

Short reports

Immunohistochemical analysis of human CD5 positive B cells: mantle cells and mantle cell lymphoma are not equivalent in terms of CD5 expression

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Abstract

CD5 is expressed by most T cells and a subset of B cells. Human CD5 positive B cells are present in fetal lymphoid tissue, their frequency decreasing with fetal age. In adult human tissues, CD5 positive B cells have been reported to be present in the germinal centre and mantle zone. Malignancies of CD5 positive B cells include mantle cell lymphoma and chronic lymphocytic leukemia. This report describes an immunohistochemical staining technique used to visualise the expression of CD5 by B cells in human fetal intestine, tonsil, and mantle cell lymphoma. B cells in fetal intestine, tonsillar epithelium, and mantle cell lymphoma all had a similar high intensity of CD5 expression. In contrast, CD5 B cells in the mantle and germinal centre expressed very small amounts of CD5, below the threshold of the technique. Therefore, mantle cells and mantle cell lymphoma are not equivalent in terms of CD5 expression.

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The CD5 antigen is expressed by most T cells and a subset of B cells. In mice, CD5 positive B cells appear to be an independent B cell lineage capable of self renewal. It is not known whether CD5 is associated with B cell lineage in humans, where CD5 has been shown to be an inducible B cell activation antigen. Functionally, CD5 positive B cells in humans and mice tend to produce polyspecific antibodies that might provide a first line of defence against bacterial pathogens.¹

CD5 positive B cells constitute a large proportion of the B cells in human fetal lymphoid tissue, although the percentage decreases with fetal age.¹ In postnatal human lymphoid tissues, CD5 positive B cells are thought to be located predominantly in the mantle zone. This is based on flow cytometric analysis, which showed that up to approxi-

mately 30% of B cells of the mantle zone phenotype expressed CD5.^{2,3} CD5 is also expressed by tumour cells in mantle cell lymphoma and chronic lymphocytic leukaemia (CLL). The expression of CD5 by mantle cell lymphoma cells is thought to reflect their origin from CD5 positive mantle zone B cells.^{4,5}

The original description of normal CD5 positive B cells in humans included the demonstration of CD5 positive B cells in a germinal centre.⁶ However, we are unaware of any immunohistochemical demonstration of CD5 expression by mantle cells. One study using immunofluorescence presents a schematic representation of the location of CD5 positive B cells around the periphery of the germinal centre, but with no supporting photographic evidence.⁷ We have developed an immunohistochemical staining technique that allows us to observe CD5 expression by B cells, and have used this technique to compare the expression of CD5 by B cells in fetal and postnatal lymphoid tissue and mantle cell lymphoma.

Materials and methods

TISSUES

Normal human tonsils from three patients with sleep apnoea and two patients with mantle cell lymphoma were received in the laboratory within four hours of surgery. Intestine from three fetuses (19 weeks gestation) from therapeutic terminations were collected with ethical committee approval. All tissues were snap frozen and stored in liquid nitrogen.

IMMUNOHISTOCHEMISTRY

All immunological reagents were purchased from Dako (Ely, Cambridge, UK), and chemical reagents from Sigma (Poole, Dorset, UK), unless stated otherwise. Frozen sections (8 µm) were air dried and fixed in fresh acetone for 30 minutes. Sections were incubated with mouse monoclonal anti-CD3 primary antibody and binding was detected using rabbit antimouse immunoglobulin conjugated to horseradish peroxidase. Staining was visualised using 3,3'-diaminobenzidine (DAB). Sections were then washed in running tap water for at least 30 minutes and re-equilibrated in Tris buffered

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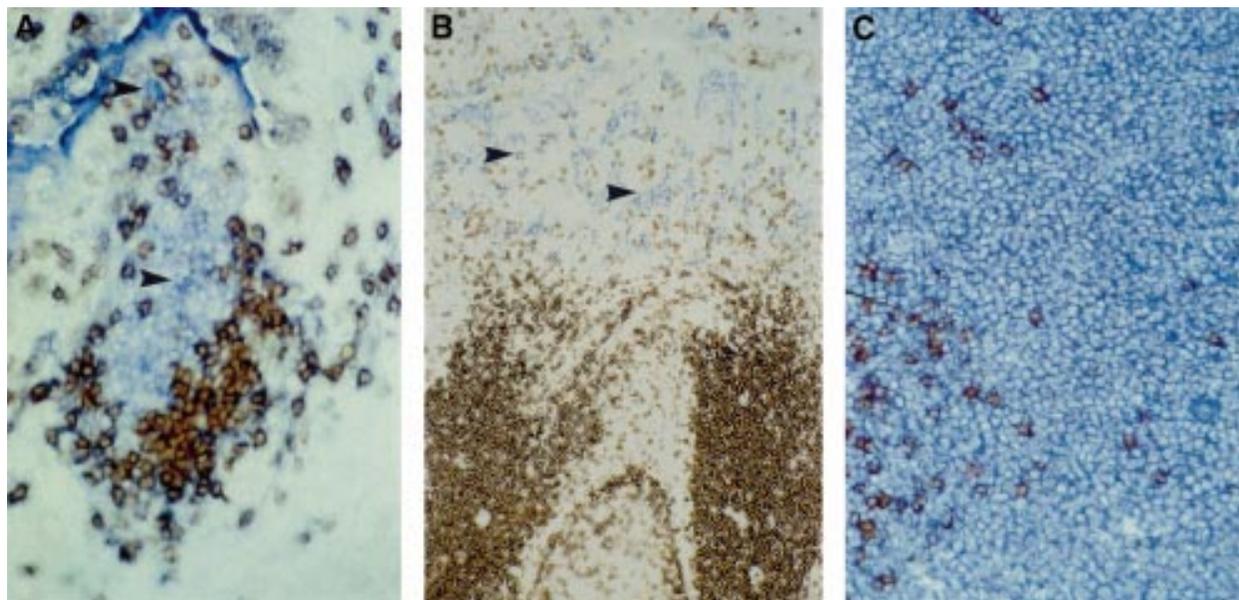


