Immunosuppressive Therapy for Chronic Liver Disease

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The finding of autoantibodies in the sera of many patients with active chronic hepatitis and primary biliary cirrhosis (Doniach *et al.*, 1966) and the presence of a positive LE cell test in about 15 per cent of patients with active chronic hepatitis (Mackay *et al.*, 1956) were the first pieces of evidence linking autoimmunity with these diseases. It is not surprising, therefore, that various immunosuppressive drugs have been used in their treatment. Although some benefit often seems to follow their use, it is only recently that the real effects of therapy have been evaluated.

ACTIVE CHRONIC HEPATITIS

Autoimmune Mechanisms

Before discussing the immunosuppressive agents that have been used in the treatment of active chronic hepatitis, it is obviously necessary to examine the evidence for this being an autoimmune disease. The circulating autoantibodies are not organ-specific but are capable of reacting with nuclei, mitochondrial or smooth muscle components in almost any organ, so it is unlikely that they are directly involved in the pathogenesis of a disease largely confined to the liver. However, liver-specific immunological reactions have been detected recently in patients with active chronic hepatitis and these could be more important in the production of liver damage. Meyer zum Buschenfelde, using indirect immunofluorescence, demonstrated liver-specific autoantibodies in 10 per cent of his patients with active chronic hepatitis and isolated the liver-specific antigens from human liver to which these antibodies were directed (Meyer zum Buschenfelde and Kossling, 1971). When these antigens were injected repeatedly into rabbits, liver lesions were produced which closely resembled chronic aggressive hepatitis in man.

Our own studies of the immunological reactions which accompany rejection of some human liver transplants (Eddleston *et al.*, 1971) focused our attention on cell-mediated immunity. Using a leucocyte migration test as an assay of this type of immunity, we were able to demonstrate in about half our cases of

active chronic hepatitis that there was evidence of hypersensitivity to antigens in a liver homogenate (Smith *et al.*, 1972). As with humoral antibodies this sensitisation would be of more relevance to the pathogenesis if it could be shown to be liver-specific. We have recently isolated from human liver one of the liver-specific antigens of Meyer zum Buschenfelde, a lipoprotein thought to be a normal constituent of the hepatocyte plasma membrane (Hopf *et al.*, 1973), and have used it as antigen in the leucocyte migration test (Miller *et al.*, 1972). With this system, liver-specific cell-mediated immunity has been demonstrated in 60 per cent of patients with active chronic hepatitis, in none of the control subjects, and in only 7 per cent of those with chronic liver disease not thought to be autoimmune (Fig. 1).

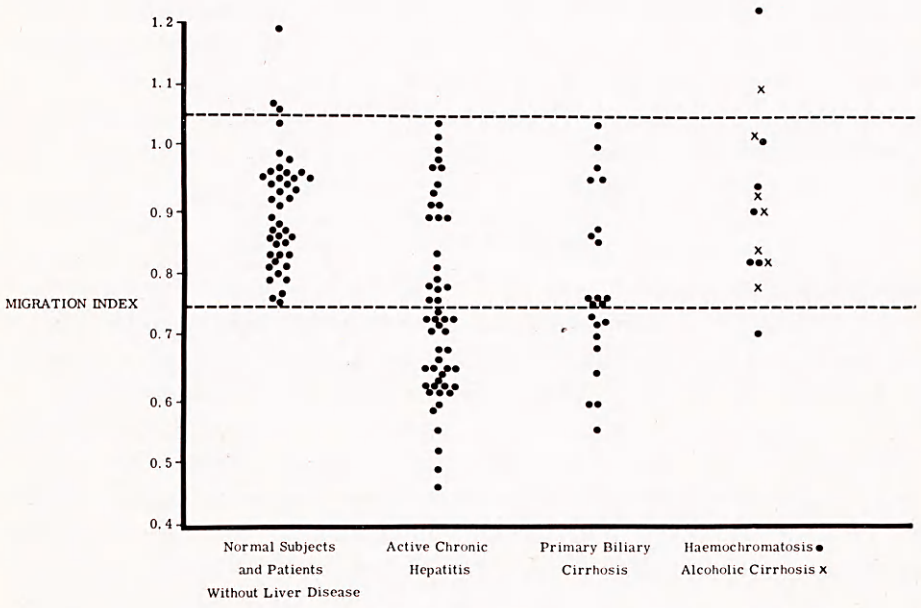

Fig. 1. Results of the leucocyte migration test, expressed as a migration index, using a liver specific lipoprotein as antigen. Inhibition of migration, indicating cell-mediated immunity to the antigen, is present in 60 per cent of patients with active chronic hepatitis and in 42 per cent of those with primary biliary cirrhosis. The results in the other groups are almost all within the normal range.

