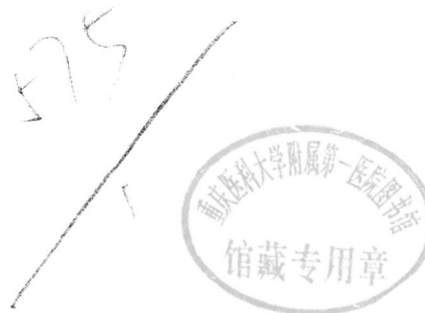
# Immune Reactions in Liver Disease

Edited by
A L W F Eddleston
J C P Weber
Roger Williams

# IMMUNE REACTIONS IN LIVER DISEASE

*Edited by*

A L W F Eddleston
J C P Weber
Roger Williams




PITMAN MEDICAL

*First published* 1979

*Reprinted* 1980

Catalogue Number 21-0798-81

*Pitman Medical Publishing Co Ltd*
P O Box 7, Tunbridge Wells,
Kent, TN1 1XH, England

*Associated Companies*

UNITED KINGDOM
Pitman Publishing Ltd, London
Focal Press Ltd, London

CANADA
Copp Clark Ltd, Toronto

USA
Fearon Pitman Publishers Inc, California
Focal Press Inc, New York

AUSTRALIA
Pitman Publishing Pty Ltd, Carlton

NEW ZEALAND
Pitman Publishing NZ Ltd, Wellington



British Library Cataloguing in Publication Data

Immune reactions in liver disease.
1. Liver—Diseases—Immunological aspects—Congresses 2. Immune response —Congresses
I. Eddleston, A L W F II. Weber, J C P
III. Williams, Roger, b. 1931
616.3'62'0795 RC846

ISBN 0-272-79509-7

Printed and bound in Great Britain
at The Pitman Press, Bath

## PARTICIPANTS

Alberti, A, University of Padova, Italy
Allison, AC, Northwick Park Hospital, Harrow, UK
Batchelor, JR, Blond Laboratories, Queen Victoria Hospital, East Grinstead, UK
Berg, P, Medizinische Universitätsklinik, Tübingen, Germany
Bernardi, M, University of Bologna, Italy
Berthowe, F, Hopital Herriot, Lyon, France
Bianchi, FB, University of Bologna, Italy
Calne, RY, University of Cambridge Clinical School, Cambridge, UK
Cannady, WG, Harvard Medical School, Boston, USA
Cantell, K, Central Public Health Laboratory, Helsinki, Finland
Chen, T, College of Medicine and Dentistry of New Jersey, Newark, USA
Chiarmonte, M, University of Padova, Padova, Italy
Chisari, FV, Scripps Clinic, La Jolla, USA
Cochrane, AMG, King's College Hospital, London, UK
Colombo, M, Mount Sinai School of Medicine, New York, USA
Davis, M, King's College Hospital, London, UK
de Groote, J, University of Leuven, Belgium
Desmyter, J, Rega Institute for Medical Research, Leuven, Belgium
Doniach, D, Middlesex Hospital, London, UK
Dubey, DP, Harvard Medical School, Boston, USA
Eddleston, ALWF, King's College Hospital, London, UK
Edginton, TS, Scripps Clinic, La Jolla, USA
El Sheikh, N, King's College Hospital, London, UK
Falchuk, KL, Harvard Medical School, Boston, USA
Fernandez, L, King's College Hospital, London, UK
Fitzpatrick, D, Harvard Medical School, Boston, USA
Galambos, JT, Emory University School of Medicine, Atlanta, USA
Galbraith, RM, King's College Hospital, London, UK
Gillette, JR, National Institutes of Health, Bethesda, USA
Good, RA, Memorial Sloan-Kettering Cancer Center, New York, USA
Guarriero-Bobylera, V, University of Bologna, Italy
Henning, H, University of Tubingen, Tubingen, Germany
Hodgson, HJF, Royal Postgraduate Medical School, London, UK
Holborow, EJ, The London Hospital Medical College, London, UK
Hütteroth, TH, Freie Universität, Berlin, Germany
Jain, S, Royal Free Hospital, London, UK
Jewell, D, Royal Free Hospital, London, UK
Kanagasundaram, N, College of Medicine and Dentistry of New Jersey, Newark, USA
Leevy, CM, College of Medicine and Dentistry of New Jersey, Newark, USA
Liebe CS, Veterans Administration Hospital, New York, USA
McFarlane, IG, King's College Hospital, London, UK
Mackay, IR, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
MacSween, S, Harvard Medical School, Boston, USA
Matsumoto, K, College of Medicine and Dentistry of New Jersey, Newark, USA
Meyer zum Büschenfelde, KH, Freie Universität, Berlin, Germany
Miller, JO, Yale University School of Medicine, New Haven, USA
Moussouros, A, King's College Hospital, London, UK
Nielsen, JO, Copenhagen Kommunes Hvidovre Hospital, Denmark
Noreen, H, Harvard Medical School, Boston, USA
Paronetto, F, Mount Sinai School of Medicine, New York, USA

