METHODS FOR QUANTITATING HLA GENE PRODUCT EXPRESSION IN THE LIVER DURING HEPATITIS B VIRUS INFECTION

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HLA antigens are human glycoproteins regulating many immune responses which are encoded for by genes mapped on the short arm of chromosome 6. HLA class I (A,B,C) antigens are composed of 43,000 mol. wt. glycosylated polypeptides carrying the polymorphic determinants bound to a 12,000 mol. wt. non-glycosylated polypeptide (β₂-microglobulin) coded for by a gene on chromosome 15. Class I antigens are present on the majority of mononucleated cells, although recent studies have indicated that their distribution is somewhat more restricted. In the liver HLA class I antigens are mainly present on the endothelial cells, Kupffer's cells and bile duct epithelial cells, whereas normal hepatocytes display low amount of these antigens.

HLA class II (DR, DP and DQ) antigens are composed of two glycosylated polypeptides of mol. wt. 33,000 (α) and 28,000 (β). These antigens have a more restricted distribution being present only on monocytes, B lymphocytes and some endothelial cells, but in the liver both hepatocytes and bile duct epithelial cells are negative.

Early studies in mouse have demonstrated that HLA antigens play an important role in the initiation of the immune response to a virus. The association of viral antigens with the Ia antigens (equivalent of HLA class II antigens in mouse) on the surface of specialized macrophages is thought to be recognized by inducer (helper) T lymphocytes which then assist in priming the cytotoxic T lymphocytes and in initiating the production of sensitized B lymphocytes. The elimination of the virus-infected cells is then dependent on the recognition by sensitized cytotoxic T cells of the viral antigens in association with the H-2K and H-2D antigens (equivalent to human HLA class I) on the cell membrane. Subsequently this mechanism was shown to be operative in several animal and human virus systems. Thus, it has been proposed that defects in HLA display could lead to a failure of the immune system to recognize and eliminate virus-infected cells.

Key-words: Collagenase; Hepatitis B virus; HLA; Immunofluorescence; Microphotometer; Monoclonal antibodies; Radioimmunobinding.

In the last few years there has been a great deal of interest in studying the changes in the HLA expression during a variety of liver diseases of various aetiology such as autoimmune, alcoholic, neoplastic, rejection after orthotopic liver transplant and bone marrow transplantation, as well as viral infections. All these studies have been performed mainly by using immunocytochemical techniques, such as immunofluorescence and immunoperoxidase.

Monoclonal antibodies to different HLA loci are now widely available and have been proved to be useful tools in studying the HLA tissue distribution. Even more of interest, there are now a few monoclonal antibodies to the HLA-D region (e.g., TAL 1B5) and to β2-microglobulin (e.g., BBM1) which can be used on paraffin-embedded tissue. This property is of course particularly useful because the liver architecture is better preserved in paraffin than it is in frozen sections. In addition we now have available different techniques to increase the sensitivity by 100-1,000 fold using an immunocomplex enzyme anti-enzyme linked to an antibody bridge (peroxidase-antiperoxidase). Furthermore, we can use the high-affinity avidin for the vitamin biotin to enhance the staining. Several molecules of biotin can be coupled to an antibody bridge and so the final addition of a labeled avidin results in a firm bound with exceedingly bright staining. The various techniques are described in details by Polack and Van Noorden. Quantitative assessment however of HLA expression can be particularly useful when patients with different liver pathology are compared or when the effects of drugs such as interferons, which are potent HLA inducers, are studied.

In the last few years we have been particularly interested in studying the expression of HLA antigens in the liver during hepatitis B virus (HBV) infection. HBV causes one of the most common infections in man; 90% of patients develop an acute illness from which they recover, but 10% develop a chronic infection which may lead to chronic liver disease and primary liver cell cancer. The persistence of HBV in some individuals after acute infection may be due to a selective impairment of the immune system in recognizing and eliminating virus-infected cells.

The following discussion is a summary of some techniques which we have used to study and quantitate the changes of HLA antigen expression in the liver occurring during various phases of HBV infection and the way in which we have attempted to identify possible mechanisms leading to chronicity.

**Technique for quantitating HLA antigen expression on frozen sections from liver biopsy**

Cryostat sections (5-μ thick) are cut from frozen biopsies and air-dried at room temperature overnight. Sections are then fixed for 5 min in chloroform:acetone solution (1:1) and, after an extensive wash in phosphate buffered saline (PBS), pH 7.6, can be either stored at -70 °C or stained immediately. The fixation in chloroform:acetone or alternatively in acetone only is particularly useful for detecting cell surface antigens.

Sections are then incubated with specific monoclonal antibodies recognizing HLA gene products for 60 min at room temperature at saturating concentrations, determined by titration on known positive specimens. Specificity controls are performed using nonimmune mouse serum at same protein concen-