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Intra- and extrathymic B cells in physiologic and pathologic conditions

Immunohistochemical study on normal thymus and lymphofollicular hyperplasia of the thymus*, **

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Summary. Normal thymuses and thymuses with lymphofollicular hyperplasia have been examined immunohistologically using immunoenzymatic single and double labelling methods and a panel of monoclonal antibodies against B lymphocyte differentiation antigens (CD19-, CD20-, CD21-, CD22-, CD23- and CD37ag) and human immunoglobulins (IgM, IgD) for the presence and localisation of B lymphocytes and cells expressing B cell differentiation antigens. The numerous hyperplastic lymph follicles which occur in the pathological condition of lymphofollicular hyperplasia of the thymus were found to originate in the extrathymic compartment of the interlobular septal space. This area was found to be blown up by the growing lymph follicles with exactly the same cellular composition as their counterparts in the peripheral lymphatic tissue. Some of the B lymphocytes expressing the immunophenotype of follicular mantle zone lymphocytes which were detected in the thymic medulla probably infiltrated through discontinuities of the border between the perivascular space and the thymic medulla. Apart from this primarily extrathymic B cell compartment, B lymphocytes and cells expressing B cell antigens were found within the thymus medulla of normal control thymuses of different ages from fetal to adult life. These cells were detected as a small subpopulation in normal fetal, juvenile and adult thymuses. Morphologically they could be subdivided into small, round lymphoid cells accounting for less than 1% of medullary lymphoid cells, and into a larger variant, asteroidally shaped because of short cytoplasmic processes. These asteroid cells were even more infrequent than the lymphoid variant. Immunophenotype (CD19ag+, CD20ag+, CD22ag+, CD37ag+, IgM+, IgD+) and morphology of the first cell type led to the conclusion that the lymphoid cells were in fact B lymphocytes. They were scattered throughout the medulla of fetal and juvenile and adult thymuses alike. The second, the asteroid cell type, constantly expressed CD20ag and inconstantly IgM, CD22ag and CD37ag; furthermore, CD23ag was detected in a subset of the asteroid cells either restricted to the perinuclear zone or expressed in the entire cytoplasma and on the plasma membrane. The asteroid cells were located in the corticomedullary region of the fetal thymuses but were randomly distributed with a tendency to Hassall's corpuscles in juvenile and adult thymuses. They often formed rosettes with non-B lymphocytes. It can be concluded that a small number of B cells and asteroid cells of still uncertain origin, but expressing B cell antigens, are constitutive elements of the fetal and adult thymic medulla. It can be assumed that the asteroid cell might represent a novel type of thymic accessory cell and that the rosetting of non-B lymphocytes around this asteroid cell might simulate or in fact be the earliest B cell interaction of maturing T cells.

Key words: Thymus – Lymphofollicular hyperplasia – B lymphocytes

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** Dedicated to Prof. Dr. V. Becker on the occasion of his 65th birthday

Abbreviations: mAb(s): monoclonal antibody(-ies); CDxxag: antigen defined by the mAb cluster xx; CDxx(mAb): mAb of the cluster xx

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Introduction

The thymus is the central organ of T lymphocyte maturation and differentiation (for review see

Haynes 1984; Haynes 1986) where immature thymocytes, identifiable by their characteristic immunophenotype, acquire the qualities of the mature, peripheral T lymphocytes with their distinct immunophenotype (Tidman et al. 1981). Among the events occurring within the thymus is the acquisition of MHC-restriction and tolerance induction to self antigens of maturing T cells and the elimination of autoreactive T cell clones (Kappler et al. 1987; Lo et al. 1986; Tutschka 1987).

There are, however, some pathological conditions under which B lymphocytes do occur within this domain of T lymphocytes. Lymphofollicular hyperplasia of the thymus (Castleman 1966) and the state of so called "thymitis" (Otto 1984; Kirchner et al. 1986) illustrate this. Moreover, there is an entity of B cell lymphomas occurring in the anterior mediastinum, which have been previously described (Möller et al. 1986) and which already have been considered to be of thymic origin (Addis and Isaacson 1986).

Concerning lymphofollicular hyperplasia, it is still uncertain whether the lymph follicles belong to the extrathymic or intrathymic compartment. Furthermore, the cellular composition of the lymph follicles has up to now not been examined with a large panel of clustered monoclonal antibodies to B cell differentiation antigens, in order to compare them with the lymph follicles of the peripheral lymphatic tissue. A prerequisite for a thymic origin of mediastinal clear cell lymphoma is the occurrence of thymic B lymphocytes or thymic cells of B lymphocyte lineage. This has not been investigated systematically so far.

We therefore examined lymphofollicular hyperplasia of the thymus and normal control thymuses from the fetal to adult periods by means of immunoenzymatic methods, using a panel of monoclonal antibodies to investigate firstly, the cellular composition and the localisation of the lymph follicles of lymphofollicular hyperplasia, and secondly the occurrence of B lymphocytes or cells of B lymphocyte lineage within the normal thymus.

Materials and methods

Tissue

Four fetal thymuses were obtained from iatrogenic abortions carried out between the 19th and 27th week of gestation for medical reasons. Five normal juvenile/adult thymuses (age range 17–46 years) were resected together with extrathymic primary or metastatic mediastinal tumours (benign mediastinal cyst, benign mediastinal teratoma, teratocarcinoma, chondrosarcoma, osteosarcoma). In addition, one thymus was removed from a 49-year-old female and showed numeric hyperplasia (10 cm × 5 cm × 2 cm; 44 g). 10 sections from different regions of the organ as proposed by Grody et al. (1986) were examined by routine histology using one haematoxylin-eosin stained section and one stained with Schiffs' reagent (PAS) to exclude the presence of lymph follicles and other pathological conditions.

Two thymuses with lymphofollicular hyperplasia were removed from a 23-year-old female suffering from myasthenia gravis and from a 19-year-old man suffering from a metastasizing osteosarcoma of the right femur. Both thymuses revealed abundant hyperplastic lymph follicles histologically, with germinal centers within the thymus region.

Immediately after removal, the tissue was snap frozen in liquid nitrogen and stored at −70° C. Cryostat sections with a thickness of 4–6 µm were prepared, air dried overnight at room temperature and subsequently fixed in acetone for 10 min at room temperature.

Immunoreagents and chemicals

Monoclonal antibodies (mAbs) against B cell differentiation antigens used in this study are listed in Table 1. Nomenclature of antigens/antibodies was used as proposed by the nomencla-

Table 1. Primary mAbs used in this study

A MAbs against B cell differentiation antigens

Antibody cluster	Clone	Reference	Antigen distribution in B cells[a]
CD19	HD37	Pezzutto et al. (1986)	pan-B and B-progenitors
	B4	Nadler et al. (1983)	
CD20	B1	Stashenko et al. (1980)	pan-peripheral B
CD21	B2	Nadler et al. (1981) Tedder et al. (1984)	restricted B
CD22	HD6	Moldenhauer et al. (1986) Pezzutto et al. (1987)	restricted B
	HD39	Dörken et al. (1986)	
CD23	HD50	Dörken et al. (1987)	restricted B
	B-LAST-2	Thorley-Lawson et al. (1987)	
CD37	HD28	Schwartz et al. (1987) Moldenhauer et al. (1987)	pan-B

B Other mAbs

Clone	Reactivity	Source
Na1/34	CD1ag	Dakopatts
R4/23	follicular dendr. cells	Dakopatts
To5	C3b-receptor	Dakopatts
R1/69	human IgM	Dakopatts
IgD26	human IgD	Dakopatts
KL1	Cytokeratin	Dianova