Pattison, J, King's College Hospital, London, UK
Perlmann, P, Wenner-Gren Institute, Stockholm, Sweden
Popper, H, Mount Sinai School of Medicine, New York, USA
Preisig, R, University of Berne, Berne, Switzerland
Read, A, University of Bristol, Bristol, UK
Realdi, G, University of Padova, Italy
Redeker, AG, University of Southern California, Los Angeles, USA
Revillard, JP, Hôpital Herriot, Lyon, France
Roitt, IM, The Middlesex Hospital Medical School, London, UK
Scheuer, P, Royal Free Hospital, London, UK
Schuff-Werner, P, University of Tubingen, Tubingen, Germany
Scullard, G, King's College Hospital, London, UK
Sherlock, S, Royal Free Hospital, London, UK
Sullivan, S, King's College Hospital, London, UK
Talal, N, University of California School of Medicine, San Francisco, USA
Thomas, H, Royal Free Hospital, London, UK
Thomas London, W, The Institute for Cancer Research, Philadelphia, USA
Trepo, C, Hôpital Herriot, Lyon, France
Trey, C, Harvard Medical School, Boston, USA
Tsantoulas, DC, King's College Hospital, London, UK
Vergani, D, King's College Hospital, London, UK
Vernace, S, Mount Sinai School of Medicine, New York, USA
Vogten, AJM, Academisch Ziekenhuis Utrecht, Utrecht, The Netherlands
Walton, B, The London Hospital, London, UK
Wands, JR, The Massachusetts General Hospital, Boston, USA
Wansborough-Jones, M, King's College Hospital, London, UK
Wilkinson, S, King's College Hospital, London, UK
Williams, RM, Harvard Medical School, Boston, USA
Williams, Roger, King's College Hospital, London, UK
Wojcicka, B, King's College Hospital, London, UK
Wright, R, University of Southampton, Southampton, UK
Yunis, EJ, Harvard Medical School, Boston, USA
Zauli, D, University of Bologna, Italy
Zuckerman, AJ, London School of Hygiene and Tropical Medicine, London, UK

## CONTENTS

*Part VII – IMMUNOTHERAPY*

# INTRODUCTION

*Roger Williams*

It is about eight years since our last meeting on immune reactions in liver disease. Some of you who were at that meeting will know only too well what an enormous amount has been added to our knowledge since then. We would not be able to cover it all in this book, and we have therefore tried to identify certain main areas of importance. Active chronic hepatitis is at the centre of the stage, but in alcoholic liver disease and hepatic drug reactions there is undoubtedly, at least in some patients, an autoimmune component. The scope too has been widened by the finding of a relationship to certain histocompatibility antigens and familial influences are also more clearly apparent. We hope that our discussions here will give some perspective to these new areas of knowledge.

There are three questions too, to which I hope we will address ourselves in this book, even if we may not be able to answer them exactly as yet.

1. Can we ever prove that autoimmunity is damaging to the liver in man?
2. Can we relate the defects in immunoregulation to a genetic background?
3. Can specific immunotherapy for the abnormalities found be developed?