Although cell-mediated immunity has become the new fashion in autoimmunity, it is likely that both humoral and cellular effector mechanisms co-operate in the production of tissue damage. Such co-operation has been described *in vivo* in experimental autoimmune orchitis (Brown *et al.*, 1967)

and in some *in vitro* studies of cytotoxicity (MacLennan *et al.*, 1969). For immunosuppressive therapy to be effective it may therefore be necessary to suppress both limbs of the immune response.

Action of Immunosuppressive Drugs

Many immunosuppressive drugs have been used in the treatment of active chronic hepatitis, but their modes of action are often distinct, and without full understanding of the immunological reactions involved in the production of the disease it is difficult to choose the most suitable therapeutic regime. The usual division of immunosuppressive drugs into antimetabolites such as azathioprine and 6-mercaptopurine, alkylating agents such as cyclophosphamide, and corticosteroids, conceals the large differences in action that exist between drugs of similar type. An alternative classification into three types has been proposed, based on the relative effectiveness of the drugs at different stages in the development of an immune response (Makinodan *et al.*, 1970). Type 1 compounds such as corticosteroids are most effective when given before the administration of antigen, Type 2 drugs such as azathioprine when given after the antigen, and Type 3 drugs such as cyclophosphamide seem capable of suppressing the immune response whenever they are given. Although the differences are of obvious importance in a situation such as organ transplantation, where the introduction of foreign antigens occurs at a clearly defined moment in time, in autoimmune disease drugs of Types 2 and 3 would appear to be the only possible choices. However, the situation is further complicated by consideration of two other variables in the mode of action of immunosuppressive agents, namely, the selectivity of their effects on humoral or cellular immunity and the extent of any co-existing anti-inflammatory properties (Stevens and Willoughby, 1969). In these respects, the action of each drug is likely to be unique (Makinodan *et al.*, 1970; Steinberg *et al.*, 1972) (Table 1). Indeed, there is some evidence that in man the anti-inflammatory rather than the immunosuppressive effects of these agents are responsible for

TABLE 1. An example of the different effects on immunological reactions of two commonly used immunosuppressive drugs

	Cyclophosphamide	Azathioprine
Primary immune response	↓↓	↓↓
Secondary immune response	↓↓	↓
Inhibition of mitosis	+	++
Delayed hypersensitivity	↓↓	↓
Anti-inflammatory	±	++

the symptomatic improvement that often follows their use in autoimmune diseases (Dodson and Bennett, 1969; Denman *et al.*, 1970). On the other hand, in experimental autoimmune diseases in animals, the therapeutic value of immunosuppressive drugs given in larger doses often correlated better with their effects on immune responses. Allergic encephalomyelitis, induced in animals by injection of antigens from neural tissue, is often fatal but can be prevented by many drugs when they are given before the onset of clinically apparent disease. Once encephalitis has appeared, it is much more difficult to control, and most immunosuppressive drugs, even those with marked anti-inflammatory properties, are without effect. However, dramatic reversal of this disease has been achieved with cyclophosphamide (Paterson and Drobish, 1969). This drug also appears to be most successful in the treatment of the haemolytic anaemia that develops in New Zealand Black mice (Lemmel *et al.*, 1971). Although cyclophosphamide has been shown to be effective in man in controlling Wegener's granulomatosis (Novack and Pearson, 1971) and rheumatoid arthritis (Co-operating Clinics Committee of the American Rheumatism Association, 1970), it is more toxic than some of the other immunosuppressive drugs and may produce alopecia and haemorrhagic cystitis. It has not been used extensively in active chronic hepatitis.

Immunosuppressive Drugs and Active Chronic Hepatitis

Corticosteroids have been used since the 1950s and the first use of 6-mercaptopurine was reported by Dameshek and Schwartz in 1960. Since then there have been numerous poorly controlled observations on treatment with azathioprine (Mistilis and Blackburn, 1967; Mackay, 1968; Gross and Zwirner, 1969), 6-mercaptopurine (Mistilis and Blackburn, 1967) and chlorambucil (Phlippen *et al.*, 1969). Although many reports stress that the patients showed clinical and biochemical improvement, proper assessment of therapy is possible only in a prospective randomised trial. The unpredictable and fluctuating clinical course of this disease makes less stringent enquiry almost useless.

