The incidence of germinal centres in thymus glands of patients with congenital heart disease

J. M. VETTERS AND R. S. BARCLAY

From the University of Glasgow, Department of Pathology, Western Infirmary, Glasgow, and the Thoracic Unit, Mearnskirk Hospital, Glasgow

SYNOPSIS  The incidence of thymic germinal centres in normal individuals is disputed by various authors. The present study gives the results of a detailed histological assessment of 75 thymic biopsies obtained during operations to correct congenital heart lesions. Serial sectioning showed structures previously of disputed significance to be obliquely sectioned germinal centres. Comparison with the data of Hammar (1926) showed that there was some stress involution of the glands studied. Despite this 40% of the subjects had thymic germinal centres. Germinal centres were not detected in subjects under 4 years of age and there were insufficient subjects over 16 years of age to allow assessment of the frequency in adult thymus glands.

It is now recognized that the thymus is involved both in the maintenance of immune competence and the genesis of autoimmune diseases. Consequently, much attention has been paid to thymic histology in diseases, such as myasthenia gravis, in which thymic germinal centres are common. However, information on the incidence of germinal centres in the thymus gland of normal individuals is fragmentary and often contradictory (table I).

The descriptive terms used in the publications on this subject vary and it is clear that similar terms are used to refer to different types of structure. Where reference is made to another publication the descriptive term employed, 'lymphoid follicle', 'germinal centre', etc, is that used by that author; the definitions stated by the various authors are given in the discussion.

Although Sloan (1943) described thymic germinal centres in approximately 10% of a group of subjects who died suddenly, Castleman and Norris (1949), Anderson (1956), Burnet (1962), and Burnet and Mackay (1965) stated that thymic germinal centres did not occur in normal subjects. Interest in the topic was rekindled in 1967 by Middleton's observation that 51% of a group of patients dying shortly after accidents had structures in the thymus which he identified as lymphoid follicles (the incidence in subjects under 40 years of age was 72%). Middleton also noted that subjects who died after a terminal illness of less than three days' duration had a much higher incidence of thymic lymphoid follicles than those who had more protracted illnesses. Middleton also stated that many of the thymic lymph follicles detected in accidentally killed subjects were of the same size as those found in thymus glands removed surgically from patients with myasthenia gravis.

Studies of patients with congenital heart diseases by Bhatthal and Campbell (1965) and by Henry (1968) have reported germinal centres in approximately 20% of individuals. However, Goldstein and Mackay (1967) in a study of thymus glands similarly biopsied during operations to correct congenital heart lesions or obtained at necropsy after brief illnesses detected thymic germinal centres in only two of 94 individuals. Okabe (1966) detected only 18 subjects with thymic germinal centres in a study of 1356 necropsies whilst Habu, Kameya, and Tamaoki (1972) detected thymic lymph follicles in approximately 17% of subjects dying rapidly from accidental causes.

The present study was performed in an attempt to resolve these widely divergent findings and to determine the following points: (1) Do the thymus glands of patients with congenital heart disease show stress involution, i.e., do the percentages of the glands occupied by cortex and medulla differ from Hammar's (1926) data on normal subjects? (2) In what proportion of patients operated on for congenital heart disease are germinal centres present in the thymus gland? (3) Does the age affect the incidence of thymic germinal centres?

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Materials and Methods

Biopsies were obtained from 75 patients during operations to correct congenital heart disease. Cases of rheumatic heart disease were excluded because rheumatic fever is believed to have an immunological basis. The ages of the subjects ranged from 13 days to 39 years of age; 36 were males. The age and sex distributions are shown in figure 1.

The operations took place between November 1968 and February 1971.

Technical Methods

PROCESSING FOR HISTOLOGICAL EXAMINATIONS

All thymic tissue was placed in 10% formal saline immediately after excision. After fixation blocks were trimmed, processed, and embedded in paraffin wax using conventional techniques. Sections were cut at 6 μ thickness and stained with haemalum and eosin.

MENSURATION

The proportions of each thymus gland occupied by cortex, medulla, and interstitial tissue were determined by point counting. The minimum number of points counted in any case was 500. If the tissue sections were too small the slides were turned round and recounted and the two scores summated.

