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Serological cross-reaction between *Legionella pneumophila* and campylobacter in the indirect fluorescent antibody test

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SUMMARY

Sera from 50 patients with culture-proven campylobacter gastroenteritis were examined for the presence of antibodies to *Legionella pneumophila*. Ten patients (20%) had a positive titre ($\geqslant 16$) as measured by indirect immunofluorescence. Antibodies were detected in only 1 of 36 acute sera but in 10 of 14 (71%) sera obtained more than 10 days after the onset of symptoms. All positive sera contained specific IgM antibodies but specific IgG or IgA could not be detected in any sample. No legionella antibodies could be detected in sera from 42 similar patients with salmonella gastroenteritis. These results were shown to be due to serological cross-reaction between *L. pneumophila* and campylobacter.

INTRODUCTION

Following the description of the Indirect Fluorescent Antibody Test (IFAT) [1] many cases of *Legionella pneumophila* infection have been diagnosed by serology. When heat-killed antigens are used, false-positive titres can occur in a variety of infections including psittacosis and bacteroides septicaemia [2, 3]. However the problems of cross-reactivity have generally not been encountered with the use of a formalinized yolk-sac antigen of *L. pneumophila* serogroup 1 in the IFAT [4]. Since 1978 only one case has been reported, as far as we are aware, in which a diagnostic rise in antibody to legionella was shown to be caused by a cross-reaction with another organism [5].

This study was initiated after the discovery of high antibody titres to legionella in a patient who presented with campylobacter gastroenteritis. We report on the results of 50 patients with culture-proven campylobacter infection who were tested for the presence of legionella antibodies.

PATIENTS AND METHODS

Patients

Sera were obtained from 50 patients hospitalized with gastroenteritis, whose stool cultures yielded campylobacter. Where possible convalescent samples were obtained. These ranged from 11 to 61 days after the onset of illness which was defined as the start of gastrointestinal symptoms. In total 55 sera were available

from 50 patients for analysis although an exact date of onset was not known for 5 patients.

Forty-one acute and 6 convalescent sera from 42 similar patients hospitalized with salmonella gastroenteritis acted as a control.

Indirect Fluorescent Antibody Test (IFAT)

The standard IFAT was performed as previously described using formalinised yolk-sac antigen of *L. pneumophila* serogroup 1 [6] (Division of Microbiological Reagents, CPHL, Colindale).

Class specific antibody titres were determined by using IgM-, IgG- or IgA-specific FITC-conjugates (goat antihuman, Sigma) in the second incubation instead of the antihuman whole globulin FITC-conjugate (MFO1, Wellcome). The serum incubation time was extended from 30 to 90 min for IgM and IgA tests to allow for maximum binding.

Absorption with killed campylobacter

Campylobacter isolates were available from two of the patients who subsequently developed legionella antibodies.

These were grown on nine 5% horse blood agar plates (Advanced Protein Products Ltd) for 3 days at 42 °C in an atmosphere of reduced oxygen. The bacterial growth from all plates was harvested and dense suspensions of each isolate prepared in phosphate-buffered saline (PBS) pH 7·2.

The organisms were washed once and then killed by exposing to 0·08% sodium azide or 1% formalin in PBS pH 7·2 for 4 h at room temperature. The killed organisms were then washed a further three times in PBS. A 3 ml aliquot of each suspension was centrifuged at 1200 ***g*** for 10 min. The supernatants were discarded and each pellet re-suspended in 200 μl of a 1:8 dilution of the corresponding patients serum in PBS. These mixtures were then shaken for 18 h at room temperature and finally centrifuged; 200 μl of absorbed and unabsorbed (1:8 dilution) serum were tested in parallel by IFAT.

To determine whether absorption with campylobacter could remove genuine legionella antibody a convalescent serum from a patient with culture-proven *L. pneumophila* serogroup 1 pneumonia was processed similarly at the same time.

RESULTS

Table 1 shows the legionella antibody titres in the 50 patients with campylobacter infection. Forty patients had no detectable antibody by IFAT but 10 patients (20%) had a titre of 16 or above. Six of these (12%) had titres above 32 and 3 (6%) had titres greater then 256.

There was no distinguishable difference in the quality or pattern of bacterial fluorescence in the IFAT when these sera were compared with control sera from a genuine case of legionnaires' disease.

Figure 1 shows the results of 50 sera from 45 patients for whom the date of onset of gastroenteritis was accurately known. Only one (2·8%) of the 36 acute specimens (days 0–10) was positive at a titre of 16. In contrast 10 of 14 (71%) convalescent samples (days 11–61) representing 8 of 11 patients were positive with titres of up to 2048.

Table 1. *Legionella antibody titres in 50 patients with campylobacter gastroenteritis*

Indirect fluorescent antibody	Number of patients
< 16	40
16 – 32	4
64 – 256	3
512 – 2048	3

* Maximum titre if more than one serum per patient.

