized and irradiated rats

Cells transferred	Time of transfer of cells after Tx-X (days)	No. of rats	Thyroid pathology: Mean score ±s.e.m.	Thyroid pathology: Incidence (%)	Antibody to rat thyroglobulin: Mean titre* ±s.e.m.	Antibody to rat thyroglobulin: Incidence (%)
Lymph node cells 10^8 i.p.	1	6	0	0	0	0
Lymph node cells 10^8 i.p.	14	5	1·2±0·3	80	5·8±2·9	60
Lymph node cells 10^8 i.p.	28	3	3·5±0·2	100	10·0±4·0	100
None	—	10	2·5±0·4	90	7·3±1·0	100
Thymocytes 10^8 i.v.	1	10	0·8±0·4	25	1·6±0·9	25
Thymocytes 10^8 i.v.	14	4	1·3±0·3	50	1·0±1·0	25
Thymocytes 10^8 i.v.	14, 28, 42	5	1·2±0·4	80	7·0±2·0	80
Thymocytes 10^8 i.v.	42	6	2·7±0·6	83	8·2±2·3	83
None	—	5	3·2±0·2	85	7·6±1·1	81

* log 2.

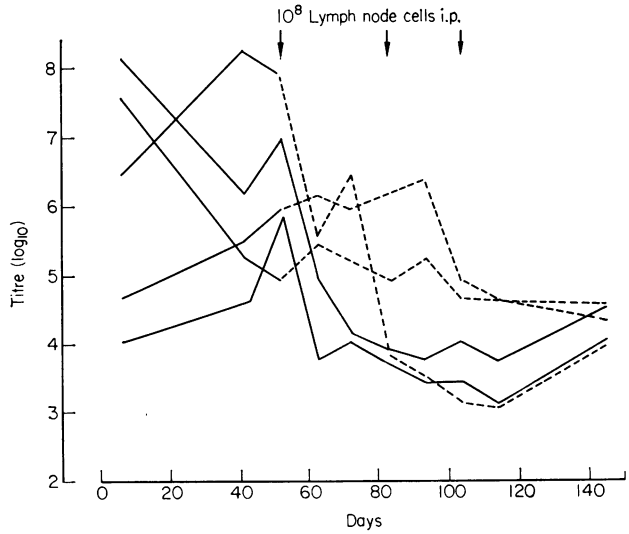

FIG. 4. Reconstitution of Tx-X rats with long-standing autoimmune responses. The titres (log_{10}) of antibodies to thyroglobulin are shown in individual animals before (——) and after (- - -) repeated reconstitution with 1×10^8 lymph node cells at the intervals shown. Two of the five animals were not reconstituted and served as controls (solid line throughout). The rats had all received their final irradiation 150 days earlier. Note that the rats given cells had a similar response profile to the non-reconstituted animals.

autoregulatory cell is of similar origin in this particular instance. In order to investigate this possibility further, lymph node cell suspensions were selectively depleted of T cells by incubation with an anti-T-cell serum and guinea-pig complement before use in transfer studies. Following treatment with the anti-T-cell serum the suspension was adjusted to contain the same number of viable cells (1×10^8) as the control suspension incubated with medium and complement only prior to administration to Tx-X recipients. Moderate to severe thyroid change and antibodies to thyroglobulin were found in two of four rats receiving cells subjected to anti-T-cell serum treatment (Table 6). This is in contrast to the

TABLE 6. Reconstitution of Tx-X rats with lymph node cells treated with anti-T-cell serum and complement

Treatment	Rat no.	Thyroid pathology	Antibody to rat Tg (log_2 titre)
1×10^8 LNC after treatment with anti-T-cell serum+ complement	1	+++*	8
	2	+	4
	3	0	0
	4	0	0
1×10^8 LNC after treatment with medium+complement	5	0	0
	6	0	0
	7	0	0
	8	0	0
	9	0	0
Tx-X only	10	0	0
	11	+++	7
	12	+++	7
	13	++++	13
	14	+	0

* For details of assessment of thyroid pathology see Materials and Methods section.