[a] According to Ling et al. 1987

Table 2. Expression of B lymphocyte differentiation antigens on the cells of the lymph follicles of lymphofollicular hyperplasia of the thymus investigated immunohistologically by means of the mAbs listed in Table 1

Cell type	CD19ag MW 95 KD	CD20ag MW 35 KD	CD21ag MW 140 KD	CD22ag MW 135 KD	CD23ag MW 46 KD	CD37ag MW 40–45 KD
Follicular mantle cells	+[a]	+	+	+	+ ≪ −	+
Germinal centre B cells	+	+ ≫ −	−	(+) ≫ −	−	+ ≫ −
DRC[b]	+	+ ≫ −	+	(+)	+ ≫ −	+

[a] Scoring of antigen expression: + = strong expression; (+) = weak expression
+ = all cells positive; − = all cells negative; + > > − = considerable more positive than negative cells; + < < − = vice versa
[b] Follicular dendritic reticulum cells

Table 3. Expression of B lymphocyte differentiation antigens on medullary cells of normal fetal, juvenile and adult thymus investigated immunohistologically by means of the mAbs listed in Table 1

Cell type	CD19ag MW 95 KD	CD20ag MW 35 KD	CD21ag MW 140 KD	CD22ag MW 135 KD	CD23ag MW 46 KD	CD37ag MW 40–45 KD	IgM MW 900 K	IgD MW 180 KD
Fetal thymus								
B lymphocytes	−[a]	+ ≫ −	−	+ ≫ −	−	+	+ ≫ −	+ ≪ −
asteroid cells	+ ≪ −	+	−	−	+ ≪ −	+ ≫ −	+	−
Juvenile and adult thymus								
B lymphocytes	+ ≫ −	+	−	+ ≫ −	−	+	+ ≫ −	+ ≪ −
asteroid cells	−	+	−	+ ≪ −	+ ≪ −	−	+ ≫ −	−

[a] Scoring of antigen expression: + = strong expression; (−) = weak expression
+ = all cells positive; − = all cells negative; + ≫ − = considerably more positive than negative cells, compared with that/those antigens with the quantitative highest expression on the respective cells; + ≪ − = vice versa

ture committee of the Third International Workshop and Conference on Leucocyte Differentiation Antigens (Oxford 1986). MAbs CD22(HD6), CD22(HD39), CD23(HD50), CD37(HD28) were generous gifts of the producers B. Dörken (Dept. of Internal Medicine, University of Heidelberg) and G. Moldenhauer (German Cancer Research Center, Heidelberg, FRG); CD23(B-LAST-2) was a kind gift of L. Nadler (Dana-Farber Cancer Institute, Boston, MA). CD19(B4), CD20(B1), CD21(B2) were purchased from Coulter (Hialeah, FL, USA); a mAb against the T cell differentiation antigen CD1 (clone Nal/34) and mAbs against follicular dendritic cells (clone R4/23), against the C3b-receptor (To5), against human IgM (clone R1/69) and IgD (clone IgD26) were obtained from Dakopatts (Copenhagen, Denmark). Cytokeratin reactive monoclonal antibody (KL1) was purchased from Dianova (Hamburg, FRG).

Biotinylated goat anti-mouse Ig (final dilution 1:50) and streptavidin-biotinylated peroxidase complex (1:100) were purchased from Amersham (High Wycombe, UK). Pooled human gammaglobulin ("Gamma-Venin") was supplied by Behring (Frankfurt a.M., FRG). 3-amino-9-ethylcarbazole (AEC) and N'N-dimethyl-formamide (DMF) were obtained from Sigma Chem. Co. (St. Louis, MO, USA) and hydrogen peroxide and sodium acetate from Merck (Darmstadt, FRG). Rabbit anti-mouse Ig (final dilution 1:50) was purchased from Dakopatts (Copenhagen, Denmark); monoclonal (mouse) alkaline phosphatase complex (1:40) was supplied by Dianova (Hamburg, FRG). Fast Blue BB salt was obtained from Serva (Heidelberg, FRG) and naphthol AS-bi-phosphate and Levamisole from Sigma Chem. Co (St. Louis, MO, USA).

Staining procedures

Acetone-fixed rehydrated serial cryostat sections were incubated with the primary mAb in appropriate dilutions for 1 h at room temperature. All dilutions and washing steps were carried out in phosphate buffered saline pH 7.4; the biotinylated anti-mouse reagent contained 5% pooled human IgG to inhibit cross reactions with human Ig. Incubation time was 30 min for both the secondary antibodies and the streptavidin-biotinylated peroxidase complex. Using AEC as the chromogen (0.4 mg/ml in 0.1 mol/l of acetate buffer pH 5.0 with 5% DMF and 0.01% H_2O_2 for 10 min), the peroxidase reaction resulted in an intense red precipitate. The sections were counterstained with Harris' haematoxylin and mounted with glycerol gelatin.

The above described ABC method was combined with the APAAP method according to Cordell et al. (1984). After incubation with the first monoclonal antibody followed by the biotinylated goat anti-mouse Ig and the streptavidin-biotinylated peroxidase complex, incubation with the second monoclonal antibody followed by the rabbit anti-mouse Ig and the APAAP complex was carried out. The latter steps were rerun to enhance the following enzyme substrate reaction. The peroxidase substrate reaction was then developed using AEC as the chromogen. Finally, the alkaline phosphatase substrate reaction was developed using naphthol-AS-bi-phosphate as substrate and Fast Blue BB salt as coupling reagent (0.025% NAS-bi-PO_4, 0.05% FB (w/v), 0.005% DMF (v/v) in 0.7% Amino-methyl-propandiol buffer (w/v), pH 8.7).

Results

Lymphofollicular hyperplasia of the thymus

The cellular composition of the lymph follicles in lymphofollicular hyperplasia was examined with monoclonal antibodies against B cell differentia-

