Immunohistochemical method for identification of ebstein barr virus from the tons...

Science, Chemistry

IMMUNOHISTOCHEMICAL METHOD FOR IDENTIFICATION OF EBSTEIN BARR VIRUS FROM THE TONSIL TISSUE

INTRODUCTION:

Epstein Barr Virus, commonly referred to as ‘ EBV’ belongs to the herpes virus family. It is one of the most commonly found human viruses. It has a worldwide occurrence and most people become infected with it at one or the other time of their lives. This essay describes an immune-histo-chemical method for the identification of Epstein Barr Virus from the tonsil tissue removed from a patient.

METHOD:

Here we will discuss a method used for detection of Epstein Barr Virus by Tetsuya Ikeda, Ryo Kobayashi, Manabu Horiuchi, Yoshifumi Nagata, Makoto Hasegawa, Fumio Mizuno and Kanji Hirai from the Departments of Microbiology, Tumor Virology and Otolaryngology of Tokyo Medical University, Tokyo Medical and Dental University and Kyorin University School of Medicine, Japan, respectively. The main objective was the detection of lymphocytes productively infected with Epstein Barr Virus in non-neoplastic tonsils.

This study used the samples of tonsils from patients suffering with the chronic tonsillitis. So, a total of fifteen tonsillar tissues were obtained from eight donors. After performing a series of tests on these samples, it was confirmed that none of these patients had any sort of malignancy or Infectious Mononucleosis. Also, almost all of them had antibodies to EBNA indicating that they were not in acute phase of EBV infection.

As there is no in vitro natural system for EBV replication, most of the studies

have relied upon the semipermissive EBV carrying Burkitt’s lymphoma cell lines. In this technique, various means are used to induce the reproductive cycle in the virus. The EBV gene products obtained as a result include a protein product of the BZLF 1 gene, called as ZEBRA (BamHI Z fragment, EBV replication activator). This protein plays a role in switching the virus from latency to the lytic cycle.

Moreover, another protein called as the viral capsid antigen (VCA) is expressed only in the virus producing cells. This one is in close relation with the capsid of the viral particles.

Thus keeping the above information in mind, the following study can be understood.

In order to determine the presence of the lytic proteins of this virus in the formalin-fixed tonsils, immunohistochemistry was performed. The steps were as follows:

1. Serial sections of the paraffin embedded tonsils were deparaffinized.

2. Rehydration was done and 0. 2% trypsin was applied to retrieve the antigen.

3. Reaction of these sections was then performed with the anti-ZEBRA, -VCA and –cytokeratin (marker for the epithelial cells, clone AE1/AE3) monoclonal antibodies.

This immunohistochemical detection revealed the expression of ZEBRA and VCA in about 7-10 of a total of 700 tonsillar mononuclear cells. Three of the eight donors showed the cytoplasmic immunoreactivity. Morphologically, these ZEBRA and VCA-positive cells had a resemblance with the lymphocytes. Also they were found in the same area as the tonsillar

mononuclear cells.

ASSESSMENT OF THE RELATIONSHIP BETWEEN EPITHELIUM AND EBV LYTIC PROTEINS:

In order to assess the relationship between the epithelium and EBV lytic proteins, antibodies to ZEBRA, VCA and cytokeratin were obtained and the serial tissue sections were reacted with them. These sections did not have ZEBRA and VCA-positive cells localized in them. As a control, the samples which lacked the primary antibody did not show any immunoreactivity.

The donors which did not have ZEBRA-positive cells by immunohistochemistry, did not show the BZLF 1 transcripts.(1)

In this study, in addition to the immunohistochemistry, some other tests were also performed. The results suggested that the tonsillar lymphocytes are among the EBV replication sites and serve as a reservoir for the EBV in normal individuals.

REFERENCES

1. Detection of lymphocytes productively infected with Epstein-Barr Virus in non-neoplastic tonsils [online] 2000 [cited 2008, November 16]. Available from:

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