IMMUNE REACTIONS IN ACUTE VIRAL HEPATITIS

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CONTENTS

Summary

The work described in this thesis was carried out in the University Department of Medicine at The Queen Elizabeth Hospital, Woodville, South Australia during the period January 1970 - November 1973.

The thesis is introduced by a general review on humoral and cellular immune reactions in Hepatitis A and Hepatitis B. The second chapter concerns the methods used in the study. They include a lymphocyte culture microtechnique using whole blood rather than purified lymphocytes and immunofluorescent and autoradiographic techniques for the identification of B & T lymphocytes and lymphocytes undergoing blast transformation. Other methods used in the study which were in routine operation in the University or Hospital laboratories are not described in detail. These include detection of serum autoantibodies and Australia antigen, the measurement of serum immunoglobulins and complement and biochemical liver function tests.

The whole blood technique described is a modification of that reported by Junge, Hoekstra, Wolfe & Deinhardt (1970). Chapter III describes the comparison of this method with culture of purified lymphocyte preparations. This validation was considered necessary

because most studies of lymphocyte function in patients with hepatitis and in normal subjects have been performed with purified lymphocyte preparations. In normal subjects there was a good correlation between the two methods for measuring phytohaemagglutinin (PHA) induced tritiated thymidine (^{3}HT) uptake when the cultures were done in autologous serum but not when they were done in foetal calf serum (FCS). There was also a high degree of correlation when the two methods were used to measure spontaneous lymphocyte transformation in peripheral blood. The reproducibility of the results as assessed by the coefficient of variation was better for whole blood cultures than for purified lymphocyte cultures. This chapter also includes a description of the studies undertaken to determine the optimal culture conditions and preliminary work on the influence of diet and the time of collection of blood samples on PHA induced and spontaneous lymphocyte uptake of ^{3}HT. The important implications in regard to reproducibility of results are discussed.

Chapter IV concerns a study of patients with acute viral hepatitis. Serial measurements of humoral and cellular immune reactions were performed in 26 patients, of whom 17 had hepatitis A and 9 had hepatitis B. The patients were studied at weekly intervals during the

acute and convalescent periods of the illness. They were compared to normal control subjects and to 11 persons who had previously had hepatitis. With regard to humoral immunity the frequency of autoantibodies and serum levels of immunoglobulins and complement were similar to those reported by other workers. Studies of lymphocyte function showed a marked impairment of the lymphocyte response to PHA during the first two weeks after the onset of jaundice. A less marked impairment of the lymphocyte response was found to persist for at least 6 - 10 weeks. Investigation of the 11 persons with a previous history of hepatitis showed that the impairment was not permanent. The two main possible reasons for the impaired response, a direct effect on the lymphocyte by the viruses of hepatitis or the production of a serum inhibitory factor were further investigated and the results are detailed in Chapter V.

At the same time as the lymphocyte response for PHA was impaired during the first few weeks of hepatitis spontaneous lymphocyte transformation was increased. This response also occurred in other viral infections such as infectious mononucleosis and after smallpox vaccination. It seemed paradoxical that there should be a rise in spontaneous transformation at the time when PHA induced transformation was impaired. The immunofluorescent and

autoradiographic studies in Chapter VI demonstrated that there was no increase in the number of circulating B cells in the circulation during the acute phase of hepatitis. Dividing lymphocytes were present in the circulation in increased numbers and were shown by autoradiography to be large cells, probably the "atypical lymphocytes" seen in the blood films. It was concluded that these cells were T cells, that they accounted for the increase in spontaneous ^{3}HT uptake in the lymphocyte cultures and that the impaired response of the lymphocytes to PHA might at least in part, be attributed to commitment of an abnormally large proportion of the T cells as a result of hepatitis.

The effect of serum factors on the lymphocyte response to PHA was next investigated and is described in Chapter V. Serum taken from patients during the acute phase of hepatitis inhibited PHA induced ^{3}HT uptake by normal lymphocytes whilst convalescent serum did not. Moreover, lymphocytes from patients with hepatitis were also inhibited by autologous acute phase serum but not by autologous serum taken later in the course of the disease. Since washing the lymphocytes did not completely restore their response to normal, a defect in the lymphocyte itself, possible induced by virus, cannot be excluded.

It has been suggested that the competence of the

immune system in the host determines the outcome of infection with hepatitis B virus. The hypothesis suggests that in acute hepatitis the T cells react specifically against Australia antigen (Au) incorporated in the liver cell membrane with consequent liver damage but with ultimate elimination of the virus. If this is so it should be possible to detect specific cell mediated immunity to the antigen and the work of Yeung Laiwah (1971) suggested that this could be done. For this reason a study was made of the response of lymphocytes from patients with hepatitis to various Au preparations including pooled Au containing serum, a partially purified preparation and commercially prepared purified Au particles (Chapter VII). These preparations only rarely stimulated lymphocyte transformation, as measured by an increase in ^{3}HT uptake, in patients with hepatitis B and lymphocytes from patients with hepatitis A reacted in a similar manner.