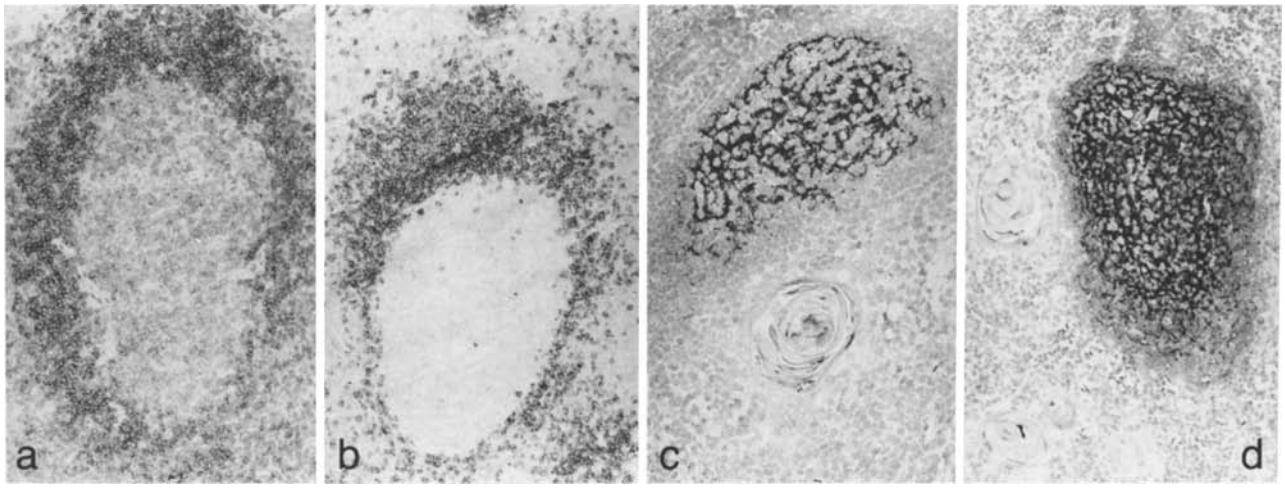

Fig. 1 a–d. Cryostat sections of lymphofollicular hyperplasia of the thymus stained with monoclonal antibodies against B cell differentiation antigens, against follicular dendritic reticulum cells and human IgD. Thymic lymph follicles with germinal center and follicular mantle zone. Hassall's corpuscles in the neighbourhood. **a** Lymphocytes of the follicular mantle zone express the CD22ag; the weakly stained germinal center lymphocytes and follicular dendritic reticulum cells are hardly visible. CD22(To15), ×89.5. **b** Lymphocytes of the follicular mantle show strong expression of surface IgD; the cells of the germinal center are negative. MAb IgD26, ×71.5. **c** The follicular dendritic reticulum cells of the germinal center show a very strong staining with the mAb R4/23. ×143. **d** The CD21ag is shown on follicular dendritic reticulum cells and on some mantle zone lymphocytes. CD21(B2), ×89.5.

tion antigens, antigens of follicular dendritic cells and the C3b-receptor and against human IgM and IgD (Table 2). Immunohistology with these reagents elucidated the structure of the lymph follicles, which could be divided into a germinal center and a follicular mantle zone (Fig. 1). The lymphocytes of the follicular mantle zone expressed the B cell differentation antigens, of the cluster CD19, CD20, CD21, and CD22 (Fig 1a) and only some of them also expressed the CD23ag; the B cell associated CD37ag was also expressed by all lymphocytes of the follicular mantle. All lymphocytes of the follicular mantle zone expressed surface IgD (Fig. 1b). The lymphocytes of the germinal centers expressed the CD19ag, the majority of which also expressed the CD20ag, the CD22ag and the CD37ag; in addition a faint surface expression of IgM was observed. The CD21ag and the CD23ag were not found on the germinal center lymphocytes.

Follicular dendritic cells of the germinal centers were positive for the CD19ag, the CD21ag (Fig. 1d) and weakly for the CD22ag; the CD20ag and the CD23ag were also expressed by most of them. The follicular dendritic cells of the germinal center bound the monoclonal antibodies against DRC (Fig. 1c) and the C3b-receptor. Strong reactivity of the follicular dendritic cells was observed with mAb against human IgM.

In order to examine the exact localisation of these lymph follicles, double labelling experiments with the CD37(HD28) antibody and with antikeratin antibody (KL1) were performed. These experiments revealed a distinct separation of the lymphofollicular hyperplastic tissue from the thymic medulla by a continuous row of epithelial cells and a connection with the surrounding connective tissue of the interlobular septal space (Fig. 2). Besides the above mentioned B lymphocytes within the lymph follicles, B lymphocytes and cells expressing B lymphocyte antigens were also found within the thymus medulla. Some of them may have migrated from the B cell follicles of the interlobular septae through discontinuities in the border between the perivascular space and the thymus medulla (not shown). Others, however, were found within medullary regions distant from the described lymph follicles.

Normal thymus

Normal control thymuses were examined with the same panel of monoclonal antibodies to investigate whether these B lymphocytes and cells expressing B lymphocyte antigens within the thymus medulla belong to a reactive infiltrate and are therefore part of the state of the so-called "thymitis", or whether they are constitutive elements of the normal thymus.

Against the background of the thymic epithelial framework given by immunostaining with antikeratin antibodies which clearly defined extra- and

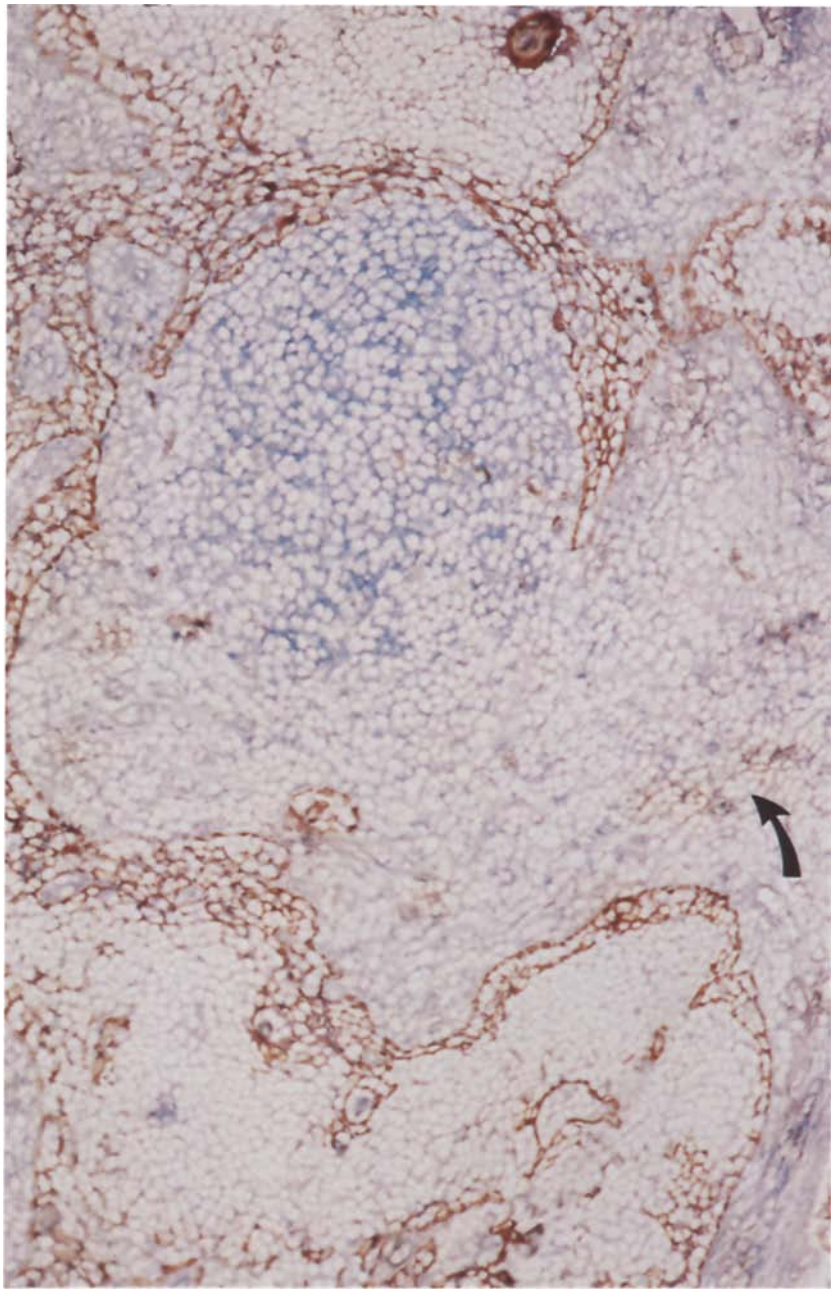

Fig. 2. Cryostat section of lymphofollicular hyperplasia of the thymus double-immunolabelled with mAbs against keratin (*red*) and CD37ag (*blue*). A lymph follicle with CD37ag positive cells is shown, which is situated outside the thymus medulla having broad connections to the tissue of interfollicular septae (*arrow*). A continuous row of epithelial cells appearing to be compressed separates the follicle from the medulla, the border is indented at one side only. mAb KL1, CD37(HD28), ×280

intrathymic compartments, immunostained serial sections revealed the existence of medullary cells expressing B cell antigens both in pre- and postnatal thymuses.