An outline drawing of each histological section was obtained using a Wild M20 microscope with drawing tube. The apparatus was calibrated to give a linear magnification of ×10. The area of each outline drawing was determined with a Haff 317 planimeter.

Each section was systematically scanned using the ×10 objective. Germinal centres were identified by the following criteria: large pale cells in a discrete mass in which mitotic figures and phagocytosed nuclear debris (tingible bodies) were identified. The cluster of pale cells was usually surrounded by a cuff of lymphocytes (see figs 2 and 6).

In addition to classical germinal centres, other structures were present in the medulla which had some of the features of germinal centres. Various authors have interpreted these structures differently and have used different names; in this study to avoid confusion they have been called 'rounded lymphoid clusters' (see figs 3-5).

Three appearances were recognized: (1) Sharply circumscribed clusters of lymphocytes, the peripheral cells of which were in clearly defined rows. (2)
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Fig 2  Germinal centre. The lymphocytic cuff surrounding the pale central cluster of reticulum cells and macrophages is clearly seen. The pale centre contains mitotic figures (M) and tingible bodies. HE × 400.

Fig 3  Rounded lymphoid cluster. This consists of a large dense cluster of lymphocytes. HE × 400.

Fig 4  Rounded lymphoid cluster. In this example small numbers of large pale reticulum cells are present in the cluster of lymphocytes. HE × 250.
Clusters of lymphocytes in the centre of which there were pale cells reminiscent of those seen in germinal centres but lacking both mitotic figures and tingible bodies. (3) Structures closely resembling germinal centres but lacking either tingible bodies or mitotic figures.

The total numbers of germinal centres and rounded lymphoid clusters were ascertained in each section. Their sizes were determined using a calibrated eyepiece graticule; measurements of each structure were made in two axes at right angles to each other and the mean was calculated.

Results

Comparison with Hammar’s data

The percentages of thymic tissue occupied by cortex and medulla were determined in subjects in the following age groups: birth-5 years, 6-10 years, and 11-15 years. There were insufficient biopsies from subjects over 16 years of age to allow meaningful statistical evaluation. The results are shown in table II. It will be noted that in the first group there is a highly significant diminution in the amount of cortex compared with that in Hammar’s subjects. In addition, the birth-5 years and 11-16 years age groups show a significant increase in the percentage of the thymus occupied by medulla. These figures indicate stress-induced change of the thymus; the changes are most severe in the youngest subjects.

The implication is discussed below.

Incidence of germinal centres and rounded lymphoid clusters

Twelve individuals (16%) had classical germinal centres in the thymus gland. Of these, seven (9.3%) also had rounded lymphoid clusters. Of the remain-
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<table>
<thead>
<tr>
<th>(Age yr)</th>
<th>Percentage Gland Occupied by Cortex</th>
<th>Percentage Gland Occupied by Medulla</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Hammar</td>
<td>Present Series</td>
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<tr>
<td>0-5</td>
<td>56.82 ± 1.73</td>
<td>49.61 ± 1.26</td>
</tr>
<tr>
<td>6-10</td>
<td>48.56 ± 1.51</td>
<td>44.45 ± 2.67</td>
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<tr>
<td>11-16</td>
<td>45.5 ± 1.26</td>
<td>41.37 ± 2.63</td>
</tr>
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</table>

Table II Comparison of Hammar's data with those of the present series

Neither germinal centres nor rounded lymphoid clusters were found in children under 4 years of age. However, they occurred both in children over that age and in adults. In subjects under 16 years the combined incidence is approximately 38% (26/29) and in subjects between 17 and 39 years the incidence of rounded lymphoid clusters is 4/6. No true germinal centres were seen in this group. There is a significant relationship between the ages of those with and without germinal centres and rounded lymphoid centres; the average age of those without the structures is 6 years and those with the structures is 8.6 years (Student's t = 1.739, p = 0.043). This is because of the absence of the structures in subjects under 4 years of age.

