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Thyroid transplantation in rats developing autoimmune thyroiditis following thymectomy and irradiation

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SUMMARY

Post-irradiation transplantation of normal thyroids under the renal capsule of syngeneic thymectomized and irradiated (Tx-X) rats leads to the development of thyroiditis in the ectopic grafted thyroids. A close correlation was observed between the extent of the lesions in the grafted and recipient's own thyroid. The histopathology of both grafted and recipient thyroid was similar and was characterized by infiltration with mononuclear cells together with some plasma cells. Conversely, grafting of affected thyroids from Tx-X rats to normal animals resulted in the regression of the lesion in the graft and no evidence of thyroiditis was observed in either the graft or the recipient's thyroid when these were examined 60 days post-grafting. Thyroids derived from normal animals grafted to syngeneic normal rats were found to remain healthy and intact over a 60-day period. In contrast to normal animals, Tx-X rats were unable to reject totally the transplanted allogeneic thyroids by 28 days post-grafting, suggesting that some impairment of cell-mediated immunity follows this treatment. These findings indicate that the pathological change occurring in the thyroid gland of Tx-X rats is not attributable to the local effect of irradiation of the thyroids and adds further support to the concept that the process is immunologically mediated by thyroid-specific circulating components in the absence of normal immune regulatory function.

INTRODUCTION

Experimentally, chronic lymphocytic thyroiditis can be regularly induced in certain inbred rat strains by a combination of early thymectomy together with a series of sublethal irradiations (Tx-X) (Penhale *et al.*, 1973; Penhale, Farmer & Irvine, 1975). This phenomenon is considered to be of autoimmune origin because of the characteristic cellular infiltration and the accompanying high concentrations of antibody to thyroid components (Penhale *et al.*, 1973, 1975). It has been postulated that the Tx-X procedure leads to the elimination of the radiation-sensitive natural autoregulatory suppressor T cells whilst sparing the more radiation-resistant populations of autoreactive helper T and B cells (Penhale *et al.*, 1973).

Evidence has been assembled to eliminate the possibility that irradiation plays a direct role in causing the thyroid damage and includes irradiation shielding experiments (Penhale *et al.*, 1973), neonatal thymectomy alone (Penhale, unpublished observations), and reconstitution of Tx-X rats with normal syngeneic lymphoid cells (Penhale *et al.*, 1976).

Another approach to this problem is by transplantation of normal syngeneic thyroid lobes to TxX rats after these have been subjected to the irradiation process. Moreover, this procedure also

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enables sequential observations of the development of the lesion in the individual animal as well as other studies on basic pathogenesis to be made.

In this article preliminary studies are reported in which thyroid lobes from normal syngeneic donor rats have been transplanted to Tx-X recipients and conversely thyroid lobes from autoimmune Tx-X donors have been transplanted to normal animals. In addition, thyroid allografting studies are presented which compare the autoimmune and rejection processes and provide a method of assessment of the level of cell-mediated immune (CMI) competence of Tx-X rats.

MATERIALS AND METHODS

Rats

PVG/c strain rats were bred and maintained within our Animal House. They were fed a commercial solid pellet diet (Milne Feeds, Perth, Western Australia) and watered *ad libitum*. Inbred Wistar rats were obtained from another animal facility. Female animals were used exclusively in this study.

Surgical procedures

Thymectomy. Weanling rats (21 days old) were subjected to thymectomy by the technique described earlier (Penhale *et al.*, 1973).

Grafting of thyroids. Whole thyroid lobes were implanted under the renal capsule by the following technique. Donor rats were killed and their thyroids were carefully removed from the larynx and placed immediately in cold MEM medium (pH 7·4) and transplanted to recipient animals within 5 min of removal from the donor. The recipient rats were anaesthetized by parenteral injection of sodium pentobarbitone and a flank incision was made. After the abdominal muscles were incised the kidneys were gently lifted out and held in position by partly closing the wound lips with Allis tissue forceps. The peritoneum covering the kidneys was peeled off, and using microscissors a small portion (2 mm) of the renal capsule beneath was cautiously removed. The capsule was carefully lifted thereby forming a pouch, into which whole thyroid lobes were inserted and gently pushed away from the line of incision. Extreme care was taken to avoid renal bleeding. Abdominal muscles were sutured by 3-0 catgut and skin lips were closed by 3-0 silk sutures.