In the fetal thymuses a small subset of cells within the medulla were found to express B cell antigens. On morphological grounds these cells could be subdivided into two types, one small, round and distinctively lymphoid type, the other larger with short cytoplasmic extensions, thus not being definitely lymphoid but rather asteroid in shape. The lymphoid type expressed CD20ag (Fig. 3a, b), CD22ag (Fig. 3f), CD37ag, IgM (Fig. 3c, d) and IgD. The CD37ag was expressed by a greater number of lymphoid cells than were the CD20- and CD22ag and IgM as could be

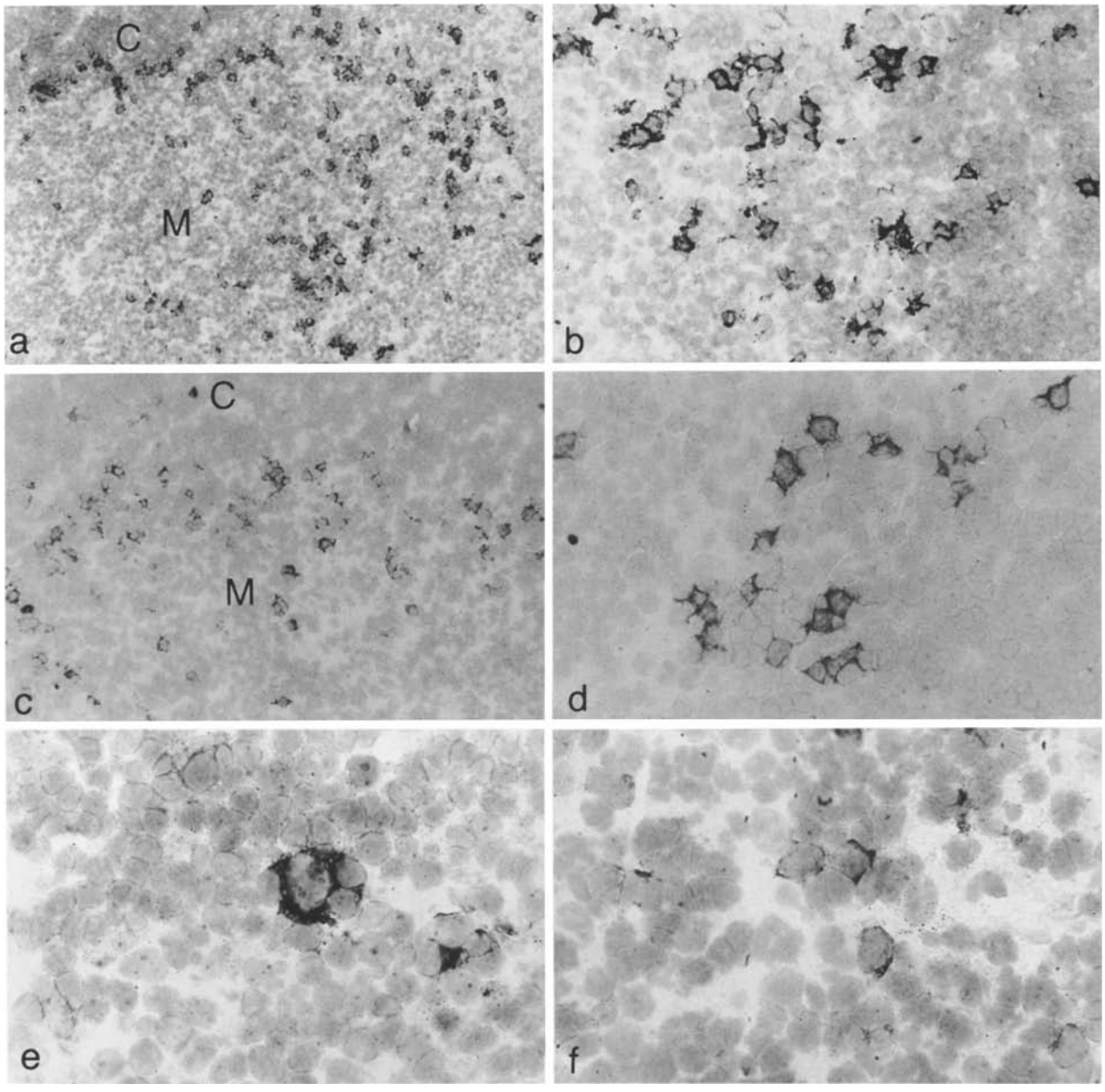

Fig. 3a–f. Cryostat sections of a fetal thymus stained with mAbs against B lymphocyte differentiation antigens and human IgM and counter-stained with haematoxylin. *M*, medulla; *C*, cortex. **a** Overview. Cells expressing the CD20ag are mainly scattered in the corticomedullary region. CD20(B1), ×115. **b** Same as **a** at higher magnification showing the two cell types characterized by lymphoid and asteroid morphology. ×276. **c** MAb R1/69 demonstrates IgM expression in the same population. ×184. **d** Same as **c** at higher magnification. ×230. **e** Only asteroid cells express the CD23ag displaying a rosette formation with the surrounding lymphocytes CD23(HD50), ×580. **f** Medullary region. Only few lymphocytes express the CD22ag, CD22(HD6), ×580

shown by serial sections. Only a small subset of this cell type additionally expressed IgD. The lymphoid cell type failed to express CD19ag, CD21ag and CD23ag. They were scattered throughout the medulla and amounted for about 1% of medullary lymphoid cells.

The asteroid cells expressed the CD20ag (Fig. 3b) and IgM (Fig. 3d). The CD37ag was only detectable in a subpopulation of these cells, and an even smaller subpopulation was found to express the CD19ag and CD23ag (Fig. 3e). The asteroid cells did not express CD21ag, CD22ag and IgD; the antigen of follicular dendritic reticulum cells, detected by the mAb R4/23 could not be found on those cells nor on any other intrathymic cell. With respect to morphology and immunophenotype, these cells will be referred to as "asteroid cells expressing B-cell antigens". This cell type,

