Immunological evidence of chlamydial infection in Reiter’s syndrome

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This paper summarizes our investigations of the immunological reactivity to *Chlamydiae* of patients with Reiter’s syndrome (RS) and other rheumatic diseases.

**Subjects, methods, and results**

We examined 163 patients and 80 controls by means of antichlamydial antibody titres, lymphocyte transformation tests, and, when appropriate, urethral scrapings for the detection of inclusion bodies.

In a healthy population (Table 1) there was a low reactivity towards chlamydial antigens, whether or not the subjects carried the B27 marker, and cell-mediated and humoral reactivity were present together in only 4% of the 80 controls. Likewise in patients with various rheumatic diseases other than RS there was a low prevalence of positive reactivity towards *Chlamydiae*, and the results were not significantly different from those of the controls.

Patients with RS were divided into three groups (Table 2): (1) ‘Complete’ RS, with three characteristic clinical signs (usually urethritis, conjunctivitis, and peripheral arthritis). (2) ‘Incomplete’ RS, with only urethritis and polyarthritis but with HLA-B27. (3) ‘Incomplete’ RS in patients without HLA-B27.

Table 1 shows that the incidence of positive tests for humoral immunity to chlamydia is similar in patients with complete or incomplete RS whether or not the latter were B27-positive. However, the incidence of positive tests in all patients with RS was significantly greater than in patients with ankylosing spondylitis, patients with other rheumatic diseases, or in healthy controls. In this connection it is interesting to recall that patients with ankylosing spondylitis without urethritis, conjunctivitis, or diarrhoea do not differ significantly from control healthy subjects in their reactivity towards chlamydial antigens (Table 1).

Table 2 shows that evidence for chlamydial inclusions and specific immunity in RS is unrelated to clinical symptoms, duration of illness, or carriage of HLAB27.

**Significance of Chlamydia in RS**

Evidence of chlamydial involvement must be viewed in terms of the results of both microbiological and clinical investigations.
immunological studies. Chlamydial isolations were first claimed by T'ang et al. who identified the trachoma agent by yolk-sac inoculation. By the same technique, chlamydiae were recovered from the genital tracts of the parents of some babies with neonatal chlamydial eye infections and in some adults with chlamydial ocular genital disease. Chlamydia were first isolated from the human urethra by Jones et al. and Dunlop et al. In a first study two positive isolates were obtained from nine patients with non-gonococcal urethritis, while in a second study 19 positive isolates were obtained from 89 patients with non-gonococcal urethritis. A lower prevalence of positive isolations was reported by Ford and McCandlish, who, using the same techniques, recovered chlamydia in 15 out of 133 men with non-gonococcal urethritis.

The yolk-sac inoculation technique, which we have also used, is usually disappointing because of the paucity of positive isolates, even when inocula are taken in the first days of an acute non-gonococcal urethritis. Advances in chlamydial isolation techniques, using irradiated McCoy cells or HeLa cells, have been further developed by Gordon and Quan, who centrifuge inocula for one hour on to the cell monolayer to enhance adherence and endocytosis of potentially infective agents. By this technique positive results have been obtained in 32–34% of non-gonococcal urethritis and in 20% of gynaecological infections with a particular higher prevalence in cervicitis (68%). Sexual partners of patients with chlamydia isolate-positive non-gonococcal urethritis are generally also infected, and the serological type of chlamydia is usually the same. To this extent chlamydial infection can be considered as a sexually transmitted disease with specified serological types according to the Wang classification.

In contrast, in RS the prevalence of positive chlamydial isolations is usually quite low. Gordon et al. obtained two chlamydia isolates from the conjunctiva and urethra of one man with RS, the isolates being obtained one month before the development of arthritis. But in 54 other attempts, with a range of patients, they obtained negative results using synovial fluid, synovial membrane, urethra, and conjunctiva. Others have described similarly negative series. Admittedly patients usually consult when polyarthritis appears and generally some weeks after the onset of urethritsis and conjunctivitis, yet techniques of isolation by cell culture are more efficient when the infection is active and in possibly extracellular form.

It is of interest to note that the strains isolated by Gordon and Quan, and termed NMR 21 and NMR 22, had little or no pathogenicity for yolk-sac or for mice. It may be concluded that the isolation of chlamydia in RS is difficult in current practice and therefore its diagnostic value must be regarded as limited.

Immunological evidence of chlamydial infection consists of looking at humoral and cell-mediated immunity towards chlamydia antigens. We find that the results of complement fixation tests with group antigens are discordant and little different between patients with non-gonococcal urethritis, with or without RS. Immunoassay for chlamydia has been demonstrated antibodies against C. trachomatis in 21 out of 74 patients with non-gonococcal arthritis whereas they were present in only 4% of healthy control subjects. Holmes et al. have implicated IgM followed by IgG antibodies in the sequence of positive serology in non-gonococcal urethritis. Our experience (Tables 1, 2) reveals greater prevalence and specificity of antichlamydial antibodies in RS than in other rheumatic diseases. Possibly the use of several different antigenic strains may enhance the incidence of antichlamydia serology, although Wang and Grayston showed that there is a fair degree of cross-reactivity between various chlamydia strains.

