antibodies against *H pylori* are indirect methods for detection of the micro-organism. Seropositivity, therefore, can reflect active infection as well as (especially if the antibody titres are low) previous infection (a so-called serological scar). Although this phenomenon can interfere with the diagnosis of acute *Helicobacter* infection, it is of no relevance in our study. Seropositivity thus indicates present or previous infection with *H pylori*. Because blood group is genetically determined, this does not interfere with the aim of the study, nor influence the final results.

We confirm the data from the previous biopsy study. Our data also agree with those of a smaller serological study published recently.⁷

It is concluded that although blood group O is a risk factor for duodenal ulcers, it is not a risk factor for acquiring *H pylori* infection.

Blood group O and *H pylori* are independent risk factors for duodenal ulcer disease.

- 1 Tytgat GNJ, Axon ATR, Dixon MF, Graham DY, Lee A, Marshall BJ. *Helicobacter pylori*, causal agent in peptic ulcer disease. *Working party reports 1990*. London: Blackwell Scientific Publications, 1990:36-45.
- 2 Wyatt JT. *Campylobacter pylori*, duodenitis and duodenal ulceration. In: Rathbone BJ, Heatley RV, eds. *Campylobacter pylori and gastroduodenal disease*. London: Blackwell Scientific Publications, 1989:117-24.
- 3 Marshall BJ, Goodwin CS, Warren JR, et al. Prospective double blind trial of duodenal ulcer relapse after eradication of *Campylobacter pylori*. *Lancet* 1988;ii:1467-9.
- 4 Clarke CA, Evans DAP, McConnel RB, Sheppard PM. Secretion of blood group antigen and peptic ulcer. *Br Med J* 1959;i:603-7.
- 5 Loffeld RJLF, Stobberingh E, Flendrig JA, van Spreeuwel JP, Arends JW. Diagnostic value of an immunoassay to detect anti-*Campylobacter pylori* antibodies in non-ulcer dyspepsia. *Lancet* 1989;i:1182-5.
- 6 Levi S, Davies KAA, Playford RA, et al. Antral *Campylobacter* colonization, ABO blood group, and secretor status. *Gastroenterol Clin Biol* 1989;13:1095.
- 7 Hook-Nikanne J, Sistonen P, Kosunen TU. Effect of ABO blood group and secretor status on the frequency of *Helicobacter pylori* antibodies. *Scand J Gastroenterol* 1990;25:815-18.

Anticardiolipin antibodies in leptospirosis

F P Rugman, G Pinn, M F Palmer, M Waite, C R M Hay

Abstract

The clinical course and serology of 16 cases of leptospirosis in an area with an unusually high endemic infection rate were studied to gain further insight into the pathology of the secondary immune phase that is typical of the disease. IgG anticardiolipin antibody concentrations were measured by immunoassay and found to be increased in eight serologically confirmed cases with severe complicated disease, compared with eight patients with relatively uncomplicated leptospirosis who had IgG anticardiolipin concentrations within the control reference range.

This previously unreported association suggests that leptospira may induce vascular endothelial injury in severe cases and expose crypt antigens or induce conformational change of cell surface phospholipids. Leptospirosis may provide a model for an infective origin of some cases of the antiphospholipid syndrome.

Syphilis can induce the cross-reaction of

antibodies with synthetic cardiolipin. More recently, IgG and IgM anticardiolipin antibodies have been reported in patients with Lyme disease, another spirochaetal disease.¹ The antigenic stimulus responsible for the induction of these antibodies in spirochaetal infection, and their relation with the complications of the disease is poorly understood.

Leptospirosis is a spirochaetal infection with important animal reservoirs in rodents, dogs, cattle and pigs. The initial septicaemic phase of the infection is characterised by fever and malaise. Although severe cases may progress to hepato-renal disease (Weil's syndrome), most are anicteric, and without confirmatory serology, may remain undiagnosed.² The second or "immune phase" occurs about two weeks later and is associated with a rise in circulating IgM leptospira antibodies and recurrence of fever. Encephalitis, neuritis, thrombocytopenia, heart-block and cardiac failure may manifest at this time, suggesting that an immunological mechanism rather than a direct cytopathic effect may be responsible for these complications.^{3,4}

University
Department of
Haematology, Royal
Liverpool Hospital,
Prescot St, Liverpool
L69 3BX
F P Rugman
M Waite
C R M Hay

Bundaberg Base
Hospital, Bundaberg,
Queensland, Australia
G Pinn

PHLS Leptospira
Reference Laboratory,
County Hospital,
Hereford
M F Palmer

Correspondence to:
Dr F P Rugman

Accepted for publication
6 December 1990

Methods

The clinical course and serology of 16 cases of severe leptospirosis requiring hospital admission were studied. All patients were from the Seychelles, an area with an unusually high endemic rate of infection.