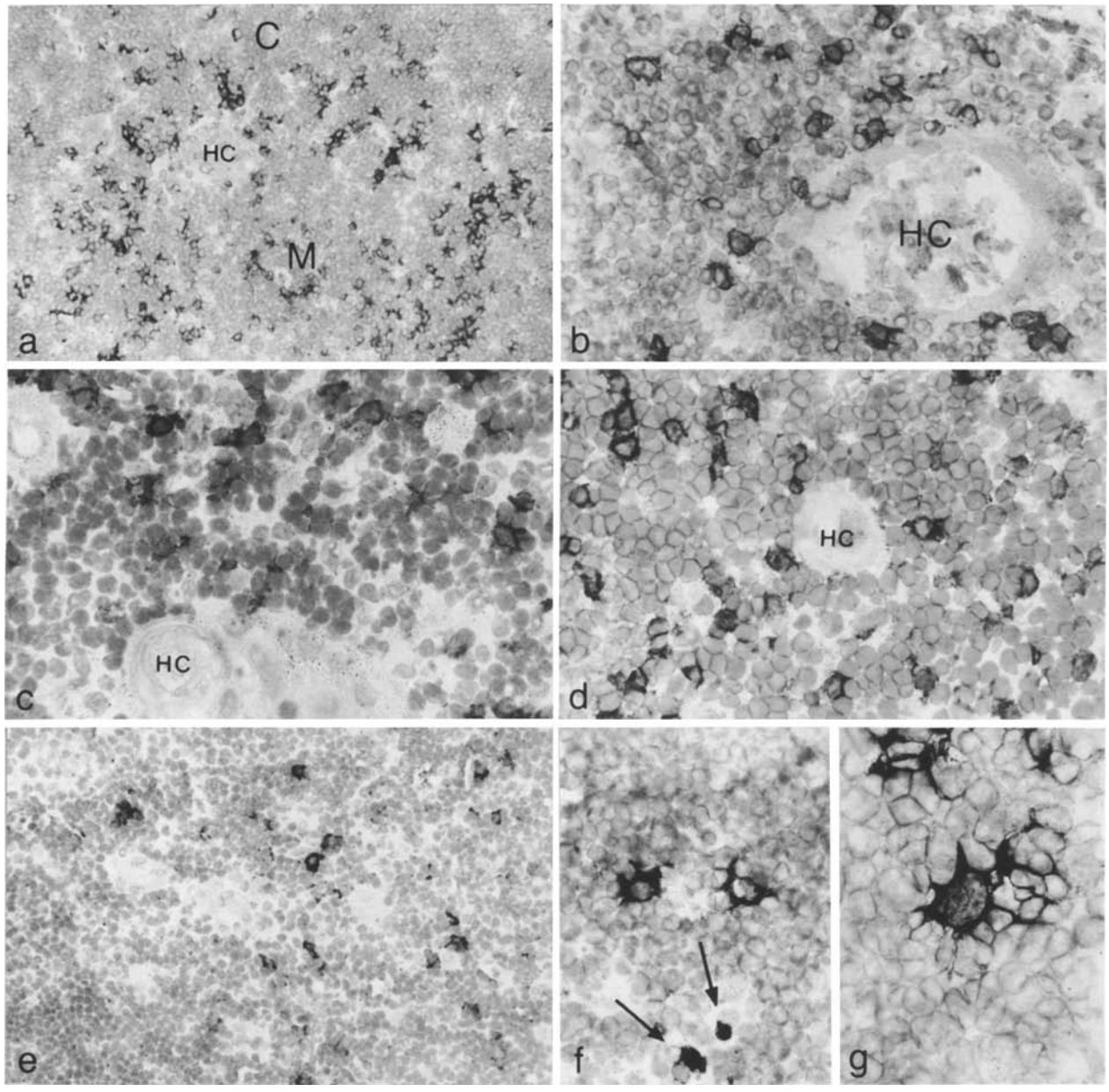

Fig. 4a–g. Cryostat section of juvenile and adult thymuses stained with mAbs against B lymphocyte differentiation antigens and human IgD. Counterstaining with haematoxylin. *M*, medulla; *C*, cortex; *HC*, Hassall's corpuscle. **a** Overview. CD20ag positive cells of asteroid and lymphoid shape are localized within the medulla at the corticomedullary region and around the HC. CD20(B1), ×144. **b** B lymphocytes situated in the vicinity of a HC expressing IgM. mAb R1/69, ×288. **c** Only few B lymphocytes between HC express the CD22ag. CD22(HD6), ×363. **d** CD37ag positive lymphocytes are scattered within the medulla. CD37(HD28), ×363. **e** Only few B lymphocytes within the thymus medulla express IgD. MAb IgD28, ×184. **f, g** The CD23ag expressed by asteroid cells showing a rosetting phenomenon with the surrounding lymphocytes. The CD23ag is also demonstrated in the paranuclear zone of two large (asteroid?) cells (*arrows* in **f**). CD23(HD50), f ×363, g ×580

less frequent than the lymphoid cell type, was located in the corticomedullary region. It was further characterized by the formation of rosettes with non-B lymphocytes (Fig. 3e).

These cell types expressing B-cell antigens could also be found in the juvenile and adult thymuses. Although the number of the cells was quite similar to that in the fetal organs, there were slight differences in tissue distribution and immunophenotype: The lymphoid cell type was scattered throughout the medulla as in the fetal thymuses, and constantly coexpressed CD20ag (Fig. 4a) and CD37ag (Fig. 4d). Furthermore, a subpopulation of them expressed the CD22ag (Fig. 4c), IgM

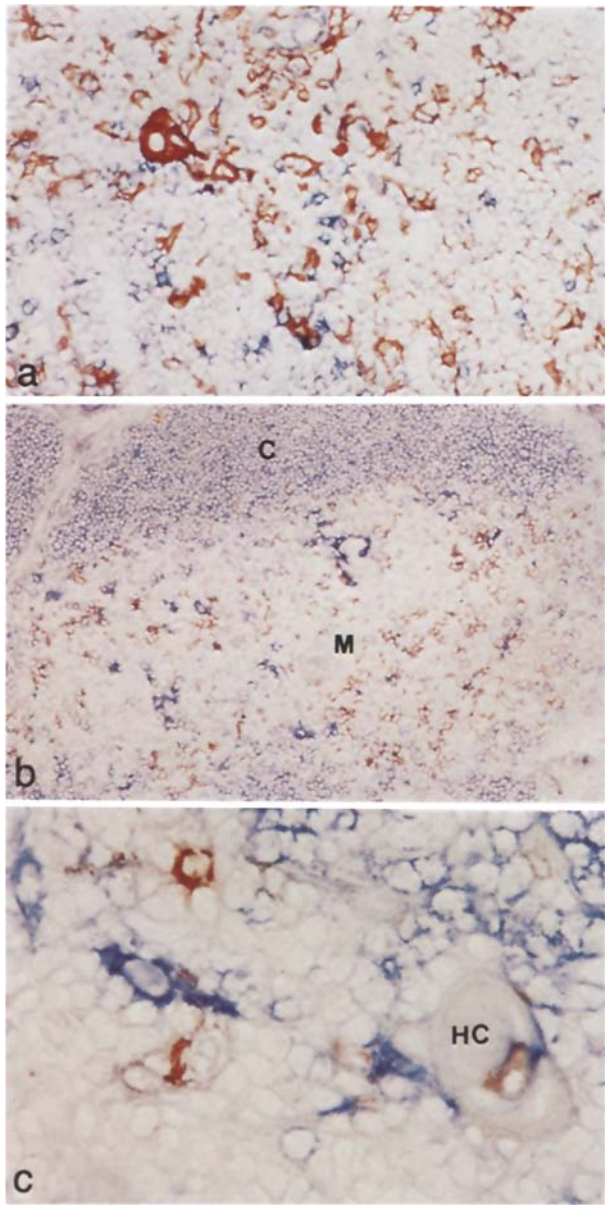

Fig. 5a–c. Double-immunolabelling experiments on cryostat sections of normal juvenile and adult thymuses. **a** Double labelling with anti-keratin mAb (KL1) (red) and CD37(HD28) (*blue*). The CD37ag positive lymphoid and asteroid cells are intermingled within the epithelial framework of the thymus medulla. ×226. **b** Double labelling with CD20(B1) (*red*) and CD1(Na1/34) (*blue*). The CD20 positive lymphoid and asteroid cells are localized within the medulla and are surrounded by CD1 negative lymphocytes. Note the CD1 positive dendritic reticulum cells of the thymus medulla. ×113. **c** Same as **b** at higher magnification. ×571

(Fig. 4b) plus CD19ag which was not found on the corresponding cells of the fetal organs. Only a small subset of the lymphocytic type also expressed IgD (Fig. 4e).