# IMMUNE RESPONSES TO THE LIVER-SPECIFIC MEMBRANE LIPOPROTEIN

*A L W F Eddleston*

In this presentation, I shall review some of the evidence that our group and others have obtained relating to the role of immunity to a liver-specific membrane lipoprotein in the pathogenesis of chronic active hepatitis. Our interest in this unusual antigen was entirely due to Professor Deborah Doniach who pointed out the importance of the work of Professor Meyer zum Buschenfelde and his colleagues at the end of the 1960s. He had discovered two liver-specific antigens in the supernatant of a human liver homogenate and had partially separated them by column chromotography [1]. Immunofluorescent studies indicated that one was found in the cytoplasm of hepatocytes while the other was an unstable high molecular weight lipoprotein derived from the hepatocyte surface membrane [2]. Antisera to these antigens were prepared in rabbits, and of considerable importance was the finding that these immunised animals developed inflammatory lesions in the liver [3], akin to those of chronic aggressive hepatitis in man (Figure 1). After prolonged immunisation, some of the animals progressed to cirrhosis [4], another characteristic of the human disease. At this time the membrane lipoprotein could not be prepared in a stable purified form but Meyer zum Buschenfelde and his colleagues were able to show that immunisation with the cytoplasmic antigen alone was relatively ineffective in inducing the liver damage [3], thus implicating the membrane lipoprotein in the pathogenesis of the experimental lesion.

We began to look for immune responses to the liver membrane lipoprotein (LSP) in patients with chronic active hepatitis in 1970 after first purifying the relevant antigen. This difficult task was undertaken first by Dr Joanna Miller [5] and later by Dr Ian McFarlane who has since gone on to explore the structure and properties of LSP [6].

The method of purification consists essentially of a series of gel chromogography columns through which the supernatant of a human liver homogenate is passed [6]. The membrane lipoprotein appears in the void volume on chromoto-

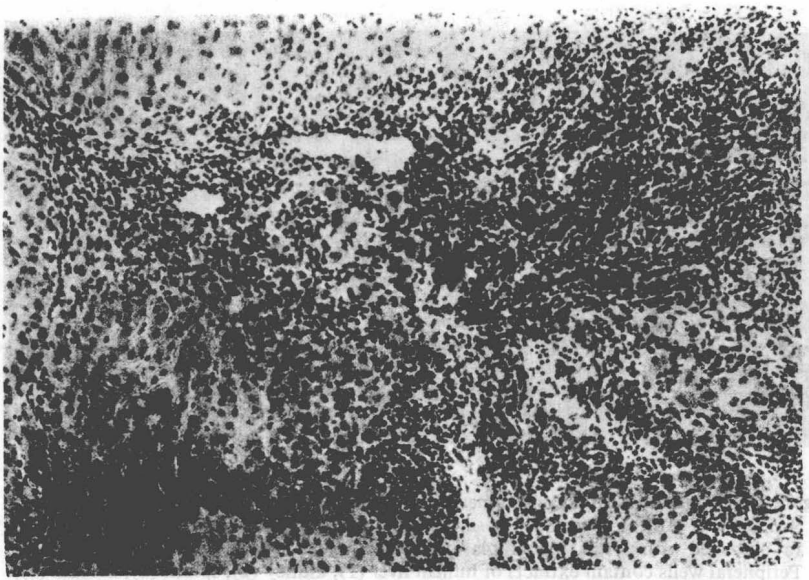


Figure 1 The histological features of chronic aggressive hepatitis induced in the liver of a rabbit by multiple injections of human liver-specific antigens.
(By courtesy of Professor Meyer zum Büschenfelde)

graphy over Sepharose 4B indicating an apparent molecular weight of greater than 20 million but, since hydrophobic molecules behave abnormally in gel chromotography, this estimate may not be accurate. Dr McFarlane has prepared the apoprotein by treatment with sodium deoxycholate and this is included in Sepharose 4B, producing a single peak [6]. Unfortunately the immunological reactivity is lost after this treatment [6].

The organ specificity of LSP has been confirmed by immunodiffusion (Figure 2). Polyvalent antiserum raised in guinea pigs against a crude preparation of LSP (the first peak from Sephadex G–100) and absorbed with normal human plasma gave two lines on immunodiffusion against liver homogenate (Figure 2a). One of these appeared to be liver-specific, the other showed complete identity with a line obtained against kidney, spleen, adrenal, thyroid and submaxillary gland homogenates. This second line was distinct from another line obtained against an adrenal homogenate. After further absorption of the polyvalent antiserum with purified freeze dried LSP the liver-specific line disappeared while the other reaction with the liver homogenate and those with all the other organ extracts were not affected (Figure 2b). The same antiserum showed surface immunofluorescence when incubated with isolated rabbit hepatocytes, a phenomenon which was not observed when the antiserum was first absorbed with LSP [6].