SEASONAL VARIATION IN INCIDENCE OF REACTIVE LYMPHOID STRUCTURES

It was found that the incidence of lymphoid structures varies with the season. The highest incidence occurred during the months November to February. When subjects under 4 years of age are excluded, 60 subjects remain. The monthly incidence in these subjects is shown in table IV; this variation is not statistically significant.

COMPARISON OF SIZE OF GERMINAL CENTRES WITH THOSE IN A GROUP OF PATIENTS WITH MYASTHENIA GRAVIS

The sizes of the all true germinal centres detected in the present series were compared with those found in comparable sections of thymus glands removed surgically from a group of 14 patients with myasthenia gravis. The mean size of the germinal centres in the present series was 217 ± 19.9 µ and the mean size of germlal centres in the patients with myasthenia gravis was 304 ± 11.4 µ. The difference between these groups is statistically highly significant (t = 3.6715, p < 0.001).

Table III Incidence of germinal centres and rounded lymphoid clusters at different ages

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of Subjects</th>
<th>Number with Germinal Centres</th>
<th>Number with Rounded Lymphoid Clusters Only</th>
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<tr>
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<td>3</td>
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Table IV Monthly incidence of thymic reactive structures

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<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
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<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Negative</td>
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<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
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<td>0</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Table IV Monthly incidence of thymic reactive structures
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Fig 7 Serial sections were made of thymic tissue removed from an apparently normal male. The illustrations are intended to demonstrate the similarity of the appearances in some levels to those seen in figures 2-5.

(a) A distinct cluster of lymphocytes is present. ×100.

(b) At one end the lymphocyte cluster contains a circular clump of reticulum cells 12 μ from (a) ×100.

(c) The cluster of reticulum cells is now more distinct but is still very small 30 μ from (a) ×100.

(d) Detail of (c). The pale centre contains a mitotic figure and tingible bodies. This is therefore a true germinal centre. ×400.

(e) The pale centre is appreciably larger 72 μ from (a) ×100.

(f) Detail of (e). Only tingible bodies are seen in the pale centre; in the present study structures like this were counted as rounded lymphoid clusters. ×400.

(g) The pale centre has now disappeared and only a dense cluster of lymphocytes remains 156 μ from (a) ×100.
Discussion

Comparison with Hammar's (1926) data
From the observations of Sloan (1943) and Middleton (1967) it is known that stress causes a marked reduction in the incidence of thymic germinal centres. Further, study of the copious data obtained by Hammar (1926, 1929) shows that subjects who die after stressful illnesses have relatively less cortex and more medulla in the thymus gland than subjects who died suddenly after accidents. Therefore, in a study of the incidence of reactive structures in thymus glands it is best to use unstressed glands, ie, from subjects dying rapidly from accidental causes. Because of Scottish legal practice it was not practical to obtain such a series. Instead glands biopsied during operations to correct congenital cardiac lesions were used. In this case it is necessary to assess whether there is alteration of the percentage of the thymus glands occupied by cortex and medulla by comparing the results with those of Hammar (1926) who painstakingly quantitated glands of subjects who died suddenly from accidental causes. This comparison makes it possible to assess whether the glands are normal or show signs of stress-induced change.

The methods used by Hammar, although tedious, are accurate and differences between the present studies and his reports cannot be attributed to technical causes. It is true that Hammar obtained his material over 50 years ago when malnutrition and infectious diseases were more prevalent in European urban communities than today. Additionally, Hammar's subjects were exposed to the severe Scandinavian winters. Since malnutrition, cold, and infection are all causes of stress it is probable that any influence they would have had would have been to reduce the differences between the groups rather than accentuate them.

It is reasonable to conclude that since there is stress-induced atrophy in the thymus glands of patients with congenital heart disease the incidence of germinal centres is lower in them than in healthy subjects.

Incidence of Germinal Centres
The nomenclature and identification of reactive lymphoid structures (lymph follicles and germinal centres) in human thymus have been a source of difficulty to many workers, eg, Burnet and Mackay (1965), Middleton (1967), and Goldstein and Mackay (1969). Basically the problem is the classification of structures which do not fulfil all the histological criteria of germinal centres. Goldstein and Mackay (1967) discounted such structures whilst Burnet and Mackay (1965) considered them to be immature germinal centres. Middleton (1967) attempted to resolve the problem by cutting several sections when circumscribed clusters of medullary lymphocytes were found. When he found a cluster of pale central cells by this technique he counted the structure as a lymphoid follicle; mitotic figures and tingible bodies were not considered essential.

