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Immunoperoxidase labelling of previously stained tissue sections: application in oral histopathology

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SUMMARY

We have investigated by the avidin-biotin peroxidase technique a wide variety of recognized cellular antigens in paraffin sections of both normal and pathological material which have been previously stained with hematoxylin and eosin. The intensity of peroxidase reaction was adequate to permit the diagnosis and the background of the immunoreaction minimal. This technique therefore, appears useful either for retrospective analysis of cellular antigens or when limited unstained material is available.

KEY WORDS:

Immunohistochemistry - Peroxidase - Hematoxylin - Eosin stained sections.

RÉSUMÉ

Nous avons étudié, au moyen de l'immunoperoxydase, la présence de nombreux marqueurs cellulaires sur des coupes en paraffine de tissus normaux et pathologiques, colorés préalablement avec l'hématoxyline-éosine. L'intensité de la réaction était tout à fait adéquate pour permettre le diagnostic avec un moindre background. Cette technique apparaît donc très utile pour des analyses rétrospectives de certains antigènes cellulaires, ou lorsque le matériel enrobé en paraffine est disponible en quantité limitée.

MOTS CLEFS:
Immunohistochimie - Peroxydase - Hématoxyline - Eosine.

INTRODUCTION

Immunoperoxidase technique has permitted great advance in diagnostic histopathology showing the direct visualization of specific antigens in tissue (Hsu et al., 1981). This method needs of unstained sections usually from formalin fixed and paraffin embedded block tissue. Retrospective investigations carried out on archived material are also possible. However, sometimes residual unstained tissue is not available for these studies or the area of interest cut through or yet the archived block may have been lost by serial sectioning. In these conditions it would be useful to carry out immunohistochemistry analysis on tissue sections previously stained with the routine stains.

Recent studies (Walder et al., 1984; Milios and Leong, 1987; Crooks and Altini, 1989) have shown that immunoperoxidase technique may be used successfully on routinely stained sections. These investigations reported, however, variable results.

Because of the potential interest of this technique, we have decided to test a range of specific diagnostic antibodies on both normal and pathological Bouin's fixed tissue and to compare the immunostaining with sections that had been previously stained with hematoxylin and eosin.

MATERIALS AND METHODS

Paraffin wax blocks containing normal tissue was sampled from area away from lesions removed for a wide variety of pathological conditions. The material used included parotid glands (n = 2), tonsil (n = 1), healthy dental pulps (n = 4), gingivae (n = 3) and buccal mucosae (n = 2). The tissues were Bouin's fixed, and dated back to 1988. Sections were of 4 μm of thickness. The coverslip from slides stained with hematoxylin-eosin were removed by treatment with xylene and the sections taken through serial change of absolute alcohol before being decolorized by 1% acid alcohol for 5 min. The decolorized tissue sections, and controls were then stained using the avidin-biotin peroxidase technique (Vector Laboratories, Burlingame, Ca, USA). The antibodies used were: PCK (pancytokeratin antigen from Amersham undiluted), S-100 (1:200), EMA (1:200), Vimentin (1:250) and LCA (1:50) purchased from Dakopatts. For the lymphoid tissues all monoclonal antibodies (see Table I) were used at concentration of 100 μl.

TABLE 1
Antibodies used to detec the presence of lymphoid tissues antigens.
Anticorps utilisés pour détecter la présence d'antigènes des tissus lymphoïdes.

Antibodies	Antigens labelled	Source
LN-1	Antigen on B-cells	Techniclone
LN-2	Antigen on B cells	Techniclone
UCHL 1	T cells, Thymocytes	Dako
L 26	Antigen on B cells	Dako
MT 1	Membrane bound on T cells	Biotest
MT 2	Cytoplasmic on B cells excepting plasma cells	Biotest
LEU-22 (L 60)	T cells, Monocytes	Dako
Leu M1	Granulocytes, macrophages	Becton

The avidin-biotin-peroxidase technique consisted in submerging the sections in 0.3% hydrogen peroxide in methanol for 20 min. Sections were then rinsed in phosphate buffered saline (PBS), pH 7.4, before incubation with the appropriate non immune serum for 30 min. prior to incubation with the specific primary antisera for 30 min. The sections were then washed in PBS and the corresponding biotinylated secondary antibody was applied for 30 min. Then, after washes in PBS, preformed avidin-biotin peroxidase complex was applied for 30 min. After washes in PBS the reaction was developed by incubating the sections in diaminobenzidine hydrochloride at a concentration of 50 mg per 100 ml of PBS containing 50 μl of 30% hydrogen peroxide. The sections were washed in PBS counterstained with hematoxylin and mounted. Omission of the primary antibody and its replacement by nonimmune sera or PBS constituted the control.