In order to assess the histopathological status of the thyroid lobes of the Tx-X donors at transplantation, the contralateral lobe was immediately fixed and examined histologically. Similarly, the non-transplanted lobe of thyroids of normal donors was also examined histologically to confirm their normal status.

Irradiation

A standard schedule of irradiation was employed as described earlier (Penhale *et al.*, 1973). However, the divided doses of irradiation were slightly increased from 200 to 250 rad; thus the total irradiation was 1,000 rad instead of 800 rad. Rats were restrained in perspex boxes and subjected to whole-body irradiation using X-irradiation (Toshiba cobalt-60 source) at 84 rad/min at a distance of 90 cm from the source without using any filters.

Histology

Larynxes and kidneys with thyroid grafts were fixed in 10% buffered formal saline and stained by haematoxylin & eosin. The slides were examined without prior knowledge of their origin and the severity of thyroiditis was scored as described earlier (Penhale *et al.*, 1973).

Serology

Autoantibodies to thyroglobulin were detected by enzyme-linked immunosorbent assay. Briefly, 1 μg of rat thyroglobulin was coated onto polystrene microtitre plates (Dynatech Cook Microtitre M129) for 120 min at 37°C. Rat antisera with appropriate controls were layered for 90 min. These were later overlaid by anti-rat IgG–alkaline phosphatase conjugate for 60 min. The plates were washed three times following the addition of each reagent. The development of colour was observed visually 30 min after the addition of the *p*-nitrophenylphosphate substrate.

RESULTS

Syngeneic thyroid grafting

The immunopathological events following grafting of thyroids (normal and autoimmune diseased) in syngeneic normal and immunologically aberrant Tx-X rats are summarized in Table 1.

Normal thyroids grafted to normal animals. Normal thyroids implanted under the renal capsule of syngeneic normal animals were found to remain healthy when examined at various intervals up to 60 days post-grafting. Histologically, the thyroid follicles were found to be intact with no evidence of any lymphocytic infiltration. No autoantibodies to thyroglobulin were detected in these animals.

Table 1. Summary of results of syngeneic thyroid grafting in Tx-X and normal rats

Status of donor	Status of recipient	No. of animals	Immunopathology of donor thyroids: Mean thyroid pathology		Immunopathology of recipient thyroids	
			Before grafting	60 days post-grafting	Mean thyroid pathology	Antibodies to thyroglobulin (mean $\log_2$ scale)
Normal	Normal	16	0	0	0	0
Normal	Tx-X					
	(a) Thyroiditis-positive	7	0	1·9±0·3	1·7±0·2	9·07±0·7
	(b) Thyroiditis-negative	8	0	0	0	0
Tx-X						
(a) Thyroiditis-positive	Normal	9	2·6±0·4	0	0	0
(b) Thyroiditis-negative	Normal	6	0	0	0	0

Normal thyroids grafted to Tx-X animals. It was observed that normal thyroids grafted to Tx-X rats subsequent to irradiation developed thyroiditis to an extent similar to that which occurred in recipient thyroids (Fig. 1a & b). The mean thyroid pathology of the affected grafts and recipients was 1·9±0·3 and 1·7±0·2 respectively. Fig. 2 shows the close correlation between the severity of the lesion in the donated and recipient thyroids ($r=0·97$).

The lesion in both the graft and recipient thyroid was characterized by heavy infiltration of mononuclear cells together with destruction of follicular architecture. In addition, a small number

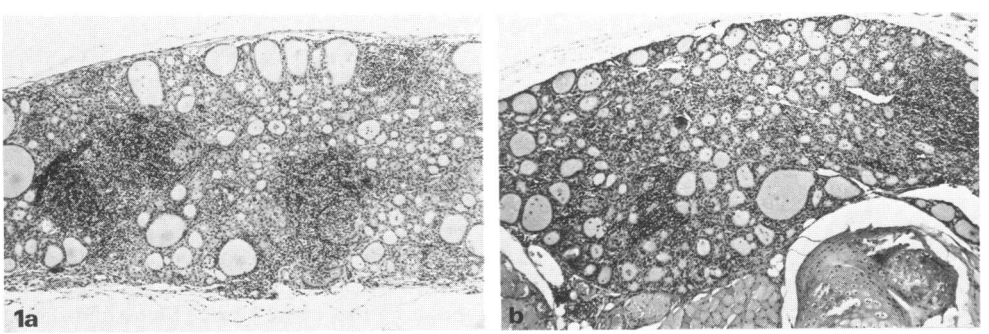

Fig. 1. (a) Graft of normal syngeneic thyroid to Tx-X PVG/c recipient showing severe mononuclear infiltration and destruction of follicles. (b) Thyroid gland of the recipient showing similar degree of infiltration and destruction. (H & E, original ×120.)