The serological diagnosis was confirmed by the Public Health Laboratory Service Leptospira Reference Laboratory, Hereford, England, using an enzyme linked immunosorbent assay (ELISA) technique (PHLS Leptospira Reference Laboratory urease ELISA) to measure specific leptospira IgM antibody. The specific microscopic agglutination technique (MAT) was used to confirm positive results.

IgG and IgM anticardiolipin antibodies were determined in the sera of 16 patients in the immune phase of the disease using a commercial ELISA kit (MELISA anticardiolipin IgG and IgM, Walker Diagnostics, Cambridge). A reference range for anticardiolipin antibody, established for our own laboratory using plasma from 23 healthy blood donors, was taken as two standard deviations from the mean (IgG 0–9 GPL units/ml, mean 4.2 (4.4) GPL units/ml; IgM 0.62–3.46 MPL units/ml, mean 2.04 (1.42) MPL units/ml). One GPL unit is defined as the cardiolipin binding antibody activity of 1 µg/ml of an affinity purified IgG ACA from a standard serum.

Results

The patients presented to hospital after a mean period of 10 days of illness. The commonest symptoms were fever (15/16) and myalgia (14/16), and the commonest signs were jaundice (10/16) (mean maximum bilirubin concentration 232 (SD 156) µmol/l) and conjunctival suffusion (7/16). Nine patients had renal impairment (mean maximum creatinine concentration 388 µmol/l).

Eight out of 16 patients had raised titres of IgG anticardiolipin antibody (mean 26.6 (16) GPL units/ml; range 10–57 GPL units/ml). All patients with raised titres of anticardiolipin antibody had severe complications of leptospirosis. By contrast, the course of the disease was uncomplicated in patients with IgG anticardiolipin antibodies within the control range (figure) (mean 6.6 (1.1) GPL units/ml; range 5–9 GPL units/ml). The eight patients with raised titres of anticardiolipin antibodies had a variety of complications including conjunctival suffusion (6/8), heart-block (2/8), congestive cardiac failure (1/8), convulsions (2/8), and severe myositis (2/8) (creatinine phosphokinase activity >1500 U/ml). One patient died suddenly from advanced hepato-renal failure four days after admission.

IgM anticardiolipin antibody titres were generally not raised (mean (SD) 4.8 (4.1); range 2–18 MPL units/ml). There was no correlation between either the anticardiolipin IgG titre and the leptospira MAT, or between the IgM anticardiolipin antibody titre and the titre of the specific leptospira IgM antibody.

IgG anticardiolipin antibody titres in complicated and uncomplicated leptospirosis.

Discussion

Anticardiolipin antibodies have not previously been reported in association with leptospirosis. As anticardiolipin antibodies also occur in association with other spirochaetal infections, Lyme disease, (*Borrelia burgdorferi*),¹ and syphilis (*Treponema pallidum*),^{5,6} they may be a feature common to many spirochaetal illnesses.

The characteristic pathological features of leptospirosis, vascular injury, haemorrhage, and oedema are all thought to result from cytotoxic and immunological mechanisms.² Similarly, the reported 40% incidence of thrombocytopenia in the absence of disseminated intravascular coagulation suggests immune peripheral consumption. Our results also suggest that these anticardiolipin antibodies cross-react with vascular endothelial cells and other cell membranes with direct pathological consequences resulting in or contributing to the complications in these patients. This is in keeping with recent observation that antibodies to anionic phospholipids frequently cross-react with endothelial cells and lipids in vitro.⁴

It is not known how anticardiolipin antibodies arise in leptospirosis. Vascular injury induced by leptospira may result in the development of such antibodies either by exposing cryptic membrane antigens or by inducing membrane remodelling with hexagonal-2 conformational change of cell-surface phospholipids. Hexagonal phospholipids are antigenic,⁷ giving rise to anticardiolipin antibodies when administered to mice, and similar mechanisms may operate in man.

Thus spirochaetal infections have the potential to induce directly or indirectly anticardiolipin antibodies. This may provide a model for an infective origin for some cases of the primary antiphospholipid syndrome.⁸ These antibodies may be transient, but if they do persist it will be important to establish whether their presence is of long term clinical importance.

We are grateful for the assistance of Dr T J Coleman, Hereford, PHLS.