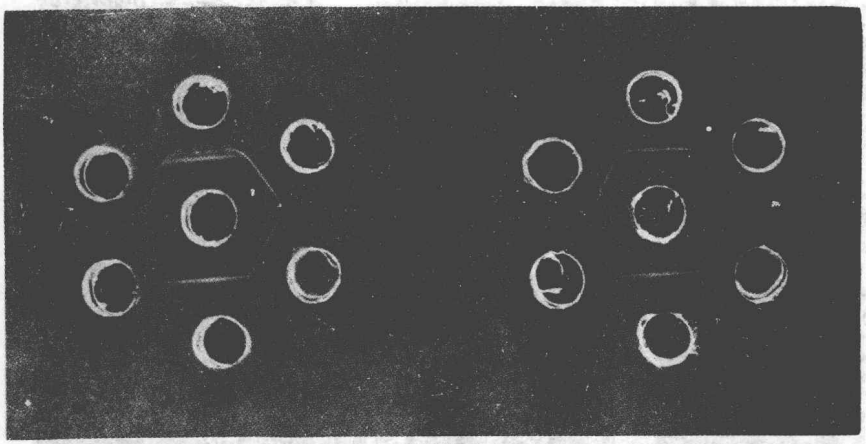


Figure 2 Immunodiffusion using guinea pig antiserum in centre wells raised against the Sephadex G–100 first peak of the supernatant of a human liver homogenate: (a) absorbed with normal human plasma and (b) further absorbed with purified LSP. Peripheral wells contain extracts of human liver (1), kidney (2), spleen (3), adrenal (4), thyroid (5), and submaxillary gland (6). (From McFarlane 1977 [6])

## Cellular Immunity to LSP

We first looked at cellular immune reactions to this antigen using the leucocyte migration test, and found that 11 of 16 patients with chronic active hepatitis showed evidence of sensitisation to LSP [5]. The number of patients who could be studied was limited at this stage because the lipoprotein antigen was only stable in solution for two or three days. Thus the discovery by Dr McFarlane that stability could be extended for up to two years by the addition of ImM EDTA to the Tris HCℓ buffer system [6] represented a major practical advance. Further studies using the leucocyte migration test showed that sensitisation to LSP was an almost universal finding in cases of untreated chronic active hepatitis while in those cases treated with prednisone with or without azathioprine the incidence of migration inhibition was lower, particularly in those with satisfactory biochemical control of disease activity (Table I).

TABLE I Cellular Immune Response to LSP in Chronic Active Hepatitis and the Effect of Immunosuppressive Therapy

| | Number tested | Inhibition of leucocyte migration with LSP as antigen |
|---|---|---|
| Untreated | 14 | 13 (93%) |
| Treated: with high serum bilirubin | 23 | 16 (70%) |
| Treated: with normal serum bilirubin | 21 | 9 (43%). |

An important further step in the investigation of the possible pathogenic role of immune responses to LSP was to demonstrate that lymphocytes from patients with chronic active hepatitis were capable of killing isolated liver cells in tissue culture. I went to Bob Good's laboratory in Minneapolis for 15 months and returned, as a determined and enthusiastic immunologist, to set up such an assay, but failed – not, I hasten to add, through any defect in an excellent training programme. Dr Malcolm Cochrane then joined our Unit and, with no previous immunological training, quickly succeeded in developing a system which has proved invaluable in further analysing lymphocyte/hepatocyte interactions in vitro. We chose rabbit hepatocytes as the target cells as these were readily available, seemed easier to maintain in short term tissue culture and, most importantly, have a surface antigen which cross-reacts with human LSP [2]. The hepatocytes were isolated by enzyme digestion and after short term culture plated into