Controlled Trials

The first controlled prospective trial was started in 1963 at the Royal Free Hospital (Cook *et al.*, 1971) and compared prednisolone with placebo. The initial dose of prednisolone was 15 mg daily but this was reduced when the disease was controlled. After the trial had been in progress for six years, 3 of the 22 corticosteroid-treated patients and 15 of the control patients had died and the difference in mortality in the two groups was highly significant. In 1968 we started a prospective controlled trial at King's College Hospital,

comparing prednisone 15 mg daily with azathioprine 75 mg daily. At the end of two years' treatment, the prednisone group (22 patients) was found to be superior to the azathioprine group (25 patients) in three respects (Murray-Lyon *et al.*, 1973). The fall in gamma globulin was significantly greater on prednisone, although the liver function tests improved in both groups. Barium swallow examination was repeated at the end of the trial period on the patients who did not have oesophageal varices at the start, and varices were shown to have developed in significantly more patients on azathioprine, suggesting that the progress of the disease had not been checked. Finally, the calculated probability of surviving for two years was 95 per cent on prednisone and 72 per cent on azathioprine (Fig. 2). Although prednisone was clearly superior to

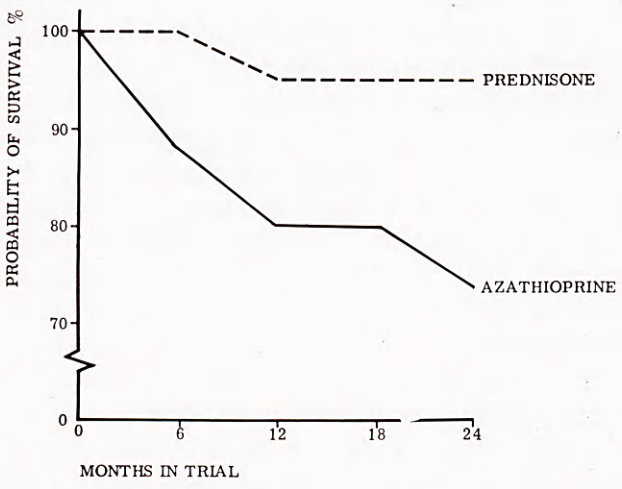

Fig. 2. The calculated probability of survival in the two groups over the two years of treatment.

azathioprine, there were serious adverse effects in 6 of the 22 patients (crush fracture of vertebrae (1), diabetes mellitus (3), and systemic hypertension (2)). This raises the question of whether a smaller dose of prednisone alone or combined with azathioprine might be sufficient to control the disease. The Mayo Clinic trial (Soloway *et al.*, 1972) showed that prednisone 20 mg, or a combination of prednisone 10 mg and azathioprine 50 mg, was superior to azathioprine alone (100 mg) or placebo in prolonging life expectation and improving the biochemical and histological abnormalities. Serious adverse effects occurred in 21 per cent of patients treated for more than 12 months with 20 mg prednisone and, although it is too early to be sure, it seems likely that

this will be less of a problem with the combination therapy. Whether or not prednisone 10 mg alone would be equally effective is also unknown.

Corticosteroid Toxicity

The reasons for the high incidence of serious steroid adverse effects have recently been clarified. At pharmacological doses many steroids, including cortisol (hydrocortisone) and prednisolone (the active metabolite of prednisone), are bound to serum albumin, and the lower the serum albumin, the higher is the percentage of unbound, biologically active steroid (Lewis *et al.*, 1971; Powell and Axelsen, 1972). In a survey of 240 medical in-patients receiving prednisone it was noted that when the serum albumin was less than 2·5 g/100 ml the frequency of prednisone adverse effects was doubled (Lewis *et al.*, 1971). Furthermore, there is also evidence that in liver disease the metabolism of prednisolone is impaired (Powell and Axelsen, 1972) resulting in prolonged high blood levels of the drug.

It had been assumed until recently that the conversion of prednisone to its active metabolite prednisolone was unimpaired in liver disease (Jenkins and Samson, 1967). However, Powell and Axelsen (1972) have recently shown that in acute liver disease conversion may be incomplete. It therefore seems logical to use prednisolone if reliable blood levels of active steroid are to be assured.