Figure 1 Frozen sections of (A) fetal intestine, (B) tonsil, and (C) mantle cell lymphoma, stained using anti-CD3 (immunoperoxidase; brown) followed by anti-CD5 (immunoalkaline phosphatase; blue). Blue CD5 positive B cells are indicated with arrowheads. They are present in the primary follicle in the fetus and the epithelium in the tonsil. Tumour cells in mantle cell lymphoma express CD5 with an intensity comparable to that seen in the fetal primary follicle and tonsillar epithelium. Note that the tonsillar mantle zone is unambiguously CD5 negative.

saline (TBS). Sections were then incubated with mouse monoclonal anti-CD5 antibody overnight at 4°C. Binding of the anti-CD5 antibody was detected using rabbit antimouse immunoglobulin conjugated to biotin, followed by avidin-alkaline phosphatase conjugate. Binding was visualised using fast blue, which contrasts with the brown DAB. Slides were washed and mounted in Aquamount (BDH, Lutterworth, Leicestershire, UK).

To confirm that the blue cells seen using this technique were B cells, for each case we included a control in which anti-CD20 was mixed with the anti-CD3 antibody in the initial immunoperoxidase stain. This consistently blocked

the blue staining considered to represent the CD5 positive B cells. To confirm the intraepithelial location of CD5 positive B cells in the tonsil, anticytokeratin monoclonal MNF116 was mixed with the anti-CD3 antibody in the initial immunoperoxidase stain.

Results

FETAL LYMPHOID TISSUE

CD5 positive B cells were found in the organised lymphoid tissue of the fetal Peyer's patches. They occurred as clusters surrounded by T cells and also as isolated cells in the dome regions (fig 1). CD5 positive B cells were also seen as isolated cells in the lamina propria.