Asteroid cells, in contrast to the fetal thymuses, were mainly distributed randomly within the medulla or in the vicinity of Hassall's corpuscles. Their immunophenotype was characterized by the expression of CD20ag (Fig. 4a); a subset of them also expressed IgM; CD22ag and CD23ag (Fig. 4f, g) were detectable in an even smaller number. CD23ag could be found in the perinuclear zone of cells which were twice as big as normal lymphocytes and which were localized in the neighbourhood of the asteroid cells expressing the CD23ag in the cytoplasm and on the cell surface (Fig. 4f). These cells did not express CD19ag, CD21ag and the antigen of follicular dendritic cells detectable by the mAb R4/23, which could not be found on any intramedullary cell.

Using anti-keratin antibodies and CD37(HD28) antibody in double labelling experiments it was shown that the asteroid cells expressing the CD37ag were localized within the epithelial network of the thymus medulla (Fig. 5a). Immunohistological double labelling with CD20(B1) antibody and CD1(T6) antibody further revealed the non-B lymphocytes (CD20ag negative) which surround the asteroid CD20ag positive cells to be CD1ag negative (Fig. 5b, c).

Another cell population expressing B-cell antigens was consistantly detected within the connective tissue of the interlobular septae of juvenile and adult thymuses (not shown). They were distinctively lymphocytic in morphology and had an immunophenotype corresponding to mantle zone B cells.

Discussion

This study describes the occurrence of B lymphocytes and cells expressing B lymphocyte differentiation antigens within the thymus under two different conditions: Firstly, B lymphocytes in the organized structures of lymph follicles which originate in the extrathymic compartment of the connective tissue of the interlobular septal spaces in the pathologic condition of lymphofollicular hyperplasia of the thymus and secondly, B lymphocytes and cells expressing B lymphocyte differentiation antigens, which are distributed within the thymus medulla in normal thymuses of different ages from fetal to adult life and which, therefore have to be considered as physiological constituents of the thymus.

Lymphofollicular hyperplasia of the thymus is a pathologic condition which is often associated with autoimmune diseases such as myasthenia gravis, systhemic lupus erythematosus, scleroderma, rheumatoid arthritis, periarteriitis nodosa, Hashimoto's thyroiditis, autoimmune anaemia, Behçet's disease and Sjögren's syndrome (Levine and Rosai 1978; Otto 1984; Rosai and Levine 1976; Tridente

1985; Wekerle and Müller-Hermelink 1986). Microscopically the striking finding of the thymus in lymphofollicular hyperplasia is the presence of lymph follicles with germinal centers that resemble those observed in any lymph node (Castleman 1966).

Examination of the cellular composition of the lymph follicles by immunohistology revealed the same constituents as in the lymph follicles of the peripheral lymphoid tissue of spleen and lymph node (Ling et al. 1987; Gerdes et al. 1983), that is to say: *follicular dendritic reticulum cells* of the germinal center staining with mAbs against DRC and C3bR express the B lymphocyte differentiation antigens CD19, CD20, CD21, CD23; *germinal center B cells* express the CD19ag and partly also the CD20ag and the CD22ag but not the CD21- and CD23ag; *B cells of the follicular mantle zone* express all the differentiation antigens tested although only few of them are positive for the CD23ag. IgM was only expressed by cells of the germinal centers whereas IgD could only be found on the lymphocytes of the follicular mantle zone.

Some of the findings presented have already been reported in the literature. Kornstein et al. (1984) also found IgM-bearing cells in the germinal center of the lymph follicles and IgD-bearing lymphocytes in the follicular mantle. Follicular dendritic cells in the germinal centers have been identified by Bofill et al. (1985) using the monoclonal antibody RFD-3. They suggested, however, the germinal centers be surrounded by a T-cell zone, calling these structures lymph-node-like areas. The distribution of the B lymphocyte differentiation antigens, however, has so far not been examined in the lymph follicles of lymphofollicular hyperplasia. Since it is identical to the lymph follicles of the peripheral lymphoid tissue these new findings – together with those mentioned above – confirm the assumption that the cellular composition of the lymph follicles in lymphofollicular hyperplasia of the thymus is the same as in the lymph follicles of other localizations of the body.

Additionally, the localization of these lymph follicles within the thymic region suggests that they belong to the extrathymic lymphoid system: The sharp delineation of the border between the lymph follicles and the thymic medulla which is formed by a continuous row of epithelial cells and the connection of the hyperplastic lymph follicles with the soft tissue of the interlobular septae in a stalk like manner strongly indicates an extrathymic origin of the lymph follicles. This assumption was made recently (Hofmann et al. 1987) on the basis of single labelling experiments and is now supported by the findings presented using double labelling experiments. The finding of "intramedullary" lymph follicles is a consequence of a two-dimensional view of the three-dimensional architecture which does not always visualize the connection of the lymphatic tissue with the interlobular septae. Tamaoki et al. (1971) assumed the existance of a "perithymic lymphoid tissue" which gives rise to the lymph follicles of lymphofollicular hyperplasia. More recently, basement membrane material like laminin was shown to surround the lymph follicles at the borders to the thymic medulla (Bofill et al. 1985; Karttunen 1987). This border was sometimes found to be interrupted and so as to facilitate the B lymphocytes of the follicular mantle zone to immigrate into the thymus medulla, a finding also referred to as "thymitis" (Kirchner et al. 1986). However, some B lymphocytes and cells expressing B lymphocyte antigens could be found at large distances from the lymph follicles in the lymphofollicular hyperplasia described above and also in normal control thymuses.

The first data on B cells within normal human thymus were published by Eimoto et al. (1986) using the mAb CD20(B1). A small number of CD20ag positive cells were situated within the medulla of normal human thymus which was used as a control specimen during the examination of thymomas. Our results clearly indicate the existence of cells expressing B lymphocyte antigens within the human thymus medulla. On morphological grounds the cells detected within the thymus medulla, expressing B lymphocyte differentiation antigens, can be subdivided into two populations: typical, small and round lymphoid and asteroid cells with small cytoplasmic processes. The cells twice as big as normal lymphocytes expressing the CD23ag in a perinuclear zone might be a transient form between the former and the latter. The morphology and immunophenotype of the lymphoid cell population suggested that these cells were in fact B-lymphocytes.

The histogenesis of the asteroid variant is still undetermined. The only cell in the peripheral lymphoreticular system that shares CD19ag, CD20ag, CD21ag, CD22ag and CD23ag with B lymphocytes is the follicular dendritic reticulum cell (Lin et al. 1987), a cell of alleged histiocytic nature (Gerdes et al. 1983). Thus it has to be discussed whether the asteroid cell type is related to the follicular dendritic reticulum cell. Follicular dendritic reticulum cells did not occur outside lymph follicles; the asteroid cells in the thymus however, lacked expression of the antigen recognized by the mAb R4/23 specific for follicular dendritic reticulum cells. Thus a relation between follicular dendritic reticulum cells and the asteroid cells of the

thymus seems unlikely. There is also no correlation between the medullary dendritic cells of the thymus which are CD1ag positive and the asteroid cells described since double labelling experiments revealed that the latter cell type is CD1ag negative. Another cell type which, despite its non-lymphoid morphology in situ, has recently been shown to be a B cell is the so-called "monocytoid B cell" of the lymph node formerly known as "immature sinus histiocyte" (de Almeida et al. 1984; Piris et al. 1986; Sheibani et al. 1984; Stein et al. 1984). It represents an alternative pathway of B cell development that, at present, cannot be located in the current physiological differentiation scheme (Nadler 1986). It can therefore be concluded that, in spite of the non-lymphoid morphology, the asteroid medullary cells described are B cells since they express B cell restricted antigens.