Pathological tissues slides previously hematoxylin and eosin stained, were retrieved to represent the range of antigens studied in normal tissue. This material was represented by pleomorphic adenomas of parotid (n = 2), mucoepidermoid carcinomas (n = 2), radicular cysts (n = 5), dentigerous cysts (n = 3), inflamed dental pulp obtained from carious teeth (n = 4), squamous cell carcinomas (n = 3) and chronic periodontitis (n = 3). The sections were decolorized and incubated by specific antibodies according the technique described above.

RESULTS

The section were practically decolorized by this method. All the antigens investigated could be demonstrated in tissue sections of normal and pathological tissues (Figs. 1-3). Immunostaining intensities comparable with those of control sections were observed in most of the previously stained hematoxylin-eosin sections. The quality of the immunostaining was adequate to permit diagnostic examination. In none of the sections, there was an increased background staining. Control sections were negative.

Fig. 1: S-100 antigen in a tissue section of salivary gland previously stained with hematoxylin and eosin. Nerve bundle showing a positive immunostaining for S-100 protein antigen is visible. × 400.
Fig. 1: Expression de l'antigène S-100 dans une section de tissu salivaire colorée au préalable avec hématoxyline et éosine. Un faisceau nerveux est marqué positivement par l'anticorps dirigé contre la protéine S-100. × 400.

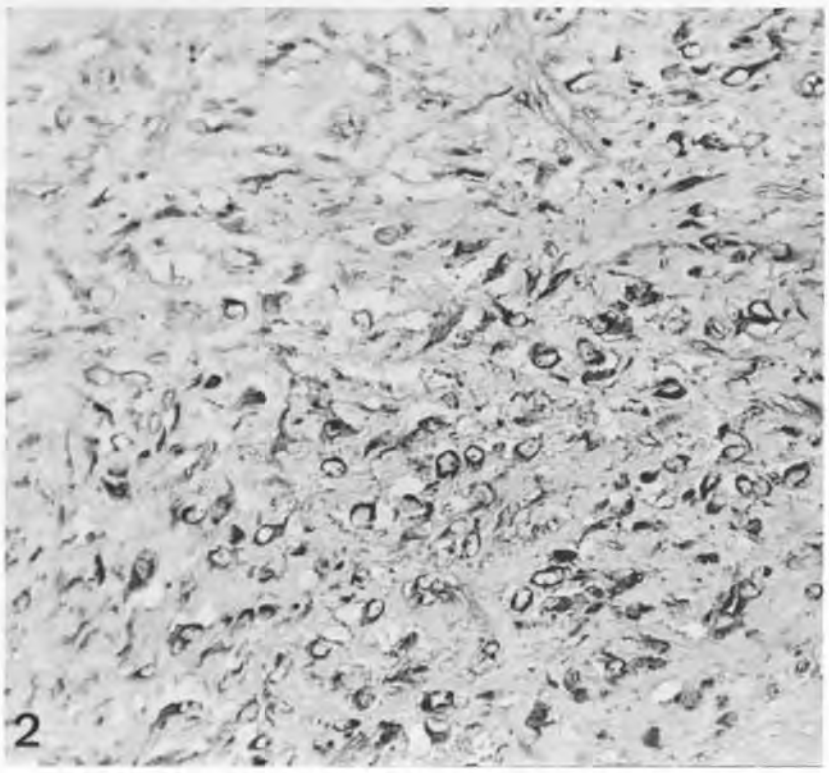

Fig. 2: Vimentin in fibroblast cells of a dental pulp section, previously stained with hematoxylin-eosin. × 250.
Fig. 2: Présence de vimentine dans des fibroblastes pulpaires colorés préalablement avec hématoxyline et éosine. × 250.

