

## Synovial fluid cells in Reiter's syndrome

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**SUMMARY** Synovial fluid cells in Reiter's syndrome were studied by cell subset specific monoclonal antibodies and avidin-biotin-peroxidase complex staining. Mean leucocyte count was  $9842/\text{mm}^3$  ( $9.842 \times 10^9/\text{l}$ ), and 71% of all cells were polymorphonuclear leucocytes.  $26 \pm 11$ (SEM)% and  $47 \pm 5$ % of all mononuclear cells in synovial fluid were  $\text{M1}^+$  monocytes and  $\text{Ia}^+$  cells, respectively.  $\text{T11}^+$  T lymphocyte was the predominant synovial fluid mononuclear cell ( $61 \pm 8$ %) but, in contrast to the inflammatory joint effusions in rheumatoid arthritis,  $\text{T4}^+$  cells clearly outnumbered  $\text{T8}^+$  cells in Reiter's syndrome. Thus the synovial fluid in Reiter's syndrome contains the immunocompetent and accessory cells required for immune response, which in fact is activated as suggested by lymphocyte Ia expression. Furthermore, in contrast with rheumatoid arthritis inducer/helper cells with T4 phenotype seem to be involved preferentially in the local pathogenetic mechanisms in Reiter's syndrome.

**Key words:** monoclonal antibodies, immunohistochemistry, reactive arthritis.

Diagnosis of Reiter's syndrome is based on a constellation of clinical findings, which sometimes includes the complete, classic triad of arthritis, urethritis, and conjunctivitis.<sup>1,2</sup> Although the disease mechanism is unknown, we now recognise several different infective agents<sup>1,3</sup> and a specific genetic background.<sup>4,5</sup> Reiter's syndrome is not an infectious arthritis, because synovial fluid microbial cultures of the relevant micro-organisms are, according to disease definition, negative.<sup>6</sup> Activation and/or abnormalities in B and T cell mediated functions have been reported in Reiter's syndrome,<sup>7-11</sup> and immunological mechanisms may be responsible for the transition of the infectious enteric or venereal action into a reactive complication located in the joints and entheses.<sup>12</sup> However, all the above-mentioned immunological studies<sup>7-11</sup> were performed with samples derived from peripheral blood. Changes in the diseased joints are largely unknown. This prompted us to study the occurrence in the synovial fluid of cells participating in inflammatory and immune responses in Reiter's syndrome.

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### Patients and methods

#### PATIENTS

All five patients studied fulfilled the diagnostic criteria of the American Rheumatism Association for definite Reiter's syndrome<sup>2</sup> (for clinical data see Table 1). They all had seronegative asymmetric arthropathy of more than one month's duration and all except one were HLA-B27 positive. Synovial fluid specimens were collected from the knee joint by needle aspiration into heparinised tubes and incubated with hyaluronidase for 30 min at 37°C. Five patients with definite or classical rheumatoid arthritis<sup>13</sup> were studied for comparison.

#### IMMUNOHISTOCHEMISTRY

Cytocentrifuge preparations obtained by spinning  $1.5 \times 10^5$  cells per slide were fixed in cold acetone for five minutes and washed with phosphate-buffered saline (PBS; pH 7.3, 0.1 M). The cytospin preparations were stained by the avidin-biotin-peroxidase complex (ABC) method introduced by Hsu *et al.*<sup>14,15</sup> Briefly, the endogenous peroxidase activity was blocked with 0.3%  $\text{H}_2\text{O}_2$  for 20 minutes. The slides were washed in PBS and then treated with: (a) mouse monoclonal antibody, (b) biotinylated horse

Table 1 Clinical data on the patients

Patient No	Age (years)	Sex	Duration of the disease (months)	B27	Urethritis/cervicitis	Dysentery	Arthritis	Differential counting of synovial fluid cells		
								leucocytes/mm <sup>3</sup> *	PMN(%)†	MN(%)†
1	35	Male	20	+	-	+	+	11 000	78	22
2	29	Male	10	+	+	-	+	20 270	30	70
3	36	Male	2	-	-	-	+	11 200	88	12
4	39	Male	2	+	+	-	+	4 230	82	18
5	36	Female	24	+	+	-	+	2 510	79	21

\* SI conversion: leucocytes/mm<sup>3</sup> × 10<sup>6</sup> = leucocytes/l.