Habu et al (1971) concentrated their attention on lymphoid follicles which they defined as 'well demarcated dense accumulations of lymphocytes . . . which seem to shrink away from the surrounding tissue'.

It is clear that because of the various criteria which have been used by different authors it is difficult to compare their data as presented. However, the demonstration by Millikin (1966) of the polar arrangement of human germinal centres helps to resolve the difficulty (fig 6). Sections of a germinal centre other than through the polar axis results in a variety of appearances, many of which do not fulfil the classical criteria used to identify germinal centres (figs 7 and 8).

In the present study these have been called rounded lymphoid clusters. Habu et al (1971) used the term lymphoid follicle to include both germinal centres and rounded lymphoid clusters. Middleton
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(1967) also used the term 'lymphoid follicle' but in a more restrictive way in that he ignored 'solid' clusters of lymphocytes. Burnet and Mackay (1965) used the term 'germinal centre' but included some structures not fulfilling all the criteria for germinal centres. Henry (1968) counted only classical germinal centres. The results of the various authors are conflicting (see table I).

Some of the variation may be due to the ages of the subjects examined. Lymphoid follicles and germinal centres are very infrequent in subjects over 40 years of age (Middleton, 1967; Habu et al., 1971). However, the principal causes of the discrepancies lie in two factors: (a) whether the glands examined came from stressed subjects, and (b) the criteria used to identify germinal centres. These will be considered separately.

Influence of stress
Following the studies of Sloan (1943), Middleton (1967), and Habu et al. (1971) (see table I) there can be no doubt that stress reduces or abolishes thymic germinal centres. When necropsy material is used failure to use only subjects who have died very rapidly has resulted in the difficulty in identifying thymic germinal centres. However, biopsies obtained during thoracic operations may also show alterations due to the stress caused by the disease (usually congenital heart disease) which necessitated the operation. The present study has shown that the percentage of the thymus occupied by cortex is decreased and the percentage occupied by medulla is increased in patients with congenital heart disease. These are the same alterations observed by Hammar (1929) in his studies of large numbers of subjects who had died from a wide variety of diseases. The alterations are most marked in the youngest subjects in our series and this is to be expected since they have the most severe cardiac lesions. It is therefore reasonable to conclude that the incidence of germinal centres and lymph follicles in patients operated on for congenital heart disease is lower than in normal subjects of corresponding age.

Criteria used to identify germinal centres
The classical features used to identify germinal centres are the presence of large cells with relatively pale cytoplasm surrounded by a cuff of lymphocytes. The pale cells must contain mitotic figures and there must also be phagocytosed nuclear debris (tingible bodies). Some authors have adhered rigidly to these criteria, e.g., Goldstein and Mackay (1967) and Henry (1968). Some have used more liberal interpretations and others have not defined the structures they called germinal centres, e.g., Bhathal and Campbell (1967). Naturally those who adopted stringent criteria found appreciably fewer cases with thymic germinal centres. However, by cutting serial sections we have shown that many structures which these authors would have discounted are in fact true germinal centres which have been sectioned obliquely so that all the histological features are not visible in one histological section. It is our view that the structures which do not meet the full criteria for germinal centres (which we have termed rounded lymphoid clusters to distinguish them from the classical germinal centres) may be legitimately counted as germinal centres. This being the case, the overall incidence of germinal centres in the group of subjects examined ranging up to 40 years of age is approximately 39%. Since we have shown that the subjects studied had stress involution of the thymus as demonstrated by alteration of the percentages of their thymus glands occupied by cortex and medulla it is reasonable to conclude that the incidence is even higher than this and that Middleton’s figure, though disputed by others, is likely to be correct.

Figure 8 was kindly drawn by Mr R. Callander, Medical Illustrations Unit, University of Glasgow. This project was aided by the Macmillan Research Funds of the University of Glasgow.

References
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