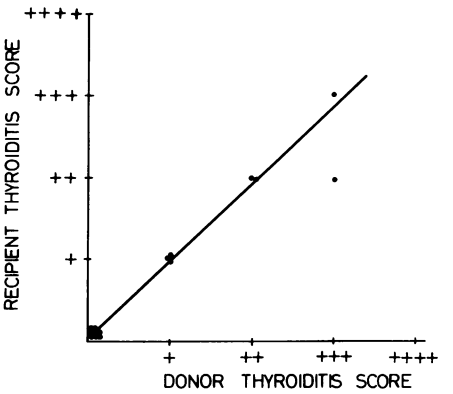

Fig. 2. The correlation between the severity of lesion within the thyroid graft and the recipient's thyroid ($r = 0{\cdot}97$).

of plasma cells were not uncommonly observed. The lesions were always accompanied by autoantibodies to thyroglobulin (mean anti-thyroglobulin titre of $9{\cdot}07 \pm 0{\cdot}7$ on a $\log_2$ scale).

Since the grafts developed thyroiditis although not exposed to irradiation it is apparent that the induced autoimmune lesion is not dependent upon the local damaging effects of irradiation and also that irradiation does not augment the severity of the lesion.

An interesting control was provided in a group of Tx-X rats which failed to develop thyroiditis. Grafting of normal thyroids to such animals failed to provoke any lesion in the ectopic grafted thyroids. These animals did not have any detectable levels of autoantibodies to thyroglobulin. Although in this instance the precise reasons for failure to induce thyroiditis either in the thyroid of the recipient or graft remain unclear, these results suggest again the absence of anti-thyroid-specific circulating components.

Autoimmune diseased thyroids grafted to normal animals. The mean thyroid pathology of Tx-X donors at grafting was $2{\cdot}6 \pm 0{\cdot}4$ and these animals also had a high titre of antibodies to thyroglobulin (mean thyroglobulin titre of $10{\cdot}1 \pm 1{\cdot}1$ on a $\log_2$ scale). Nevertheless, the autoimmune changes in such grafts were found to have regressed completely when examined 60 days post-grafting. The recipient developed neither lesions nor detectable levels of autoantibodies, which presumably reflects the normal immunoregulatory capabilities of the recipients (Fig. 3a, b).

As expected, thyroid lobes derived from Tx-X rats which had not developed thyroiditis were found to be normal when examined 60 days post-grafting.

Table 2. Summary of results of allogeneic thyroid grafting in Tx-X and normal rats

Status of donor (Wistar) ($Ag\text{-}B^2$)	Status of recipient (PVG/c) ($Ag\text{-}B^5$)	No. of animals	Days after grafting	Mean thyroid pathology of recipient	Total rejection/ No. of animals*
Normal	Normal	13	21	0	13/13
Normal	(i) Tx-X	8	21	$1{\cdot}0 \pm 0{\cdot}3$	0/8
	(ii) Tx-X	8	28	$1{\cdot}5 \pm 0{\cdot}6$	0/8

* The criterion used for total rejection was the absence of any thyroid follicles within the graft. The presence of one or more recognizable follicle whether infiltrated or otherwise was regarded as evidence of partial rejection only.