Azathioprine Metabolism

The failure of azathioprine to control the disease process is perhaps unexpected, but may be related to the role of the liver in the production of a metabolite with immunosuppressive properties. Azathioprine is converted to 6-mercaptopurine by sulphydryl compounds widely distributed in the body, and the major urinary metabolite is thiouric acid (Elion, 1972). Although more than 10 metabolites have been discovered, it is not certain which are responsible for the immunosuppressive activity of the drug (Bach and Dardenne, 1972) and this is usually monitored by the rosette inhibition test. Here the titre of immunosuppressive activity is taken as the highest dilution of serum that will inhibit the spontaneous formation of the rosettes that occur when sheep red blood cells are mixed with mouse spleen cells. Using this test, it has been clearly shown that little, if any, immunosuppressive activity is found in the blood following azathioprine in patients with hepatic parenchymal disease (Mitchell *et al.*, 1970; Bach and Dardenne, 1972; Whelan and Sherlock, 1972) and this may explain the poor results that we and others have experienced using it alone in the treatment of active chronic hepatitis.

Hepatotoxicity has been described with both 6-mercaptopurine (Mistilis

and Blackburn, 1967) and azathioprine (Starzl *et al.*, 1971) and in patients with active chronic hepatitis this may be impossible to distinguish from the deterioration in liver function that accompanies progression of the disease. There was no evidence of hepatotoxicity in our trial (azathioprine 75 mg/day) but it seems more likely to occur when larger doses are given.

Treatment Regime for Active Chronic Hepatitis

In the light of the results of the controlled trials and the new data on the metabolism of corticosteroids, it is clearly logical to use prednisolone in the smallest dose that will control the disease process. If adverse effects are troublesome it may be possible to reduce the dose of prednisolone further by adding a small dose of azathioprine (50 to 75 mg/day). Most patients will require life-long treatment, as it is the experience of most centres that even after two to three years' treatment the majority of patients will relapse sooner or later after treatment is withdrawn (Mackay, 1968). If, however, all biochemical and immunological parameters have returned to normal and the liver biopsy shows no evidence of continuing activity, it would be reasonable to try the effect of withdrawing treatment.

PRIMARY BILIARY CIRRHOSIS

Corticosteroids do not seem to improve the clinical picture, although no formal controlled trial has been conducted. Ross and Sherlock (1971) reported preliminary results of a controlled prospective trial comparing azathioprine (2 mg/kg) with placebo. There was slight temporary improvement in liver function tests in the azathioprine group. Azathioprine is also being compared with placebo in an international prospective trial that includes centres in Europe, Australia, and the USA. Whether or not this treatment improves life expectancy in primary biliary cirrhosis should be known shortly.

This article is based on a paper read at the Conference on Chronic Liver Disease held in the Royal College of Physicians in May 1973.

References

Bach, J.-F. and Dardenne, M. (1972) *Proceedings of the Royal Society of Medicine*, **65**, 260.
Brown, P. C., Glynn, L. E. and Holborow, E. J. (1967) *Immunology*, **13**, 307.
Cook, G. C., Mulligan, R. and Sherlock, S. (1971) *Quarterly Journal of Medicine*, N.S. **40**, 159.
Co-operating Clinics Committee of the American Rheumatism Association (1970) *New England Journal of Medicine*, **283**, 883.
Dameshek, W. and Schwartz, R. (1960) *Transactions of the Association of American Physicians*, **73**, 113.
Denman, E. H., Denman, A. M., Greenwood, D. M., Gall, D. and Heath, R. B. (1970) *Annals of Rheumatic Diseases*, **29**, 220.
Dodson, W. H., and Bennett, J. C. (1969) *Journal of Clinical Pharmacology*, **9**, 251.
Doniach, D., Roitt, I. M., Walker, J. G. and Sherlock, S. (1966) *Clinical and Experimental Immunology*, **1**, 237.