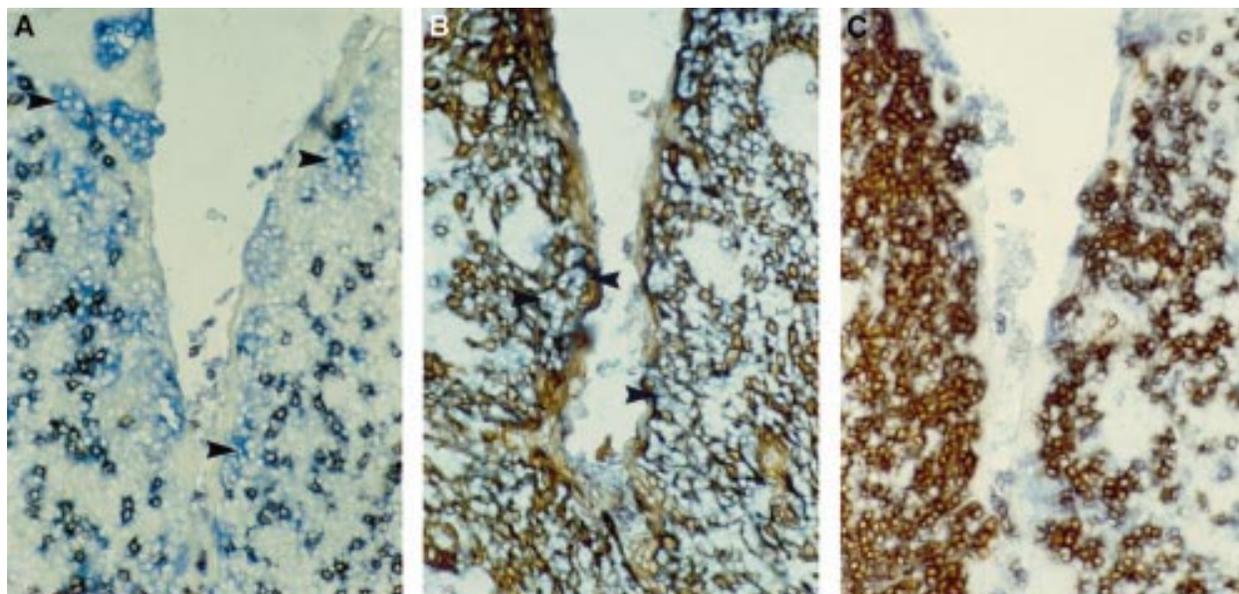


Figure 2 Frozen sections of tonsil stained with (A) anti-CD3 (immunoperoxidase; brown), followed by anti-CD5 (immunoalkaline phosphatase; blue); (B) anti-CD3 plus anticytokeratin (immunoperoxidase; brown), followed by anti-CD5 (immunoalkaline phosphatase; blue); and (C) anti-CD3 plus anti-CD20 (immunoperoxidase; brown), followed by anti-CD5 (immunoalkaline phosphatase; blue). These serial sections confirm that the blue cells identified with arrowheads in (A) are located in the epithelium because blue cells can be seen within the epithelial network in (B). They also confirm that the blue cells in (A) are B cells because in (C) the blue staining is blocked by CD20.

TONSIL

CD5 positive B cells were present within the epithelial network of the tonsillar crypts (figs 1 and 2). They expressed CD5 with approximately the same intensity as the CD5 positive B cells in the fetal lymphoid tissue. There was no evidence of CD5 positive B cells in the mantle zone or germinal centres of the tonsil using this technique.

MANTLE CELL LYMPHOMA

CD5 expression was clearly visualised in both cases of mantle cell lymphoma (fig 1). The intensity of expression was similar to that seen in the tonsillar epithelium and fetal lymphoid tissue.

Discussion

We have developed a technique that allows the visualisation of CD5 expression by B cells without double staining the B cells themselves. Therefore, this technique allows the assessment of the intensity of CD5 expression and the localisation of these cells within the lymphoid microenvironment. Within the normal tissues studied, B cells expressing similar amounts of CD5 were seen in the fetal intestine and the tonsillar epithelium. CD5 expression by fetal B cells is a consistent finding, which has been observed using several techniques, including single immunohistochemical staining.⁸ The expression of CD5 by intraepithelial B cells is a novel finding, but perhaps not surprising because CD5 is an inducible activation antigen in humans.⁹ Memory B cells are known to accumulate in the epithelium in the tonsil.¹⁰ As such, they are likely to be the first to contact antigen. The expression of CD5 by intraepithelial B cells might be the result of the induction of CD5 in response to antigenic challenge.

A surprising finding of our study was the consistent inability to identify CD5 positive B cells in the tonsillar mantle and germinal centre, despite intense CD5 expression by B cells in the intraepithelial compartment in the same section. This was confirmed using other double staining methods and other anti-CD5 antibodies, all of which were also able to identify CD5 positive B cells in rheumatoid joints, another site associated with CD5 positive B cells¹ (data not shown). This implies that amounts of CD5 expressed by the mantle zone and by germinal centre B cells, as measured by means of flow cytometry, are extremely small. Although B cells expressing CD5 in the mantle zone of secondary lymphoid follicles and CD5 positive B cells in primary follicles in the fetus have been considered to be analogous, they are certainly not analogous in terms of the amounts of CD5 that they express.

Mantle cell lymphoma is the accepted designation in consensus classifications and has replaced the terms intermediate lymphocytic lymphoma, lymphocytic lymphoma of intermediate differentiation, mantle zone lymphoma, and centrocytic lymphoma. The designation of the term "mantle cell lymphoma" is based on a number of clinicopathological features, which include the morphological and phenotypic resemblance of tumour cells to mantle cells, and

the tendency for the tumour to surround uninvolved benign follicle centres. It has been suggested that CD5 expression by mantle cell lymphoma is consistent with the origin of this lymphoma from either fetal lymphoid tissue or mantle cells.^{4,5} In our study, we found that CD5 expression by mantle cell lymphoma cells is similar to that observed in the fetus but is at a much higher intensity than that observed in the mantle zone. This dramatic quantitative difference in CD5 expression suggests that the tumour cells in mantle cell lymphoma might be more closely related to fetal B cells than to mantle zone B cells.

Molecular genetic analysis has shown that many cases of mantle cell lymphoma have the chromosomal translocation t(11;14)(q13;q32), involving rearrangement of bcl-1 to the immunoglobulin heavy chain locus. This results in overexpression of the PRAD1 gene, which encodes cyclin D1, a cell cycle regulatory protein that is not normally present in lymphoid cells. High concentrations of cyclin D1 can lead to greatly increased cell proliferation and the development of lymphoma.^{1,4,5} Because CD5 is an activation antigen, it is possible that the CD5 expression observed in mantle cell lymphoma is associated with this heightened state of activation.

In conclusion, we have found that the intensity of CD5 antigen expression by B cells in the mantle zone and germinal centre is so low that this antigen is undetectable using an immunohistochemical method that readily identifies other normal CD5 positive B cell populations and the tumour cells in mantle cell lymphoma. These data suggest that CD5 should not necessarily be considered to be a unifying characteristic of mantle cells and mantle cell lymphoma. It is possible that mantle cell lymphoma cells are more closely related to the B cells in primary lymphoid tissue in the fetus.

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