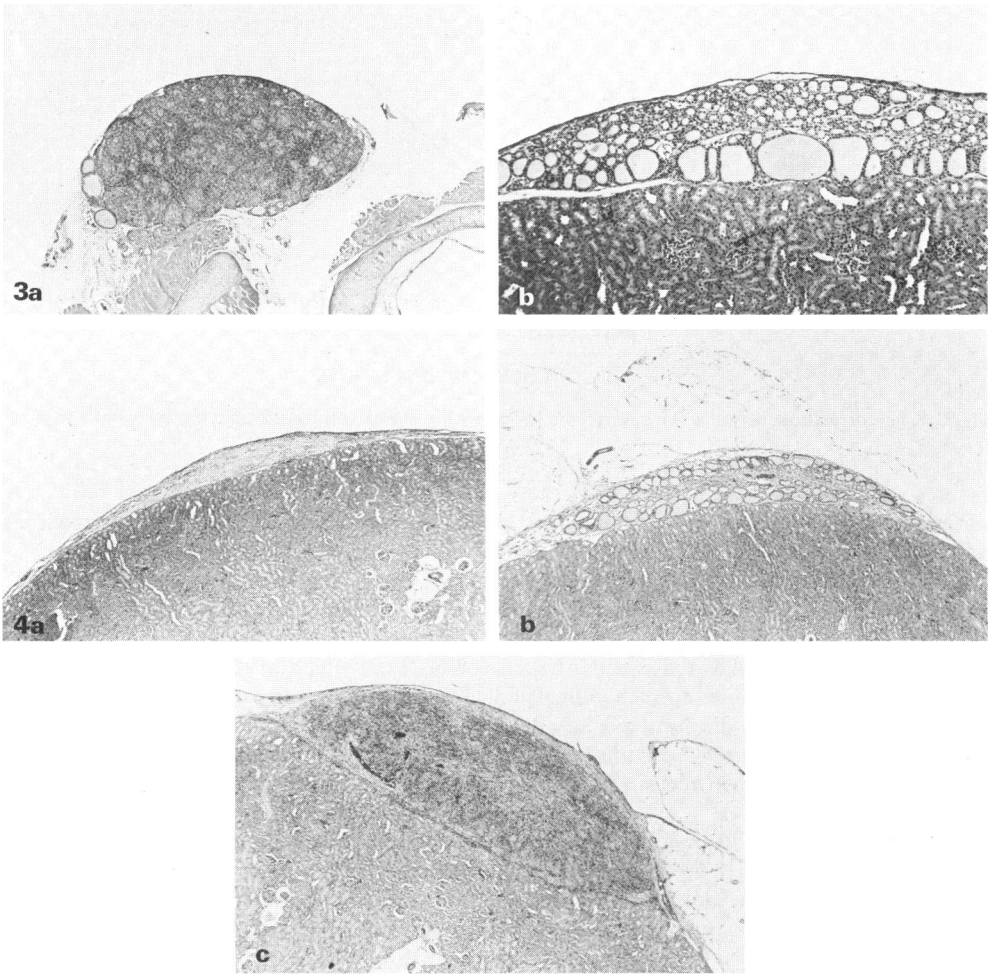

Fig. 3. (a) Donor thyroid obtained from a Tx-X rat showing severe thyroiditis at grafting. (b) Thyroid graft from the same donor removed 60 days post-grafting from a normal rat. Note the resolution of thyroid damage. (H & E, original ×48.)

Fig. 4. (a) Allogeneic thyroid graft in normal PVG/c rat showing complete replacement of graft at 21 days post-grafting by fibrous connective tissue. (b) Allogeneic thyroid graft in Tx-X PVG/c rat showing only partial rejection at 21 days. Note healthy follicles with moderate infiltration of follicles by mononuclear cells and the sparse distribution of fibrous connective tissue. (c) Allogeneic thyroid graft in Tx-X PVG/c rat showing collapsed follicles and heavy infiltration of mononuclear cells. (H & E, original ×48.)

Allogeneic normal thyroid grafts to normal and Tx-X rats

Transplantation of normal thyroid lobes obtained from Wistar strain rats (Ag-B^2) to both normal and Tx-X PVG/c strain (Ag-B^5) rats was carried out to assess cell-mediated immune function of Tx-X rats, to examine the nature of the induced lesion and to investigate the possibility that genetic differences in the MHC region might restrict its development.

As expected, normal PVG/c rats effectively rejected the allogeneic graft when examined 21 days post-grafting (Table 2). The histological picture was characterized by total destruction of the thyroid follicles and replacement by fibrous connective tissue (Fig. 4a). This lesion differed from the autoimmune lesions typically observed in Tx-X rats which are chiefly characterized by severe infiltration of mononuclear and plasma cells and destruction of the normal follicular architecture without fibrosis. In contrast to the normal rats, all Tx-X rats were found to be unable to reject their

allografts totally by day 21 (Table 2). Although partial rejection was evident histologically and atrophied and collapsed follicles were observed, it was often found that considerable areas of the graft had healthy intact follicles (Fig. 4b). In one case the graft appeared completely healthy without signs of follicular disorganization. In areas where follicular damage was observed there was moderate cellular infiltration predominantly with lymphocytes and plasma cells. Compared to the normal recipients there was little evidence of fibrous connective tissue replacement of the grafted tissue in Tx-X rats (Fig. 4c).

