Histopathological findings in the combined immunity-deficiency syndrome

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SYNOPSIS The histopathological appearances of the thymus, lymph nodes, spleen, and gut-associated lymphoid tissue (tonsil, Peyer's patches, and appendix) in cases of the combined immunity-deficiency syndrome are presented. The appearance of tissues remaining after foetal thymic transplants and the effects of such transplants on the morphology of lymph nodes are also discussed.

Although thymic appearances are remarkably constant the picture in the lymph nodes and spleen may vary considerably. The tonsils, Peyer's patches, and appendix appear to constitute one lymphoid organ in man, at least with regard to the developmental arrest that may occur in this syndrome.

In a previous study (Berry, 1968) a surprisingly high incidence of 1.8% of 1,000 thymuses showing epithelial dysplasia was demonstrated. A clinico-pathological study of these cases showed that this finding was generally associated with the so-called 'Swiss type' hypo- γ -globulinaemia (Glanzmann and Riniker, 1950) but that other varieties of combined immunity-deficiency syndromes were also seen (see Berry and Thompson, 1968).

This paper concerns the histopathological findings in the lymphoid tissues obtained by biopsy or at necropsy in a total of 31 cases of the combined immunity-deficiency syndrome seen at the Hospital for Sick Children, Great Ormond Street.

Two cases of total thymic agenesis, associated with anomalies of the aortic arch and absent parathyroid glands, are excluded. Lymphoid tissue development in these cases has been discussed recently by Dische (1968).

Materials and Methods

In 22 cases, including 14 from the original series

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(Berry, 1968), necropsy material comprising all viscera and segments of small and large intestine were available for study. In a further four cases permission for only a limited necropsy had been obtained and blocks of thoracic viscera taken. In one instance, permission to remove a grafted thymus was given but no further tissues were obtained. In five cases, surgical biopsy material was available (lymph nodes in four cases, appendix in one).

All material was formalin fixed, processed and embedded in paraffin wax and sectioned at 5 μ m.

In order to assess the normal rate of histological development of the human thymus, sections from 116 therapeutic abortions between 10 and 16 weeks' gestation were examined.

Results

THYMUS

Morbid anatomy

A consistent and striking feature of the 25 cases examined was the tendency of the small thymic remnant to be found largely above the innominate vein. In all instances the gland weighed less than 3 g; in 20 cases, less than 2 g.

Histopathology

All glands show a strikingly similar appearance. The appearance of a simple lobular pattern of the gland, with considerable amounts of interstitial tissue that is seen early in development, persists (Figure 1). The lobules are composed of mesenchymal and epithelial cells with poor or absent cortico-medullary demarcation and paucity of lymphocytes. Hassall's corpuscles are absent or grossly reduced in numbers. In some instances, a 'glandular' or 'alveolar' pattern is seen at the periphery of the lobules (Figs. 2 and 3) but formation of a lumen is never seen. Reticulin stains show some tendency to enclose small

peripheral groups of cells (Figure 4). In two cases, the absence of Hassall's corpuscles and a lobular pattern was associated with gross depletion of lymphoid tissue but with some remaining lymphocytes seen in the putative cortex (Figure 5). Mononuclear cells were seen in the medulla.

Three foetal thymuses grafted to the anterior sheath as part of reconstitutive therapy were available for study. One showed complete necrosis and organization after six weeks *in situ*. The others, after longer periods, were well maintained and histological examination suggested that further development might have occurred after implantation (Figures 6 and 7).

Fig. 1.

Fig. 3.

Fig. 1 Thymus: 'lobular' pattern and lack of cortico-medullary demarcation. Haematoxylin and eosin (H & E). $\times 40$.

Fig. 2 Thymus: alveolar pattern seen in a thymic lobule. H & E. $\times 180$.

Fig. 3 Thymus: reticular and epithelial cells and a few lymphocytes are seen. H & E. $\times 480$.

Fig. 4 Thymus: reticulin pattern of thymic lobule. Gordon and Sweet. $\times 50$.

Fig. 5 Thymus: lymphocytes are seen in the prospective cortex. H & E. $\times 50$.