- 1 Mackworth-Young CG, Harris EN, Steere AC. Anticardiolipin antibodies in Lyme disease. *Arthritis Rheum* 1988;31:1052.
- 2 Sitprija V. Leptospirosis. *Med Int* 1988;55:2291-3.
- 3 Davenport A, Rugman FP. Is thrombocytopaenia in patients with leptospirosis immunologically mediated? *J Clin Pathol* 1989;42:439-40.
- 4 Hasselaar P, Derksen RHW, Blokzijl I. Crossreactivity of antibodies directed against cardiolipin, DNA, endothelial cells and blood platelets. *Thromb Haemostas* 1990;63:169-73.
- 5 Matsuura E, Igarashi Y, Fujimoto M. Anticardiolipin cofactor and differential diagnosis of autoimmune disease. *Lancet* 1990;ii:177-8.
- 6 Koike T, Sueishi M, Funaki H, Tomioka H, Yoshida S. Antiphospholipid antibodies and false positive serological tests for syphilis in patients with systemic lupus erythematosus. *Clin Exp Immunol* 1984;56:193-9.
- 7 Rauch J, Tannenbaum M, Janoff AS. Distinguishing plasma lupus anticoagulants from anti-factor antibodies using hexagonal (II) phase phospholipids. *Thromb Haemostas* 1989;62:892-6.
- 8 Mackworth-Young CG, Loizou S, Walport MJ. Primary antiphospholipid syndrome: features of patients with raised anticardiolipin antibodies and no other disorder. *Ann Rheum Dis* 1989;48:362-7.

Cachexia and tumour necrosis factor- α in cytomegalovirus infection

H Tilg, W Vogel, M Herold, W E Aulitzky, C Huber

Abstract

Although cachexia is a common feature of cytomegalovirus infection, little is known about its cause. To explore any contributory role that tumour necrosis factor- α (TNF) might have the serum concentrations of TNF in eight patients who developed CMV disease after liver transplantation were investigated. All patients exhibited pronounced and long lasting increases in TNF serum concentrations. Increased endogenous TNF concentrations were associated with weight loss and anorexia. In contrast, liver transplant recipients without CMV disease showed no weight loss.

Cachexia has long been recognised as a prominent feature of patients with CMV infection. The cause of the profound weight loss and anorexia in these patients remains unclear. Tumour necrosis factor- α (TNF) is a macrophage-derived cytokine implicated in host defense against tumour cell growth and infection.¹ Experimental and clinical studies have suggested a role for it in inflammation, cachexia, and tissue injury.^{2,3} Recently, we have shown that enhanced endogenous production of TNF, interferon- γ , β -2-microglobulin and neopterin is a regular feature of inflammatory complications after liver transplantation.⁴ The aim of this study was to investigate TNF serum concentrations in patients with CMV infection after liver transplantation and to assess their potential role in the pathogenesis of cachexia associated with CMV infection.

Methods

Eighteen liver transplant recipients were studied. These transplantations had been performed in patients with chronic liver disease

(chronic active cirrhosis in 10, primary biliary cirrhosis in five, hepatocellular carcinoma in three). Patients received prophylactic immunosuppressive treatment consisting of methylprednisone, azathioprine, and cyclosporine A. Acute graft rejections were treated with gram bolus doses of methylprednisone.

During the early postoperative period, 10 episodes of reversible rejection were observed. Eight patients developed CMV infection. Ten patients showed no complications in the later postoperative course. These patients were used as a control group.

CMV disease was defined on the basis of serological demonstration of a CMV specific antibody response combined with clinical symptoms. Symptoms suggestive of CMV disease were cytopenia, interstitial pneumonia, liver dysfunction and otherwise unexplained fever. To corroborate the diagnosis throat swabs, urinary samples, and blood buffy coats were used for CMV cultures. Day 0 of CMV disease was defined as the first day with positive virus culture in conjunction with clinical symptoms.

Serum samples were obtained twice weekly and stored at -20°C . Serum concentrations of TNF were assayed with a commercially

Mean (SEM) TNF serum concentrations and weight loss in eight patients with CMV disease

Day*	TNF (pg/ml)	Body weight (kg)
-10	15.1 (3.3)	63.4 (4.9)
-5	27.6 (9.4)	61.9 (5.3)
0	42.3 (7.5)	60.5 (5.5)
5	48.9 (8.9)	59.5 (5.7)
10	42.3 (6.3)	60.6 (6.9)
15	67.0 (13.2)	57.9 (5.6)
20	41.7 (17.1)	56.0 (5.7)
30	52.7 (11.5)	52.3 (6.9)

*Day 0 defined as first day with positive virus culture in conjunction with clinical symptoms.

Divisions of Clinical Immunobiology and Gastroenterology, Department of Internal Medicine, Innsbruck University Hospital, 6020 Innsbruck, Austria
H Tilg
W Vogel
M Herold

Department of Haematology, Johannes Gutenberg University, Mainz, Germany
W E Aulitzky
C Huber

Correspondence to:
Dr Tilg

Accepted for publication
6 December 1990