anticomplementary. Further work to confirm the nature of immune complexes associated with parvovirus B19 infection is warranted.

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Expression of MHC class II antigens by placental villi: no relationship with villitis of unknown origin

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Abstract

The aim of the study was to determine whether immunoreactivity to major histocompatibility complex (MHC) class II antigens studied by immunohistochemistry could be used reliably to define villitis lesions in placenta. Eighteen placental sections with villitis and 32 without, as determined in a careful observer reproducibility study, were immunolabelled with a monoclonal antibody to monomorphic determinants of MHC class II antigens (CR3/43), using a standard avidin-biotin peroxidase technique. Placentas with villitis were found to express MHC class II antigens. However, some showed no immunoreactivity. Occasional villi unaffected by villitis, including those near placental infarcts, also expressed MHC class II antigens. The study therefore showed that immunohistochemistry cannot be used to define villitis of unknown aetiology. It provides further evidence of the difficulties that can arise when immunohistochemistry conflicts with previous light microscopy findings.

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Keywords: Villitis of unknown aetiology, immunohistochemistry, MHC class II antigens, placental pathology.

Villitis of unknown aetiology (VUA) is associated with intrauterine growth retardation, preeclampsia, and stillbirth and may recur in subsequent pregnancies with similar associations. The essential feature of VUA is the accumulation of chronic inflammatory cells in the placental villous stroma.¹ Because no recognised infectious agent has been identified, it has been theorised that VUA is a marker of maternal immune attack on the fetal allograft² and a recent finding that these chronic inflammatory cells are of maternal origin would

lend support to this suggestion.³ Expression of major histocompatibility complex (MHC) class II antigens by syncytiotrophoblast, macrophages, and vessels within placental villi has been cited as further evidence of immunopathology in the lesion and has been suggested as a marker for VUA, and been used to define the lesion.⁴⁻⁷ To test this hypothesis, immunohistochemistry was performed on a series of placental cases that were the subject of a study to assess observer reliability in diagnosing villitis using conventional criteria.⁸

Methods

Sequential 5 µm sections from 50 formalin fixed paraffin embedded placentas that were used for an observer reliability study⁸ were rehydrated and later subjected to immunohistochemistry using a standard avidin-biotin-horseradish peroxidase technique with 3',3'-diaminobenzidine tetrachloride as the chromogen. These slides had been carefully classified as showing villitis or not by three experienced pathologists separately on two occasions and in a third conjoint viewing. Sections were immunostained by overnight incubation with a 1:3000 dilution of a monoclonal antibody recognising the monomorphic determinants of the MHC class II antigens (CR3/43, Dako, Denmark). Negative controls were performed by replacing the primary antibody with normal goat serum and the use of irrelevant antibodies. A positive control on tonsil was run with each batch of immunostaining. The entirety of each slide was examined regardless of whether villitis was focal or diffuse, or absent.

Results

MHC class II immunoreactivity by villous components was seen in 17 of the 18 cases considered to have villitis by a consensus viewing by

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the three pathologists in the observer reliability study using conventional light microscopy (results not illustrated). Immunoreactivity was seen in villi affected by villitis and in unaffected villi. However, villitis foci also showed absent immunostaining. The one negative case resulted because the relevant focus of villitis had cut through in the deeper immunostained section. Of the 32 cases thought not to have villitis by the pathologists, 17 showed MHC class II immunoreactivity of variable intensity. Some of the positivity was seen in areas adjacent to infarcts.

Discussion

Although differences in methodology may account partly for the differing results, this study does not confirm that MHC class II immunoreactivity labels for villitis exclusively. MHC class II immunoreactivity near to infarcts indicates activation of the resident stromal macrophages and confirms their role in scavenger functions. Immunoreactivity in non-villitis areas in cases diagnosed conventionally as villitis as well as in non-villitis cases suggests immunopathology, but the pathogenesis is unclear and could be the result of infection or allogeneic recognition reactions. The possibility that villitis is a marker of a maternal immune attack on the fetal allograft² is supported by the finding of maternal inflammatory cells in VUA lesions.³ However, the alternative possibility of a fetal response to foreign antigens, maternal or infectious, should not be discounted. Firstly, MHC class II immunoreactivity by syncytiotrophoblast is analogous to aberrant expression by epithelium in graft versus host disease⁹; secondly, the majority of the intravillous inflammation is fetal in origin, the proportion of maternal cells in affected villi ranging between 30% and 54%³; and finally, in this study MHC class II immunoreactivity was detected in non-inflamed areas. Without double labelling experiments, it could not be determined whether the labelling in non-inflamed areas was present in syncytiotrophoblast and the resident stromal macrophages or confined to the latter. Labelling by syncytiotrophoblast could be the result of inducement by cytokines in the microenvironment and can lead to influx of maternal inflammatory cells and a reactive activation of resident fetal macrophages. Labelling by macrophages alone, on the other hand, could be due to activation by an as yet unidentified agent and can lead to induction of syncytiotrophoblast MHC class II labelling. Further studies will be necessary to determine the sequence and primary site of MHC class II expression in non-inflamed villi as this may

help to explain the initiating factors in the causation of villitis and in our understanding of its clinical significance. We have not excluded the hypothesis that villitis is an immunopathological lesion; we have merely found that immunopathology, if defined by MHC class II immunoreactivity, is present also in villi showing no histological evidence of villitis.

This study also questions the assumption that immunohistochemistry provides an objectivity that conventional histological methods lack. VUA is defined initially by conventional light microscopy but there can be significant intraobserver and interobserver unreliability in its diagnosis, one reason being the difficulty in distinguishing between stromal hypercellularity of the resident macrophages and true inflammation. MHC class II immunoreactivity by villitis cases led to a putative specific marker for villitis. Immunohistochemistry could then conceivably assume a precedent role over conventional light microscopy in defining the lesion. This is a paradigm of the chicken and the egg: how should the diagnosis be made and which criteria take priority? Although immunohistochemistry is being used increasingly as an aid to histological diagnosis and to define pathological lesions, cellular phenotype, and histogenesis, a dilemma can arise when there is conflict between the light microscopy and immunohistochemistry diagnoses.¹⁰ Unfortunately, attempts to resolve this using decision analysis and probability calculations may not apply always as illustrated by this study.

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