Fig. 6 Foetal thymus: implanted in rectus sheath. H & E. $\times 25$.

Fig. 7 Foetal thymus: large mononuclear cell clusters in a lobule of implanted thymus. H & E. $\times 140$.

Fig. 2.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 8 *Lymph node: reticulin structure. The subcapsular sinus is widely patent. Gordon and Sweet. $\times 50$.*

Fig. 9 *Lymph node: 'mesenchymal' appearance. Note the subcapsular sinus. H & E. $\times 60$.*

Fig. 10.

Fig. 10 *Lymph node: occasional lymphocytes seen at the periphery of the node. H & E. $\times 480$.*

Fig. 11 *Lymph node: follicular structures at the periphery of the node. H & E. $\times 50$.*

Fig. 12 *Lymph node: large reticulum cells in node. H & E. $\times 480$.*

Fig. 13 *Lymph node: reticulum pattern. Note the massive nodular proliferation of cells distorting the pattern, and poor development of the 'thymus-dependent' zone. Gordon and Sweet. $\times 7$.*

Fig. 14.

Fig. 15.

Fig. 16.

Fig. 14 *Lymph node: large reticulum cells, some with atypical features in node. H & E. $\times 480$.*

Fig. 15 *Tonsillar fossa: folded epithelium in tonsillar crypt. H & E. $\times 40$.*

Fig. 16 *Colon: small collection of lymphocytes in the submucosa. The muscularis mucosa is not interrupted. H & E. $\times 140$.*

LYMPH NODES

Twenty-four cases were examined, in two, only surgical biopsies were seen. In four cases, lymph nodes were not found at necropsy despite an extensive search and step sectioning of the 'Swiss roll' type of preparation of the mesentery in two infants. Appearances in other cases varied widely, but in general two main groups were found.

Group 1 consisted of essentially 'mesenchymal' nodes in which the reticulin pattern showed no evidence of distension of follicular proliferation of lymphocytes (Figure 8). These nodes often appeared to be masses of connective tissue and were recognizable only by virtue of the distinctive subcapsular sinus seen (Figure 9). The cellular populations of such nodes included few lymphocytes (Figure 10).

Group 2 included cases of nodes with well defined follicles in an otherwise 'hypoplastic' structure with a small cellular population and widely open sinusoids (Figure 11).

Intermediary forms were seen with varying numbers of lymphoid cells present, and both types might be seen in one case, but a prominent feature in both groups was the presence of many large cells with abundant cytoplasm and large pale nuclei with prominent nucleoli, presumed to be reticulum cells or histiocytes, (Figure 12). These cells are of interest, since in one case in which foetal thymus grafting and liver cell transfusion had been undertaken, axillary lymph nodes, previously impalpable,

Fig. 17 Spleen: absence of perivascular lymphocyte cuffs. H & E. × 160.

Fig. 18 Spleen: abundant perivascular lymphocytes in splenic arteries. H & E. × 160.

had become readily palpable and enlarged. Histological sections had shown massive proliferation of such cells with distortion of normal nodal architecture (Figures 13 and 14). Several nodes showed evidence of haemo-

phagocytosis. In no instance were two component 'reactive' follicles seen.

GUT-ASSOCIATED LYMPHOID TISSUES

Tonsil

In three instances, tonsils were absent, with sections showing folded crypt epithelium in the tonsillar fossa without evidence of lymphoid tissue (Figure 15). In four other instances hypoplastic tonsils with small two-cell component follicular structures were present.

Alimentary tract and appendix

There was a constant association between lymphoid development of the alimentary tract and appendix and the tonsil; in no instance of tonsillar hypoplasia or agenesis was lymphoid tissue present in the gut in significant amounts (Figure 16). 'Significance' was considered to be present if the lymphoid tissue muscularis mucosa was breached by the collection of lymphoid tissue at the sites of Peyer's patches. The lamina propria in these cases was hypocellular, with absence of cells having plasma cell morphology.