The functions of the cells described here has yet to be elucidated. They occur as early as in the 19th week of gestation, when the development of the thymus into the cortical and medullary compartment has just terminated (von Gaudecker 1986); they are non-randomly distributed in the fetal thymus within the cortico-medullary zone, an area which is passed by the maturing T lymphocytes (Scollay 1983); furthermore they partly display rosette formation with surrounding non-B lymphocytes, a phenomenon which was also shown for functional complexes between thymic accessory cells and immature T lymphocytes (Kyewski et al. 1982, 1986, 1987). Thus B cells and their antigens may play a functional role in T lymphocyte maturation.

Together with other antigens of the body which have so far been found to be exposed within the human thymus such as epidermal antigens (Bonnefoy et al. 1984; Lenkei et al. 1987; Singer et al. 1985), antigens of erythrocytes (Telen 1985), smooth muscle antigens (Drenckhahn et al. 1979), striational muscle antigens (Drenckhahn et al. 1979; Williams and Lennon 1986) and circulating antigens which are presented by antigen-presenting cells in the murine thymus (Kyewski et al. 1982, 1986, 1987) B lymphocytes and cells expressing B lymphocyte antigens within the human thymus might contribute to tolerance induction to self antigens of the B lymphocytes in the developing T cell population.

Tolerance of T cells towards autologous B cells, however, is not only necessary to avoid autoimmunereactions between the two cell types but can also be regarded as a prerequisite for the direct cell-cell interactions which take place in the course of immune reactions (for review see Bolhuis et al. 1986). Apart from specific cell-cell contact between T and B cells, which is mediated via foreign antigens and MHC class II antigens, a non-specific cell-cell contact without these antigens is possible (Kupfer et al. 1986) indicating that T cells are able to recognize B cells. This ability of mature T cells to recognize B cells might be a consequence of the intrathymic localization of B cells and B cell differentiation antigens.

The existence of intrathymic B cells, in addition, might explain the occurrence of primary mediastinal clear cell lymphomas of B cell type which have recently been described (Möller et al. 1986b) and which are distinct from B cell neoplasias originating in the bone marrow, lymph nodes and other extralymphatic sites (Möller et al. 1986a, Möller et al. 1987). The possibility of the lymphomas arising within the thymus has already been discussed (Addis and Isaacson 1986).

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References

Abdou NI, Lisak RP, Zweiman B, Abrahamson I, Penn AS (1974) The thymus in myasthenia gravis – Evidence for altered cell populations. New Engl J Medicine 291:1271–1275

Addis PJ, Isaacson PG (1986) Large cell lymphoma of the mediastinum: a B-cell tumour of probable thymic origin. Histopathology 10:379–390

Almeida de PC, Harris NL, Bhan AK (1984) Characterization of immature sinus histiocytes (monocytoid cells) in reactive lymph nodes by use of monoclonal antibodies. Hum Pathol 15:330–335

Berrih S, Savino W, Cohen S (1985) Extracellular matrix of the human thymus: Immunofluorescence studies on frozen sections and cultured epithelial cells. J Histochem Cytochem 33:655–664

Bofill M, Janossy G, Willcox N, Chilosi M, Trejdosiewicz LK, Newsom-Davis J (1985) Microenvironments in the normal thymus and the thymus in myasthenia gravis. Am J Pathol 119:462–473

Bolhuis RLH, Gravekamp C, van de Griend RJ (1986) Cell-cell interactions. Clin Immunol Allergy 6:29–90

Bonnefoy JY, Reano A, Schmitt D, Thivolet J (1984) Thymic Hassall's corpuscles-epidermis antigenic relations defined by a common glycoprotein in man (GP37). Thymus 6:387–394

Castleman B (1966) The pathology of the thymus gland in myasthenia gravis. Ann NY Acad Sci 135:496–503

Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulalziz Z, MacDonald S, Pulford KAF, Stein H, Mason DY (1984) Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 32:219–229

Dörken B, Moldenhauer G, Pezzutto A, Schwartz R, Feller A, Kiesel S, Nadler LM (1986) HD39(B3), a B-lineage-restricted antigen whose cell surface expression is limited to

resting and activated human B lymphocytes. J Immunol 136:4470–4479

Dörken B, Moldenhauer G (1987) Phenotypic analysis of the 3rd international workshop B cell panel. In: McMichael et al. (eds) Leukocyte Typing III. Oxford University Press, Oxford, pp 369–373

Drenkhahn D, Gaudecker B von, Müller-Hermelink HK, Unsicker K, Gröschel-Stewart U (1979) Myosin and actin containing cells in the human postnatal thymus. Virchows Arch [B] (Cell Pathol) 32:33–45

Eimoto T, Teshima K, Shirakusa T, Takeshita M, Okamura H, Naito H, Mitusi T, Kikuchi M (1986) Heterogeneity of epithelial cells and reactive components in thymomas: an ultrastructural and immuno-histochemical study. Ultrastructural Pathology 10:157–173

Gaudecker B von (1986) The development of the human thymus microenvironment. In: Müller-Hermelink HK (ed) The Human Thymus – Histophysiology and Histopathology (Current Topics of Pathology). Springer, Berlin Heidelberg New York Tokyo, pp 1–41

Gerdes J, Stein H, Mason PY, Ziegler A (1983) Human dendritic reticulum cells of lymphoid follicles: Their antigenic profile and their identification as multinucleated giant cells. Virchows Arch [B] (Cell Pathol) 42:161–172

Grody WW, Jobst S, Keesey J, Herrmann C, Naeim F (1986) Pathologic evaluation of thymic hyperplasia in myasthenia gravis and Lambert-Eaton myasthenic syndrome. Arch Pathol Lab Med 110:843–846

Haynes BF (1984) The human thymus microenvironment. Advances in Immunology 36:87–142

Haynes BF (1986) The role of the thymic microenvironment in promotion of early stages of human T cell maturation. Clin Res 34:422–431

Hofmann WJ, Möller P, Otto HF (1987) Thymic hyperplasia. II. Lymphofollicular hyperplasia of the thymus. An immunohistologic study. Klin Wochenschr 65:53–60

Kappler JW, Roehm N, Marrack P (1987) T cell tolerance by clonal elimination in the thymus. Cell 49:273–280

Karttunen T (1987) Basement membrane proteins and reticulin in the normal thymus and the thymus in myasthenia gravis. Virchows Arch [A] (Pathol Anat) 411:245–252

Kirchner T, Schalke B, Melms A, von Kügelgen T, Müller-Hermelink HK (1986) Immunohistological patterns of non-neoplastic changes in the thymus in myasthenia gravis. Virchows Arch [B] (Cell Pathol) 52:237–257

Kornstein MJ, Brooks JJ, Anderson AA, Levinson AI, Lisak RP, Zweiman B (1984) The immunohistology of the thymus in myasthenia gravis. Am J Pathol 117:184–194

Kupfer A, Swain SL, Janeway CA, Singer SJ (1986) The specific direct interaction of helper T-cells and antigen-presenting B-cells. Proc Natl Acad Sci USA 83:6080–6083

Kyewski BA, Fathman CG, Rouse RV (1986) Intrathymic presentation of circulating non-MHC antigens by medullary dendritic cells. J Exp Med 163:231–246