† PMN = polymorphonuclear cells; MN = mononuclear cells.

antimouse antibody, and (c) avidin-biotin-peroxidase complex. The ABC kit was purchased from Vector Laboratories (Burlingame, California, USA). Finally, the peroxidase binding sites were shown with diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical Co., St Louis, Missouri, USA), 50 mg/150 ml (333 mg/l) PBS, and 0.003% H<sub>2</sub>O<sub>2</sub> for five minutes. Between each step the slides were washed twice in PBS for five minutes. After the DAB reaction the slides were washed, counterstained with haematoxylin, and mounted. The following monoclonal antibodies were used in this study: OKT11, OKT4, OKT8, OKM1, OKIa (Ortho Diagnostic Systems Inc., Raritan, New Jersey, USA), and pan-B (Dakopatts, Copenhagen, Denmark).

To test the specificity of immunohistochemical staining the following controls were set up: (a) omission of the primary monoclonal antibodies from the staining sequence, and (b) use of inappropriate antibodies in the second stage (e.g., biotinylated goat antirabbit IgG). The slides were

also stained for endogenous peroxidase by the DAB reaction only.

#### EVALUATION OF THE RESULTS

Endogenous peroxidase activity was destroyed by H<sub>2</sub>O<sub>2</sub> pretreatment; exogenous peroxidase-positive, specifically stained cells were brown and thus readily distinguishable under the light microscope. The standard error of the mean (SEM) was used to express dispersion of the data. The significance of differences between mean values was tested with Student's *t* test.

#### Results

The percentages of various cell subpopulations in synovial fluid in Reiter's syndrome compared with rheumatoid arthritis are given in Table 2. Most of the synovial fluid inflammatory mononuclear cells were T11<sup>+</sup> T lymphocytes (61 ± 8 (SEM)%) or M1<sup>+</sup> monocytes (26 ± 11%) (Fig. 1), whereas there were few pan-B<sup>+</sup> B lymphocytes (1 ± 0.5%). In contrast

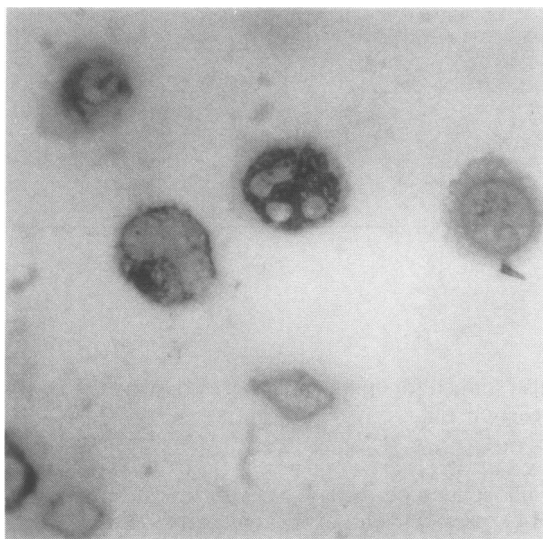
Table 2 Various cell subsets as a percentage of all mononuclear cells in synovial fluid in Reiter's syndrome compared with rheumatoid arthritis\*

Disease	Inflammatory cell subsets (%)				
	T4	T8	B	M1	Ia
Reiter's syndrome	35 ± 11	24 ± 7	1 ± 0.5	26 ± 11	47 ± 5
Rheumatoid arthritis (RA)	27 ± 6	55 ± 5	3 ± 0.8	12 ± 3	54 ± 8
p value	p > 0.05	p < 0.02	p > 0.05	p > 0.05	p > 0.05

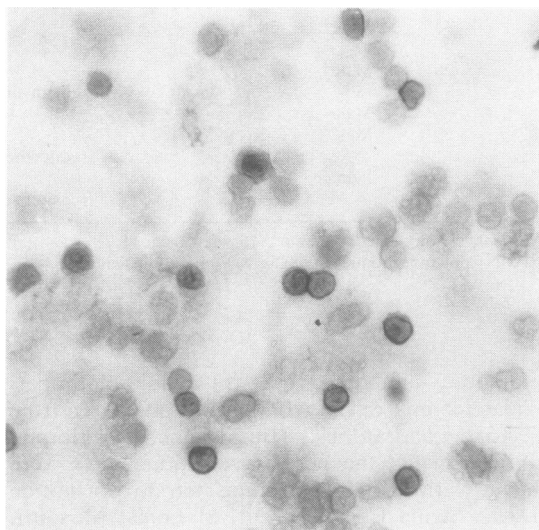
\* Values are mean ± SEM.

with the inflammatory joint effusions in rheumatoid arthritis T4<sup>+</sup> cells (Fig. 2) clearly outnumbered T8<sup>+</sup> cells (Fig. 3) in four patients with Reiter's syndrome (Table 3). The proportion of Ia<sup>+</sup> synovial fluid