Eddleston, A. L. W. F., Smith, M. G. M., Mitchell, C. G. and Williams, R. (1971) *Transplantation*, **12**, 11.
Elion, G. B. (1972) *Proceedings of the Royal Society of Medicine*, **65**, 257.
Gross, H. and Zwirner, K. (1969) *Münchener medizinische Wochenschrift*, **111**, 2481.
Hopf, U., Meyer zum Buschenfelde, K. H. and Freudenberg, J. (1973) *Clinical and Experimental Immunology* (in press).
Jenkins, J. S. and Sampson, P. A. (1967) *British Medical Journal*, **1**, 205.
Lemmel, E., Hurd, E. R. and Ziff, M. (1971) *Clinical and Experimental Immunology*, **8**, 355.
Lewis, G. P., Jusko, W. J., Burke, C. W. and Graves, L. (1971) *Lancet*, **2**, 778.
Mackay, I. R. (1968) *Quarterly Journal of Medicine*, N.S. **37**, 379.
Mackay, I. R., Taft, L. I. and Cowling, D. C. (1956) *Lancet*, **2**, 1323.
MacLennan, I. C. M., Loewi, G. and Howard, A. (1969) *Immunology*, **17**, 897.
Makinodan, T., Santos, G. W. and Quinn, R. P. (1970) *Pharmacological Reviews*, **22**, 189.
Meyer zum Buschenfelde, K. H. and Kossling, F. K. (1971) In *Immunology of the Liver*, p. 169. (Ed. M. Smith and R. Williams). London: Heinemann.
Miller, J., Smith, M. G. M., Reed, W. D., Mitchell, C. G., Eddleston, A. L. W. F. and Williams R., (1972) *Lancet*, **2**, 296.
Mistilis, S. P. and Blackburn, C. R. B. (1967) *Australasian Annals of Medicine*, **16**, 305.
Mitchell, C. G., Eddleston, A. L. W. F., Smith, M. G. M. and Williams, R. (1970) *Lancet*, **1**, 1196.
Murray-Lyon, I. M., Stern, R. B. and Williams, R. (1973) *Lancet*, **1**, 735.
Novack, S. N. and Pearson, C. M. (1971) *New England Journal of Medicine*, **284**, 938.
Paterson, P. Y. and Drobish, F. G. (1969) *Science*, **165**, 191.
Phlippen, R., Schumacher, K., Gross, R. and Eder, M. (1969) *Klinische Wochenschrift*, **47**, 524.
Powell, L. W. and Axelsen, E. (1972) *Gut*, **13**, 690.
Ross, A. and Sherlock, S. (1971) In *Immunology of the Liver*, p. 258. (Ed. M. Smith and R. Williams). London: Heinemann.
Smith, M. G. M., Golding, P. L., Eddleston, A. L. W. F., Mitchell, C. G., Kemp, A. and Williams, R. (1972) *British Medical Journal*, **1**, 527.
Soloway, R. D., Summerskill, W. H. J., Baggenstoss, A. H., Geall, M. G., Gitnick, G. L., Elveback, L. R. and Schoenfield, L. J. (1972) *Gastroenterology*, **63**, 820.
Starzl, T. E., Halgrimson, C. G., Penn, I., Martineau, G., Schroter, G., Amemiya, H., Putnam, C. W. and Groth, C. G. (1971) *Lancet*, **2**, 70.
Steinberg, A. D., Plotz, P. H., Wolff, S. M., Wong, V. G., Agus, S. G. and Decker, J. L. (1972) *Annals of Internal Medicine*, **76**, 619.
Stevens, J. E. and Willoughby, D. A. (1969) *Journal of Pathology*, **97**, 367.
Whelan, G. and Sherlock, S. (1972) *Gut*, **13**, 907.

Hail to the Chief

About the time that Hippocrates was formulating his oath, a Sanskrit medical text, the Charaka Samhita, was well read in India. It contained several rules of conduct and linked religion with the precise practice of medicine. It contained such worthy injunctions to the doctor as 'You shall not desert or injure your patient for the sake of your life or your living'. It also outlined an oath to be taken by medical students when they attached themselves to a teacher. The modern student might be encouraged by swearing that he would grow his hair and beard but this was followed by the command to live his life as a celibate. The sticking point was obviously in the commands of the teacher to the student. 'You shall dedicate yourself to me and regard me as your chief. You shall be subject to me and conduct yourself for ever for my welfare and pleasure. You shall behave and act without arrogance, with care and attention and with undistracted mind, humility, constant reflection and ungrudging obedience. . . . You shall conduct yourself for the achievement of your teacher's purposes alone, to the best of your abilities.' Well said, that man on the right.