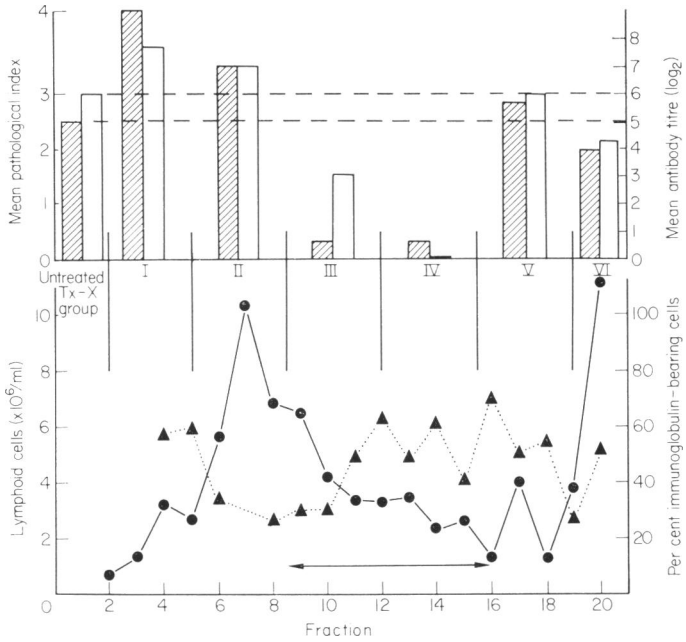

FIG. 5. Fractionation of normal rat lymph node cells by velocity sedimentation on a linear Ficoll gradient. The lower part shows the concentration of lymphoid cells (●) and the percentage of immunoglobulin-bearing cells (▲) in each of the fractions. The upper part shows the effectiveness of the different bulk fractions (depicted I–VI) in preventing the development of antibodies to thyroglobulin and thyroiditis. There were three animals in each group. Hatched columns, mean pathological index; open columns, mean antibody titres to thyroglobulin. The horizontal line (↔) indicates fractions (8–16) with suppressor activity.

complete absence of such changes in all the control Tx-X rats given the same number of cells treated with medium and complement only.

(b) *Fractionation of lymph node cells by velocity sedimentation.* Fig. 5 illustrates the distribution of cells with suppressor or autoregulatory capacity in fractions of a gradient separated lymph node suspension and compares it with the recovery of lymphoid cells and the percentage of immunoglobulin-bearing cells in each fraction. Owing to the limited number of animals available for this study it was necessary to bulk fractions as shown in the figure. Suppressor activity to thyroid autoallergy was found in bulk fraction III and particularly in fraction IV. Since fractionation by this means depends primarily upon cell diameter it would appear that autoregulatory cells are distinctly larger than most lymph node cells, the majority of which were recovered in earlier fractions. Cells in these early fractions did not possess suppressor activity but on the contrary appeared to slightly enhance the autoimmune effects when compared to the non reconstituted Tx-X group. Similarly, the distribution of suppressor activity seemed to bear no relationship to the percentage of Ig bearing B cells in these fractions.

DISCUSSION

These studies provide further evidence for the selectively depleted state of Tx-X rats. Although reduced total lymphocyte counts and the lower recoveries of cells from spleens and lymph nodes suggested that there was a general depletion of lymphocytes in the Tx-X state, the increased proportion of immunoglobulin-bearing cells, depressed PHA transformation and histological changes in the thymus-dependent areas of the lymphoid organs revealed that the effect was more pronounced on T cells than B cells. However, whilst these findings indicated a severe depletion of T cells there was some evidence that this depletion was also of a selective nature. Thus anti-T-cell serum studies revealed a substantial residual population of T cells in the spleen and the observation (Penhale *et al.*, 1975) that Tx-X rats produce IgG antibodies to homologous thyroglobulin (a thymus-dependent antigen) indicate that helper T cells

were still available in functionally effective numbers. It therefore appears likely that the procedure is depleting only the most radiation-sensitive subpopulations of T cells, presumably including those involved in suppressor or autoregulatory functions. Evidence that suppressor T cells are relatively radiation sensitive as compared to other subpopulations of T cells has been found in a number of other experimental systems (Tada, Taniguchi & Okamura, 1971; Kapp *et al.*, 1974). The abrogation of the autoimmune responses following reconstitution of Tx-X rats substantiates our previous conclusions that this process leads to autoimmunity by depleting selectively a subpopulation of autoregulatory cells. This finding also supports our conclusions from earlier studies that the thyroid lesions are not the consequence of the direct effect of irradiation on the thyroid. These studies further show that the autoregulatory cells are widely distributed in lymphoid tissue and that relatively greater numbers of active cells are found in the lymph nodes than in the spleen, thymus or bone marrow. The inability to successfully reconstitute Tx-X rats with thymocytes by the i.p. route is probably a reflection of the low number of fully differentiated or mature cells of this type in the thymus. The enhanced (but not statistically significant) changes observed in Tx-X recipients of bone marrow cells could be the consequence of the provision of additional stem cells which may subsequently differentiate to immunocompetent autoreactive B cells. Such cells are likely to be depressed to some extent in Tx-X rats following irradiation.