Spleen

In all cases the spleen was present, and in all of the 22 cases examined was within the normal weight range for the age. Histological appearances varied with sinusoidal hyperplasia and erythrophagocytosis, seen in some instances in which lymphocytes were scanty (Fig. 17), and lympho-

Case	Splenic Follicles	Lymph Nodes	Lymphoid Tissue in Gut	Tonsils
1	Present	No nodes found	Absent	Absent
2	Present	No nodes found	Hypoplastic	Absent
3	Not seen	No nodes found	Hypoplastic	Absent
4	Hypoplastic	No follicles present	Hypoplastic	Hypoplastic
5	Not seen	No follicles present	Hypoplastic	Hypoplastic
6	Present	No nodes found	Hypoplastic	Hypoplastic
7	Present	No nodes found	Present	Normal
8	Present	Single component follicles present	Present	Normal
9	Present	No follicles present	Present	Normal
10	Present	No follicles present	Present	Normal
11	Present	Single component follicles present	Present	Normal
12	Present	No follicles present	Present	Normal
13	Present	Single component follicles present	Present	Normal
14	Present	Single component follicles present	Present	Normal
15	Not seen	Single component follicles present	Hypoplastic	Hypoplastic
16	Not seen	Single component follicles present	Present	Hypoplastic
17	Present	Single component follicles present	Present	Hypoplastic
18	Present	Single component follicles present	Present	Hypoplastic
19	Present	No follicles present	Present	Hypoplastic
20	Present	Single component follicles seen	Present	Hypoplastic
21	Hypoplastic	No follicles present	Present	Hypoplastic
22	Present	No follicles present	Present	Hypoplastic

Table I Findings in other lymphoid organs in 22 cases of thymic dysplasia seen at necropsy.

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cytic perivascular cuffs and follicles seen in other cases (Figure 18).

A summary of histopathological findings in the 22 cases in which all tissues were examined is given in the Table.

Discussion

The 'high' position of the thymus in this syndrome has previously been commented on by Miller and Schieken (1967) who pointed out that the thymic remnant might be associated with the thyroid gland. This positional defect is presumably related to abnormal development of the third and fourth branchial arches, seen in its most severe form in thymic aplasia, with absent parathyroids, and with associated anomalies of the aortic arches (Dische, 1968).

The uniformity of histological appearance in the thymus in cases of thymic dysplasia is evident from previous reports (Tobler and Cottier, 1958; Gitlin and Craig, 1963; Gitlin, Vawter, and Craig, 1964; Berry and Thompson, 1968). In a light and electron microscopic study of four cases, Blackburn and Gordon (1967) also commented upon the uniformity of histological appearances, and mentioned the interesting point, also noted in the present series, that connective tissue stains revealed 'less collagen than would have been expected after viewing haematoxylin and eosin preparations' in the thymic lobules. Blackburn and Gordon also illustrated the glandular appearance of the lobules of the dysplastic gland and showed the component cells of this region to be epithelial in origin. They suggested that the appearances of the thymus were consistent with an arrest in development at the 30 mm stage, at which time corticomedullary demarcation in the human foetus becomes apparent. They considered that arrest had occurred at a stage immediately following penetration of the epithelial primordium by mesenchyme but before lymphoid differentiation.

It was in order to assess this point that aborted foetuses were examined, and a study of the thymic medulla was undertaken so as to assess the normal rate of development of the epithelial component of the thymus gland. The development of Hassall's corpuscle-like structures was preceded by the accumulation of mononuclear cells, which formed aggregates in the developing medulla of the gland (Figure 19). The cells then developed cytoplasmic inclusions in their cytoplasm, and Hassall's corpuscles gradually developed by coalescence within such cell masses.

It was found that in this group of foetuses, in which crown/rump length had been measured before fixation, the stage at which such mononuclear cells appeared in the thymic medulla varied considerably, as did the development of Hassall's corpuscles. In general, however, it

Fig. 19 Foetal thymus: mononuclear cell aggregates with fusion of cells. H & E. $\times 120$.

could be said that some evidence of thymic epithelial differentiation was present before 65 mm in the normal gland, and Hassall's corpuscles might be present by 70 mm, but were occasionally not present at the 100 mm stage.