### Table 3 Evidence of chlamydial immunity in RS patients related to clinical symptoms, duration of illness, or carriage of HLA-B27*

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>Time of occurrence in RS</th>
<th>Urethral inclusion bodies</th>
<th>Lymphocyte transformation</th>
<th>Serum antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoea (n=6)</td>
<td>At onset</td>
<td>2/6 (33%)</td>
<td>2/6 (33%)</td>
<td>2/6 (33%)</td>
</tr>
<tr>
<td>Urethritis (n=49)</td>
<td>At onset</td>
<td>18/49 (37%)</td>
<td>22/49 (45%)</td>
<td>18/49 (37%)</td>
</tr>
<tr>
<td>Arthritis and urethritis</td>
<td>Within 1 year</td>
<td>14/37 (38%)</td>
<td>11/37 (30%)</td>
<td>15/41 (37%)</td>
</tr>
<tr>
<td>Arthritis and urethritis</td>
<td>Over 1 year</td>
<td>6/23 (26%)</td>
<td>9/22 (41%)</td>
<td>9/22 (41%)</td>
</tr>
<tr>
<td>Conjunctivitis (n=5)</td>
<td>At onset</td>
<td>0/5 (0%)</td>
<td>3/5 (60%)</td>
<td>1/5 (20%)</td>
</tr>
</tbody>
</table>

*All tests in B27-positive patients = 27/43 (77%).
All tests in B27-negative patients = 8/20 (43%).
There is clearly room for standardisation in the study of strain-specific and shared antibodies to chlamydial agents in RS.

Our results of lymphocyte transformation in the study of antichlamydial reactivity run parallel, in a general sense, to those of antibody status, although the two reactions are not always positive in the same patient. There is no segregation between antigens of group A or group B strains.

Conclusions

The major problems of seeking evidence of chlamydial infection in RS are clearly those of specificity and sensitivity of the tests employed. The positivity of tests in healthy controls or in patients with other rheumatic disease may be the consequence of latent infections with micro-organisms of low pathogenicity or with antigens which cross-react with those of Chlamydiae. The negativity of chlamydia tests in 30% of patients with RS may be due to a variety of factors. Firstly, patients’ variability in immune response* may give rise to deficient responses in some. Antibody responses occur only in 30–40% of patients with acute chlamydial infections. The possibility must be also considered that other micro-organisms (for example, Shigella, Yersinia, Klebsiella) may be triggering agents in RS.

Our findings clearly distinguish positive tests for Chlamydia in RS from those in patients with other rheumatic diseases, including ankylosing spondylitis. It is of particular interest that the expression of HLA-B27 has no influence on the prevalence of these tests but that, whatever the technique employed, 30% of patients with RS are negative for all three tests. We conclude that it is appropriate to include Chlamydia among those micro-organisms which may trigger the inflammatory rheumatic disease that we know as ‘Reiter’s syndrome’ in the susceptible host. With knowledge of the triggering micro-organism in a subset of patients it may be possible to investigate the nature of host susceptibility.

*See paper on yersinia-induced arthritis and RS by D. K. Ford at p 127—Editor.

General discussion

PROF. A. T. MASI: Have you studied in the same way a control group of non-gonococcal urethritis patients without reactive arthritis?

PROF. AMOR: Yes, but the control group is small in a rheumatology unit where we see few patients with pure non-gonococcal urethritis and no arthritis. Quite a proportion have complement fixing antibodies and also a positive lymphocyte transformation reaction, and are therefore not specific for RS.

DR. F. C. ARNETT: What kind of antigen preparation were you using, and what stimulation index do you define as positive?

PROF. AMOR: We used purified antigens of two strains—a psittacosis strain, and a human trachoma strain group A (at least a strain that uses glycogen). In answer to your second question, we tried, without success, the thymidine uptake. Now we consider a stimulation positive when on a slide there are twice as many blast cells as in the control.

PROF. T. BITTER: You know, of course, that there is a whole array of antigenic specificities (labelled from A to K) in chlamydia of the kind you got from Dr. Orfila? Did you analyse the antigenic type of your strains? Indeed, some of your more disappointing results might actually be due to the fact that you used only one particular antigenic specificity out of at least 10 presently available.

PROF. AMOR: I hope we can be more specific in the future.

PROF. M. ZIFF: Were the AS controls essentially negative?

PROF. AMOR: They were the same as found in ‘normal’ blood donors.

PROF. ZIFF: So this reactivity seems to be specific for RS but not for B27 positivity?

PROF. AMOR: Yes.

DR. T. L. VISCHER: Did you have earlier data on ankylosing spondylitis with peripheral arthritis where you got an increase of lymphoblastic transformation?

PROF. AMOR: Yes, but at that time we did not have the blood donor controls. We got some stimulation but it was not different from random controls.