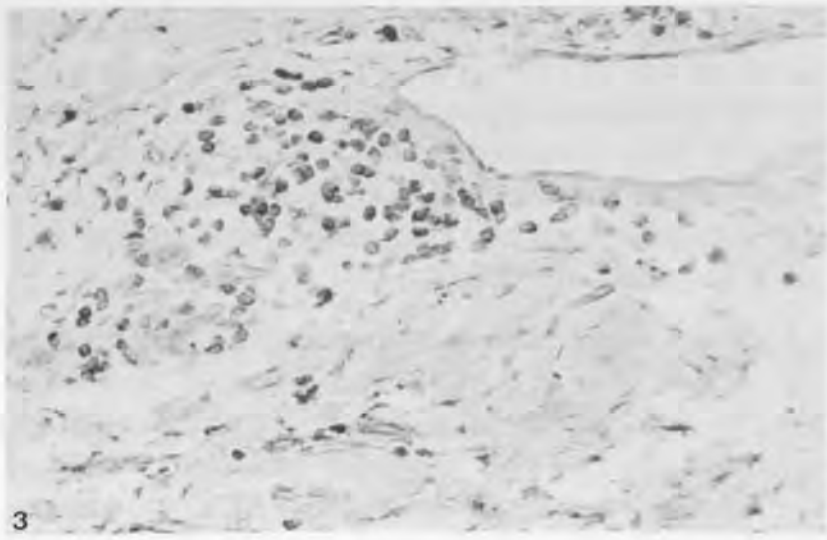

Fig. 3: Gingival tissue section previously hematoxylin-eosin stained. Numerous T cells of the cellular infiltrate located around a vessel are immunolabelled by an antibody directed against UCHL 1 antigen. × 400.
Fig. 3: Section de tissu gingivale colorée au préalable avec hématoxyline et éosine. Les nombreuses cellules T formant l'infiltrat inflammatoire qui sont situées autour d'un vaisseau, sont marquées positivement par l'anticorps UCHL 1. × 400.

DISCUSSION

Immunoperoxidase technique is an highly sensitive technique permitting to localize specific cellular antigens. One of the advantage of this technique is the possibility it offers of carrying out retrospective investigations on archived material. However, in some instances, tissue is limited or unavailable. In such cases it may be necessary to carry out immunohistochemistry analysis on the only tissue section available, that is previously stained with hematoxylin-eosin.

Few studies have reported the immunoperoxidase staining of previously stained tissue sections. The results of these investigations are variable. In fact the first publications (Walder et al., 1984) showing immunoperoxidase staining of previously stained sections stated that a higher concentration of the primary antibody were necessary in comparison with the unstained control. In addition, the quality of the immunostaining and the intensity of the staining were diminished with all the antibodies used. In the same study the authors observed an increase of the background staining and the absence of immunostaining for S-100 protein. Another report (Milios and Leong, 1987) showed that it was possible to detect a wide range of tissue antigens in previously stained sections, without loss of sensitivity and with minimal background staining.

Crooks and Altini recently reported the demonstration of tissue antigens in previously stained hematoxylin-eosin slides with little or no loss in sensitivity. They obtained variable results with PAS and Von Gieson previous stained tissue sections. On the contrary of other study they observed an intense staining for S-100 antigens.

In our investigation we attempt to localize a series of cellular antigens in Bouin's fixed material, after decolorization technique. Among the antigens investigated there were a series of cellular markers considered specific for lymphoid tissue cells. Monoclonal leucocyte antibodies localization need fresh tissue and cryostat sections. The main disadvantage of frozen sections is the generally poor morphology. Attempts to localize monoclonal antibodies directed against leucocytes antigens in paraffin sections have been disappointing without the use of special fixations and processing protocols. The introduction of antisera to leucocytes antigens resistant to formalin fixation has relaunched interest in the possibility to immunolabelling antigens of lymphoid tissues, in a series of pathological conditions (Norton and Isaacson, 1989; Epstein et al., 1984; Poppema et al., 1987).

We show here that it is possible to detect tissue antigens, including those for lymphoid tissue, in previously stained sections with no loss of sensitivity and with minimal background staining reaction. Including the antigens investigated, there was S-100 protein. The stain for S-100 protein was comparable to the unstained sections. So our results are in agreement with the others reported (Milios and Leong, 1984; Crooks and Altini, 1989). It is clear that the variable results reported in the literature can be accounted on the different technique used i.e. fixation (formalin or microwave), peroxidase technique (unlabeled peroxidase-antiperoxidase (PAP), avidin-biotin-peroxidase (ABP)), enzyme digestion prior to staining or the nature of antibodies used. On the base of our results, Bouin's fixation and ABP technique are good methods to localize cellular antigens furnishing also constant results.

Decolorization technique possesses a great potential usefulness when tissue is limited or unavailable or when tissue sections are limited. In addition with the decolorization technique, different antigens could be also demonstrated on the same slide applying different primary antibody to other sections on the slide. In conclusion the use of polyclonal and monoclonal antibodies reacting with previous stained sections provides possibilities for good preservation of morphological details associated with the immunohistological determination of cell subtypes. This method offers therefore obvious facilities for diagnostical examination and experimental investigations. Further studies will be necessary to demonstrate specific antigens, on slides previously stained with either other routine or special stain.

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