Blackburn and Gordon (1967) had suggested that there was an endodermal failure in development in these cases, and that the anomaly in thymic lymphoplasia was related to absence of Hassall's corpuscles. In a previous study (Berry, 1968), using the numbers of Hassall's corpuscles as an index of thymic epithelial development, the author has been able to select cases of the combined immunity deficiency syndrome from a blind study of a necropsy series. This finding supports the view that a defect in thymic epithelial maturation is of fundamental importance in the genesis of thymic dysplasia, but that possibly the abnormality may develop later than suggested by Blackburn and Gordon (1967).

The presence of large mononuclear cells in two cases, although not in aggregates, may indicate a less severe degree of anomaly. In both instances in which these cells were present, organized lymphoid tissue was seen in the gut, and follicular structures were present in the spleen and lymph nodes.

Reconstitution therapy at this hospital has involved the transplantation of foetal thymus and transfusion of foetal liver cells in an attempt

Fig. 20 Bone marrow: proliferation of reticulum cells and eosinophils. H & E. $\times 480$.

to repair a thymic defect and to supply stem cells. Thymic grafts may survive in these circumstances and maintain architectural and cytological patterns consistent with the stage of development that would have been reached in the period of gestation plus the time elapsed since grafting. The presence of mononuclear cell aggregates in the grafted thymus (see Fig. 7), removed from a 63 mm embryo, suggests that functional epithelial elements were present in this gland, and may relate to the appearance of lymph node enlargement, increase in peripheral lymphocyte count, and histological changes suggesting active proliferation of cells in lymph nodes and bone marrow.

Other attempts at grafting in this syndrome have generally been unsuccessful (see Kretschmer, Jeannet, Mereu, Kretschmer, Winn, and Rosen, 1969, for a bibliography), although reports of some success in this field have been made recently (de Koning, Dooren, van Bekkum, van Rood, Dicke, and Rádl, 1969). Nevertheless, the alteration in nodal histology seen in our case, together with the massive proliferation of histiocyte-like cells and eosinophils in the bone marrow (Fig. 20), is apparently unique.

The essential variability in the histological

appearances of lymph nodes in these cases is remarkable. A range of changes, including complete absence, gross hypoplasia, and some hypoplasia with primary follicle formation, is seen. No strict correlation of severity of change with duration of disease is seen, but those cases with absent lymph nodes tended to die earlier than the rest of the group (Berry and Thompson, 1968). Haemophagocytosis seen in five cases was severe in one instance in which the degree of change was reminiscent of familial haemophagocytic reticulosis (Farquhar and Claireaux, 1952). The proliferation of cells in the patient receiving the thymic transplant resembles reticulum-cell sarcoma in some respects, and is of interest in view of the higher incidence of this neoplasm in patients with immune deficiency states, and in patients undergoing immuno-suppressive therapy (Deodhar, Kuklinca, Vidt, Robertson, and Hazard, 1969). Reticulum cell hyperplasia has been noted in lymphoid organs following transplantation (Standen, Esterly, and Pearson, 1969).

The severity of splenic changes showed no correlation with the severity of the disease. In one instance, however, that case in which remarkable haemophagocytosis was seen in the lymph nodes, the splenic pulp contained large numbers of reticulum cells stuffed with red cells, and the child was noted to have a haemolytic anaemia clinically.

The finding of a constant association of hypoplasia of the tonsil with absent or grossly deficient lymphoid tissue at various sites in the gut is strong supportive evidence for the hypothesis proposed and verified by Cooper, Perey, McKneally, Gabrielsen, Sutherland, and Good (1966), Perey and Good (1968), and Perey, Cooper, and Good (1968) that such tissues represent a distinct lymphoid organ in mammals, possibly the equivalent of the avian bursa of Fabricius. These tissues should be concerned with the development of humoral rather than cellular immunity. In the six cases in which tonsillar hypoplasia has been present in this series, however, immunoglobulin levels have been normal or high in two. It is of course true that histological assessment of hypoplasia is a crude method of assessment of the potential function of an organ, and the possibility that all immuno-chemically determined immunoglobulin molecules may not be functioning antibody must be considered.

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