Direct evidence was obtained from the anti-T-cell serum studies that the suppressor cell involved in this particular model is of thymic origin. This is in keeping with studies of cell-mediated suppressor effects in other experimental systems (Gershon & Kondo, 1970; Okamura & Tada, 1971; Kerbel & Eidinger, 1972; Herzenberg *et al.*, 1973; Ha, Waksman & Treffers, 1974). Further, preliminary fractionation studies on linear Ficoll gradients revealed that these cells belong to a restricted subpopulation of T cells which are heavier than the majority of the T-cell population. This observation is in contrast to that of Ha *et al.* (1974) using thymocytes separated on a discontinuous BSA gradient. These workers again found that cells with suppressor activity in their particular system formed a small proportion of the total population but in this instance active cells were of lower density than the cells possessing activities associated with mature T cells such as helper or graft versus host competence. This difference may merely reflect basic differences in cell types, the separation procedure, or the experimental systems employed. On the other hand, it is possible that these cells are of common origin and development, but at different stages of maturation. Our finding, in contrast to that of others, that thymocytes are relatively less efficient as suppressors than cells derived from other lymphoid organs provides support for the latter possibility and suggests that further extra-thymic differentiation occurs. It can be envisaged that maturation of thymus derived cells for suppressor function may depend upon the presence of an intact thymus and thus is not possible in rats subjected to thymectomy.

The failure to reduce by reconstitution the established production of autoantibody to thyroglobulin contrasts with the studies of Okamura & Tada (1971), where the transfer of similar numbers of thymocytes to recipient rats which were producing high titres of homocytotropic antibodies rapidly depressed this response. It would seem unlikely that this difference is due to relative differences in the half lives of the respective antibodies, since in the present study there was no observable effect even after a prolonged period following repeated reconstitution. Similarly, reconstitution had no obvious resolving effect upon established thyroid lesions. Nevertheless, it was apparent that reconstitution during the development stage of the lesion arrested further change, although failing to bring about resolution.

The finding that as few as 10^6–10^7 lymph node cells when given i.p. had a marked inhibitory effect is surprising in view of the heterogeneity of these cells and also of the loss of cells which must occur within the peritoneal cavity when they are given by this route. This would appear to cast some doubt upon the specificity of this effect unless specificity can be acquired by the cells following transfer. This aspect is at present under further investigation.

The authors wish to thank Ms D. Suckling and Mr D. Doogan for their excellent technical assistance and Dr N. F. Anderson, Department of Surgery, University of Edinburgh Medical School, for kindly supplying rat thoracic duct lymphocytes. J. R. Inglis is the recipient of a Vans Dunlop Scholarship from University of Edinburgh. The work was supported by the Medical Research Council.

REFERENCES

BASTEN, A. (1974) Specific suppression by T cells. *Immunological Tolerance: Mechanism and Potential Therapeutic Applications.* (ed by B. Benacerraf and D. Katz). Academic Press, London.

GERSHON, R.K. & KONDO, K. (1970) Cell interactions in the induction of tolerance: the role of thymic lymphocytes. *Immunology*, **18**, 723.

GREAVES, M.F., TURSI, A., PLAYFAIR, J.H.L., TORRIGIANI, G., ZAMIR, R. & ROITT, I.M. (1969) Immunosuppressive potency and *in vitro* activity of anti-lymphocyte globulin. *Lancet*, **i**, 68.

GREENBERG, A.H., SHEN, L. & ROITT, I.M. (1973) Characterisation of the antibody-dependent cytotoxic cell: a non-phagocytic monocyte? *Clin. exp. Immunol.* **15**, 251.

GYÖNGYÖSSY, M.I.C. & PLAYFAIR, J.H.L. (1974) Rosette formation by mouse lymphocytes. I. Demonstration by indirect immunofluorescence of T cells binding sheep erythrocytes. *Clin. exp. Immunol.* **18**, 169.

HA, T.Y., WAKSMAN, B.H. & TREFFERS, H.P. (1974) The thymic suppressor cell. I. The separation of subpopulations with suppressor activity. *J. exp. Med.* **139**, 13.

HERZENBERG, L.A., CHAN, E.L., RAVITCH, M.M., RIBLET, R.J. & HERZENBERG, L.A. (1973) Active suppression of immunoglobulin allotype synthesis. III. Identification of T cells as responsible for suppression by cells from spleen, thymus lymph node and bone marrow. *J. exp. Med.* **137**, 1311.

KAPP, J.A., PIERCE, C.W., SCHLOSSMAN, S. & BENACERRAF, B. (1974) Genetic control of immune responses *in vitro*. V. Stimulation of suppressor T cells in nonresponder mice by the terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT). *J. exp. Med.* **140**, 648.

KERBEL, R.S. & EIDINGER, D. (1972) Enhanced immune responsiveness to a thymus-independent antigen early after adult thymectomy: evidence for short-lived inhibitory thymus-derived cells. *Europ. J. Immunol.* **2**, 114.

OKAMURA, K. & TADA, T. (1971) Regulation of homocytotropic antibody formation in the rat. VI. Inhibitory effect of thymocytes on the homocytotropic antibody response. *J. Immunol.* **107**, 1682.

PENHALE, W.J., FARMER, A. & IRVINE, W.J. (1975) 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, irradiated Wistar rats. *Clin. exp. Immunol.* **15**, 225.

TADA, T., TANIGUCHI, M. & OKAMURA, K. (1971) Regulation of homocytotropic antibody formation in the rat. I. Effect of X-irradiation. *J. Immunol.* **106**, 1012.