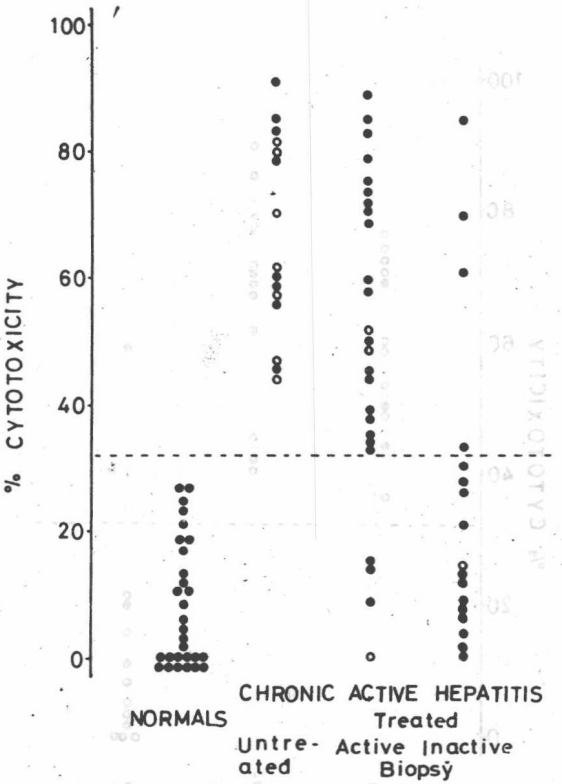



Figure 3 Percentages of hepatocytes killed after incubation for 48 hours with peripheral blood lymphocytes from normal subjects and from patients with chronic active hepatitis. The patients have been divided into three groups – those untreated at the time of the assay, those treated with prednisone with or without azathioprine whose liver biopsy still showed evidence of disease activity and those well-controlled by histological criteria. The dotted line shows the upper limit of the normal range. ○ HBsAg positive, ● HBsAg negative

microwells [7]. Peripheral blood lymphocytes were then added in the ratio of 400 to one and after 48 hours incubation at 37°C the remaining adherent hepatocytes were counted. Lymphocytes from all of 15 patients with untreated chronic active hepatitis have shown significantly increased cytotoxicity as have those from 21 of 25 treated cases whose liver biopsy still showed piecemeal necrosis of periportal hepatocytes (Figure 3). Addition of 0.5 μg of purified LSP to the incubation wells specifically inhibited the cytotoxic reaction indicating that LSP was the principal target antigen on the liver cell membrane [7].

Cytotoxic lymphocytes have been detected less frequently in treated patients whose liver biopsy no longer showed evidence of disease activity, only four of 17 showing significant lymphocyte cytotoxicity for the isolated hepatocytes (Figure 3). In fact, this assay has proved to be superior to other immunological and biochemical tests in predicting the disease activity as assessed histologically [8].

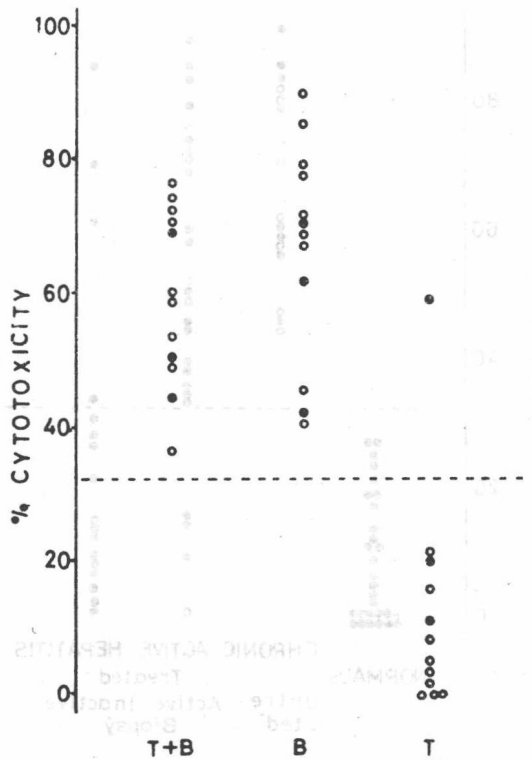



**Figure 4** Effect of removal of T cells (B cell enriched fraction) or of B and K cells (T cell enriched fraction) on lymphocyte cytotoxicity to isolated hepatocytes. The lymphocytes were from the peripheral blood of patients with uncontrolled or untreated chronic active hepatitis. (From Cochrane et al 1976 [9]).