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# Cell-mediated immunity to *U. urealyticum (Mycoplasma)* in patients with Reiter's syndrome

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Previous studies in our laboratory have shown poor correlation between serum antibody levels against *Ureaplasma urealyticum* and the presence of either non-gonococcal urethritis or Reiter's syndrome (RS).[111, 112] Most recently, the incidence of antibody to *U. urealyticum* in 40 subjects with RS was significantly greater than that in Red Cross donors or patients attending a VD clinic,[114] but the titres were low and over 50% of patients had no significant antibody. It has seemed, therefore, that the serological approach to relating *U. urealyticum* to RS, at least by current serological techniques, is unlikely to be profitable.

## Test study

The present investigation of lymphocyte reactivity to *U. urealyticum* antigen was initiated for two reasons. Firstly, it seemed possible that the standard dose-response curve, $^3$H-thymidine uptake method of demonstrating lymphocyte reactivity might provide a better tool for studying immunological relationships between *U. urealyticum* and RS. Secondly, it was thought that human lymphocytes might have the capacity to recognise a common antigenicity among mycoplasmas, dysentery bacilli, yersinia, and salmonella, all of which infective agents would seem to be precipitating causes of RS.

A patient was selected for study. He had had two episodes of RS several years previously, but no recent attacks. His antiureaplasmal serology had been investigated repeatedly between 1974–6. He had the highest titre (1:64) of many RS and Haida spondylitic patients' sera studied at that time. He was very co-operative and permitted multiple venepunctures for repeated lymphocyte testing. Table 1 shows the results of a single experiment, and Table 2 summarises the overall data of the pilot experiment.

The following preliminary conclusions can be made.

(1) The patient's lymphocytes responded with stimulation indices varying from 8–33 to two batches of *U. urealyticum* (serotype 5) antigen over a test period of five months.

(2) Lymphocyte response was cross-reactive with other serotypes and stimulation indices varying from 12–69 were found with serotypes 1, 2, and 4.

Table 1 *Sample experiment in study patient. Lymphocytes of $^3$H-thymidine uptake dose response curve for mitogen and antigen lymphocyte reactivity*

| | *cpm × 10³* | | | |
|---|---|---|---|---|
| *Dil. of stock* | *PHA* | *PWM* | *UU control* | *UU antigen* |
| *0** | *3* | *3* | *1·1* | *1·2* |
| 1:2 | 310 | 67 | 1·2 | 25 |
| 1:4 | 274 | 125 | 1·3 | 54 |
| 1:8 | 263 | 147 | 2·2 | 57 |
| 1:16 | 213 | 166 | 1·2 | 49 |
| 1:32 | 223 | 207 | 2·8 | 44 |
| 1:64 | 104 | 203 | 1·6 | 41 |

*Medium control.

Table 2 *Lymphocyte responses to U. urealyticum antigen as measured by $^3$H-thymidine uptake in study patient with previous RS and a UU serum antibody titre of 1:64*

| *Patient's lymphocyte response* | | *Antigen preparation* | | |
|---|---|---|---|---|
| *Date* | *Stimulation index* | *Mycoplasma type* | *Serum in medium* | *Date prepared* |
| 1976 | | | | |
| 2 Nov | 20 | *UU5* | Horse | 1 Nov |
| 10 Nov | 8 | *UU5* | Horse | 1 Nov |
| 18 Nov | 18 | *UU4* | Horse | 17 Nov |
| 18 Nov | 10 | *UU5* | Rabbit | 17 Nov |
| 18 Nov | 12 | *UU4* | Horse | 17 Nov |
| 1 Dec | 33 | *UU2* | Horse | 17 Nov |
| 1 Dec | 69 | *UU1* | Horse | 20 Nov |
| 1 Dec | 1 | *Hominis* | Horse | 30 Nov |
| 1977 | | | | |
| 3 Feb | 4* | *UU5* | Horse | 1 Feb |
| 17 Feb | 2·5* | *UU5* | Horse | 1 Feb |
| 13 Apr | 33 | *UU5* | Horse | 1 Feb |

* Patient had persistent, mild but definite 'chronic cold' with malaise, slight sore throat, and runny nose for several weeks at the end of January and beginning of February.

(3) Specificity of the reaction was demonstrated by: (*a*) The regular incorporation of a control antigen into each test, this antigen being the centrifuged deposit of an uninoculated incubated broth. (*b*) The demonstration that a rabbit serum broth antigen had an equal stimulating activity to those recovered from routine horse serum broth culture. (*c*) An *M. hominis* antigen gave no response, the stimulation index being 1.

(4) An interesting fall of lymphocyte reactivity occurred in February 1978 while the patient had a 'persistent cold' of several weeks' duration, during which he complained of malaise, a slight sore throat, and a runny nose. The reactivity was restored in April when the stimulation index rose to 33. Such a transient depression has been observed in our laboratory in other individuals being followed serially for lymphocyte responses to rubella antigen.

## Pilot study

After the development of this effective test system a pilot study was performed in eight patients selected because they had precisely defined clinical syndromes. Peripheral blood lymphocytes from these patients were tested against the same ureaplasmal antigen that had demonstrated responses in the lymphocytes of the study patient. If lymphocyte responses to this ureaplasmal antigen had been found it was planned to expand the studies to determine if such responsiveness characterised the clinical syndromes. In fact, no such lymphocyte stimulation was found.

Table 3 *Clinical data on eight selected patients found not to have lymphocyte responses to UU antigen*

| *Case No.* | *Sex* | *Age* | *Serum UU type 5 antibody* | *Clinical syndrome* |
|---|---|---|---|---|
| 1 | F | 54 | 1:32 | Spondylitis, Haida Indian[114] |
| 2 | M | 40 | <1:4 | Reiter's syndrome 5X, donor serotype 6, tet. resist. UU urethritis[120] |
| 3 | M | 35 | 1:4 | Reiter's syndrome 2X |
| 4 | M | 49 | 1:4 | Recurrent arthritis with urethritis |
| 5 | M | 53 | <1:4 | Recurrent UU, urethritis[115] |
| 6 | M | 30 | — | Postdysenteric Reiter's syndrome |
| 7 | F | 17 | — | Postsalmonella arthritis |
| 8 | M | 20 | — | Yersinial arthritis[116] |

Table 3 summarises the clinical data on the eight patients. It can be seen that four of them had been specifically studied and reported on previously. It would seem unlikely from this pilot study that lymphocyte responsiveness to ureaplasmal antigen would be helpful in understanding Haida spondylitis, ureaplasmal non-gonococcal urethritis, or RS whether venerally acquired or secondary to intestinal infections by dysenteric, yersinial, or salmonella organisms.

## General discussion

PROF. T. BITTER: We tried to stimulate lymphocytes from a few patients with RS using a battery of chlamydial antigens. We were disappointed.