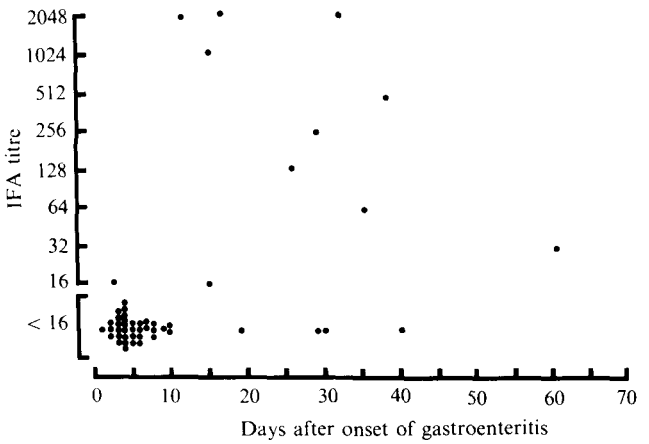

Fig. 1. Presence of antibodies to *Legionella pneumophila* detected by IFAT in 36 acute and 14 convalescent sera from 45 patients with campylobacter gastroenteritis.

Table 2. *Absorption of cross-reacting antibody by killed campylobacter*

Campylobacter killed in	Legionella IFA titre		
	Patient 1	Patient 2	Patient 3
0·08% sodium azide	< 16	64	512
1% formalin	< 16	32	512
Control (no absorption)	2048	256	512

Patients 1 and 2, Campylobacter gastroenteritis; patient 3, culture-proven *L. pneumophila* serogroup 1 pneumonia

There were five patients with no date of onset available. One of these was positive (titre 16) and the others negative.

All sera were additionally analysed for the presence of specific IgM, IgG and IgA antibodies. In all positive samples antibodies to *L. pneumophila* were of the IgM class only. IgM titres were generally two-fold greater than the total IFA titre. IgG and IgA titres were less than 16 in all samples. Low level specific IgM titres of 16 or 32 were also found in 6 of the 40 patients (acute sera) who had titres of less than 16 by standard IFAT.

Results of absorption with killed campylobacter are shown in Table 2. Absorption of both patients sera with their own isolate resulted in a significant reduction in antibody titre. All detectable legionella antibody was absorbed from

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the serum of patient 1 with campylobacter killed in formalin or sodium azide. In patient 2 a four-fold and eight-fold reduction in titre was seen with the azide-killed and formalin-killed bacteria respectively. Neither isolate when killed by either method absorbed genuine legionella antibody (patient 3 – culture-proven legionellosis).

All sera from the 42 patients with salmonella gastroenteritis had a legionella titre of less than 16 by standard IFAT. A specific IgM titre of 16 was present in one acute sample. All convalescent samples were negative.

DISCUSSION

Our results clearly demonstrate serological cross-reactivity between *L. pneumophila* and campylobacter which has not been described before. Previous work has shown the IFAT to have a diagnostic specificity of 100% [6], although occasional low titres (16 or 32) may occur in a few patients with *Pseudomonas aeruginosa* or *Mycobacterium tuberculosis* infection.

The background prevalence of *L. pneumophila* serogroup 1 antibodies in Europe is less than 3% [7]. Therefore in the absence of an outbreak of legionellosis, the finding that 20% of a group of patients infected with campylobacter had positive legionella titres suggests considerable cross-reaction. Furthermore 6% of patients had diagnostic titres greater than 512 and one patient showed a rise from < 16 to 256.

A further six patients (12%) had legionella IgM titres of 16 or 32 although these were negative by standard IFAT. This is probably due to the fact that the 90 min serum incubation time used in the IgM assay is more sensitive for detecting IgM than the 30 min used in the standard IFAT. This may also explain why the IgM titres were generally greater than the total IFA titres.

All but one positive sera (by standard IFAT) were obtained in the convalescent phase of the illness whereas the acute samples were negative. This, together with the absence of legionella antibodies in the patients with salmonella gastroenteritis, indicates the likelihood of a serological cross-reaction between *L. pneumophila* and antibodies to campylobacter. This was confirmed by the ability of killed campylobacter to absorb the cross-reacting antibody from two cases but not to absorb genuine antibody to *L. pneumophila* serogroup 1.

The finding that the cross-reacting antibody in each case was of the IgM class alone is also unusual as most patients with true legionnaires' disease who develop IgM antibodies also produce IgG or IgA antibodies (unpublished data). However as the standard IFAT utilizes a conjugate which detects IgM, IgG and IgA antibodies, this test alone cannot distinguish the class of antibody.

Gray and co-workers [5] have recently described a case in which a diagnostic rise in titre of antibody to *L. pneumophila* serogroup 1 was caused by a cross-reaction with *Citrobacter freundii*. We have demonstrated that diagnostic titres can commonly occur by cross-reaction in patients with campylobacter gastroenteritis. This is important because these infections can have similar features – campylobacter infection may present as a pyrexia and 'flu-like illness, diarrhoea may be a prominent feature of legionnaires' disease and both infections may be acquired abroad.

It would be interesting to speculate that the prevalence of legionella antibodies in healthy subjects may be related more to previous exposure to campylobacter than to legionella, bearing in mind the relative incidence of these infections. This will depend on the persistence of the IgM antibodies which may prove to be only transient, although it is recognized that genuine IgM legionella antibodies may persist for a prolonged period of time [6]. We are aiming to follow these patients in order to establish the duration of the response.

It is important to consider this potential cross-reaction in patients with positive legionella serology particularly if there is a recent history of diarrhoea, and we recommend culture of stool samples from all patients in order to avoid diagnostic confusion. Thankfully the firstline antibiotic treatment for both infections is the same!

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