A similar pattern of events was also observed in a second group of Tx-X recipients whose grafts were examined after 28 days.

The partial survival of allografts to 28 days in Tx-X recipients pointed to some impairment of CMI function in the Tx-X situation.

Unlike syngeneic grafting, no obvious relationship was apparent between the lesions in the donated graft and the recipient's thyroid and some degree of mononuclear infiltration was always noted even in the grafts of Tx-X recipients where thyroiditis was absent. However, it was generally observed that there was more extensive infiltration in the grafts of those recipients which developed moderate or severe thyroiditis. This may therefore represent an autoimmune infiltration superimposed upon a weak allograft reaction.

DISCUSSION

Thyroid grafting provides a convenient method of investigating in sequence the immunopathological events taking place in this gland during the course of an autoimmune response in an affected animal and of the process of regulation and resolution of such events in the normal animal. This approach also eliminates the direct involvement of irradiation in the pathogenesis of the lesion and complements previous evidence to support the conclusion that irradiation of the thyroid *per se* is not directly responsible for the lesion in this particular model (Penhale *et al.*, 1973, 1976).

The character of the cellular infiltration observed in the ectopic grafted thyroids appeared to be identical to that developing in the recipient's own thyroid gland and also the extent and severity of lesion in the two glands correlated directly. This observation not only eliminates direct thyroid irradiation as the primary initiator of thyroid damage but also as a potentiator of the severity of the lesion. It also suggests that circulating components specific for thyroid are involved in the genesis of the lesion. However, the nature of these components, whether humoral, cellular or both has yet to be determined in this particular circumstance.

The grafting of diseased thyroids under the renal capsule of normal animals led to the complete resolution of the thyroid lesion when grafts were examined some 2 months later. Furthermore, this procedure did not provoke thyroiditis or an autoimmune response to thyroid components in the recipient despite the presence of potentially harmful autoreactive cells within the lesion itself and the probable liberation of soluble factors such as autoantigens, autoantibodies or immune complex into the circulation of the recipient. These observations point to the operation of effective immunoregulatory mechanisms in the normal animal. They also show that the lesion is not self-sustaining and suggests that a constant influx of autoreactive components generated elsewhere is necessary for its development and maintenance.

Cell-mediated immunity has been implicated as a primary pathogenic mechanism in both clinical and certain experimental models of autoimmune thyroiditis (Brostoff, 1970; Calder *et al.*, 1972; McMaster, Lerner & Exum, 1961).

Thymectomy has been shown to cause considerable impairment of CMI in several species (Miller, 1961, 1962; Janković, Waksman & Arnason, 1962; Janković *et al.*, 1963; Cooper *et al.*, 1966). Similarly, Tx-X rats have decreased CMI function as evidenced by reduced T cell cytotoxicity, depressed lymphocyte transformation by PHA and lymphopenia (Penhale *et al.*, 1976). The reduced ability of Tx-X rats to reject allogeneic thyroid grafts as determined in this study further reflects a depressed CMI function and lends support to the hypothesis that humoral mechanisms are implicated in the effector arm of the autoimmune mechanism in this particular model (Penhale *et al.*, 1973). In this respect this model appears to be more analogous to others in

which the thyroiditis develops spontaneously without the requirement for immunization with thyroid antigens and adjuvants (Kite *et al.*, 1969; Hajdu & Rona, 1969; Silverman & Rose, 1971). In contrast, there is substantial evidence for the involvement of the CMI mechanism in the conventional antigen/adjuvant model (McMaster *et al.*, 1961; Flax, Janković & Sell, 1963; Lin & Salvin, 1976; Ben-Nun *et al.*, 1980). If, as inferred, CMI function is indeed impaired following Tx-X, then the cellular infiltrate observed in many of the allografts may be involved in the autoimmune process rather than a rejection reaction and this would clearly imply that genetic restriction does not operate in this particular model. Such a finding would once again point to the non-involvement of a CMI mechanism, many functions of which are now well known to be genetically restricted.

Further grafting studies are now underway utilizing parenteral strain thyroid grafts to F_1 hybrid Tx-X offspring in order to resolve this question more precisely.

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