Kyewski BA, Momburg F, Schirrmacher V (1987) Phenotype of stromal cell-associated thymocytes in situ is compatible with selection of the T cell repertoire at an "immature" stage of thymic T cell differentiation. Eur J Immunol 17:961–967

Kyewski BA, Rouse RV, Kaplan HS (1982) Thymocytes rosettes: Multicellular complexes of lymphocytes and bone marrow-derived stromal cells in the mouse thymus. Proc Natl Acad Sci USA 79:5646–5650

Lenkei R, Biberfeld G, Magnius LO, Fagraeus A, Biberfeld P (1985) Autoantibodies to the basal cells of squamous epithelium react with thymic epithelial cells. Clin Immunol Immunopathol 34:11–19

Levine GD, Rosai J (1978) Thymic hyperplasia and neoplasia: a review of current concepts. Human Pathol 9:495–515

Ling NR, MacLennan ICM, Mason DY (1987) B-cell and plasma cell antigens: new and previously defined clusters. In: McMichael A et al. (eds) Leucocyte typing III. White cell differentiation antigens. Oxford University Press, Oxford, pp 302–335

Lo D, Ron Y, Sprent J (1986) Induction of MHC-restricted specificity and tolerance in the thymus. Immunol Res 5:221–232

Möller P, Eberlein-Gonska M, Hermann B, Lämmler B, Momburg F, Moldenhauer G, Dörken B, Otto HF (1986a) Vergleich des Immunphänotyps der mediastinalen hellzelligen B-Zell-Lymphome mit denen typischer B-Zell-Lymphome. Verh Dt Ges Path 70:508

Möller P, Lämmler B, Eberlein-Gonska M, Feichter GE, Hofmann WJ, Schmitteckert H, Otto HF (1986b) Primary mediastinal clear cell lymphoma of B-cell type. Virchow Arch [A] (Pathol Anat) 409:79–92

Möller P, Moldenhauer G, Momburg F, Lämmler B, Eberlein-Gonska M, Kiesel S, Dörken B (1987) Mediastinal lymphoma of clear cell type is a tumor corresponding to terminal step of B cell differentiation. Blood 69:1087–1095

Moldenhauer G, Dörken B, Schwartz R, Pezzutto A, Hämmerling GJ (1986) Characterization of a human B lymphocyte, specific antigen defined by monoclonal antibodies HD6 and HD39. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID (eds) Leukocyte Typing II. Springer, New York, pp 97–107

Moldenhauer G, Schwartz R, Dörken B, Hämmerling GJ (1987) Biochemical characterization and epitope analysis of B lymphocyte-specific surface antigens defined by clustering workshop monoclonal antibodies. In: McMichael et al. (eds) Leukocyte typing III. Oxford University Press, Oxford, pp 378–382

Nadler LM (1986) B cell leukemia panel workshop: Summary and comments. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ED (eds) Leukocyte typing II. Springer, New York, pp 3–43

Nadler LM, Anderson KC, Marti G, Bates M, Park E, Daley JF, Schlossman SF (1983) B4, a human B lymphocytes associated antigen expressed on normal, mitogen – activated and malignant B lymphocytes. J Immunol 131:244–250

Nadler LM, Stashenko P, Hardy R, van Agthoven A, Terhorst C, Schlossman SF (1981) Characterization of a human B-cell-specific antigen (B2) distinct from B1. J Immunol 126:1941–1947

Otto HF (1984) Pathologie des Thymus. In: Doerr W, Seifert G, Uehlinger E (eds) Spezielle pathologische Anatomie, Vol 17. Springer, Berlin Heidelberg New York Tokyo

Palestro G, Tridente G, Micca FB, Novero D, Valente G, Godio L (1983) Immunohistochemical and enzyme histochemical contributions to the problem concerning the role of the thymus in the pathogenesis of myasthenia gravis. Virchows Arch [B] (Cell Pathol) 44:173–186

Pezzutto A, Dörken B, Feller A, Moldenhauer G, Schwartz R, Warnet P, Thiel E, Hunstein W (1986) HD37 monoclonal antibody: a usefull reagent for further characterization of "non-T/non-B" lymphoid malignancies. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID (eds) Leukocyte Typing II, Vol 2. Springer, Berlin Heidelberg New York, pp 391–403

Pezzutto A, Dörken B, Moldenhauer G, Clark EA (1987) Amplification of human B cell activation by a monoclonal antibody to the B cell-specific antigen CD22, BP 130/140. J Immunol 138:98–103

Piris MA, Rivas C, Morente M, Oliva H, Rubio C (1986) Im-

mature sinus histiocytosis a monocytoid B-lymphoid reaction. J Pathol 148:159–167

Rosai J, Levine GD (1976) Tumors of the thymus. In: Atlas of Tumor Pathology, Sec Ser, Fasc 13, Armed Forces Institute of Pathology, Washington DC

Schwartz R, Moldenhauer G, Dörken B (1987) The B cell associated GP40-45 antigen is characterized by a dominant carbohydrate determinant. In: McMichael et al. (eds) Leukocyte typing III. Oxford University Press, Oxford, pp 345–348

Scollay R (1983) Intrathymic events in the differentiation of T lymphocytes: a continuing enigma. Immunology Today 4:282–286

Sheibani K, Fritz RM, Winberg CD, Burke JS, Rappaport H (1984) "Monocytoid" cells in reactive follicular hyperplasia with and without multifocal histiocytic reactions: An immunohistochemical study of 21 cases including suspected cases of toxoplasmic lymphadenitis. Am J Clin Pathol 81:453–458

Singer KH, Harden EA, Robertson AL, Lobach DF, Haynes BF (1985) Expression of antigens by cultured epithelial cells: comparison of epidermis and thymic epithelium. J Invest Dermatol 85s:67–71

Stashenko P, Nadler LM, Hardy R, Schlossman SF (1980) Characterization of a human B-lymphocyte-specific antigen. J Immunol 125:1678–1685

Stein H, Lennert K, Mason DY, Liangru S, Ziegler A (1984) Immature sinus histiocytes. Their identification as a novel B-cell population. Am J Pathol 117:44–52

Tamaoki N, Habu S, Kameya T (1971) Thymic lymphoid follicles in autoimmune disease. Keio J Med 20:57–68

Telen MJ (1985) An antibody to human thymic Hassall's body epithelium recognizes a subset of blood group antigens. J Immunogenetics 12:3–15

Thomas JA, Wilcox HNA, Newsom-Davis J (1982) Immunohistological studies of the thymus in myasthenia gravis. J Neuroimmunol 3:319–335

Thorley-Lawson DA, Nadler LM, Bhan AK, Schooley RT (1985) B-LAST-2 (EBVCS) an early cell surface marker of human B-cell activation, is superinduced by Epstein Barr virus. J Immunol 134:3007–3012

Tridente G (1985) Immunopathology of the human thymus. Seminars in Hematology 22:56–67

Tutschka PJ (1987) The role of the thymus in regulating self and non-self. Transplant Proc 19:486–489

Wekerle H, Müller-Hermelink HK (1986) The thymus in myasthenia gravis. In: Müller-Hermelink HK (ed) The Human Thymus – Histophysiology and Histopathology (Current Topics of Pathology). Springer, Berlin Heidelberg New York Tokyo, pp 179–206

Williams CL, Lennon VA (1986) Thymic B lymphocyte clones from patients with myasthenia gravis secrete monoclonal striational autoantibodies reacting with myosin, α actinin, or actin. J Exp Med 164:1043–1059

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