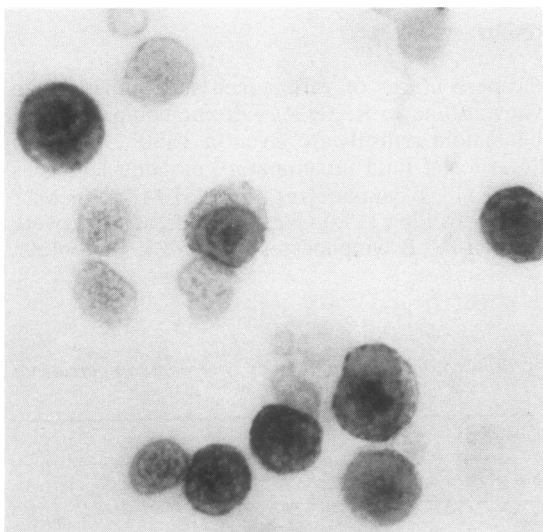
mononuclear cells was  $47 \pm 5\%$  in Reiter's syndrome (Fig. 4) and thus higher than the added proportion of monocytes and B lymphocytes. The staining controls were negative.



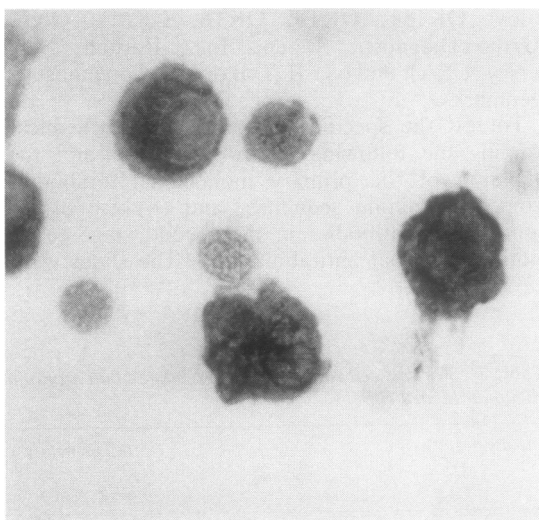
**Fig. 1** Synovial fluid cells in Reiter's syndrome stained for M1 by the avidin-biotin-peroxidase complex (ABC) method. The peroxidase staining is visible in mononuclear phagocytes and polymorphonuclear leucocytes. ( $\times 1000$ ).



**Fig. 3** Synovial fluid cells in Reiter's syndrome stained for T8 by the ABC method. T8<sup>+</sup> suppressor/cytotoxic cells are clearly visible. ( $\times 400$ ).



**Fig. 2** Synovial fluid cells in Reiter's syndrome stained for T4 by the ABC method. The majority of lymphocytes display positive peroxidase staining. ( $\times 1000$ ).



**Fig. 4** Synovial fluid cells in Reiter's syndrome stained for Ia by the ABC method. Most of the mononuclear phagocytes, B lymphocytes, and activated T lymphocytes display Ia marker. ( $\times 1000$ ).

Table 3 Various cell subsets as actual numbers of cells/mm<sup>3</sup>\* and T4:T8 ratios for each patient

Disease	Patient No	Inflammatory cell subset					T4:T8 ratio
		T4	T8	B	M1	Ia	
Reiter's syndrome	1	1355	895	73	169	1089	1.5
	2	1703	6385	114	1845	4966	0.3
	3	349	108	16	860	564	3.2
	4	137	114	5	266	495	1.2
	5	343	79	3	69	264	4.3
RA	6	583	826	65	178	1004	0.7
	7	1200	1720	40	840	2320	0.7
	8	806	7258	504	605	4536	0.1
	9	1488	2195	37	223	1711	0.7
	10	1070	2548	102	764	3007	0.4

\* SI conversion: cells/mm<sup>3</sup> × 10<sup>6</sup> = cells/l.

## Discussion

The cellular infiltrate in the early stages of Reiter's syndrome consists of neutrophilic polymorphonuclear leucocytes, lymphocytes, and plasma cells.<sup>16</sup> In chronic Reiter's syndrome lymphocytes and plasma cells abound but there is no mention of mononuclear phagocytes.<sup>16</sup> However, by histochemical and immunohistochemical methods we have observed that mononuclear phagocytes are an important cell subpopulation in the inflammatory synovial membrane in acute and chronic rheumatoid arthritis.<sup>17 18</sup> The frequent occurrence of M1<sup>+</sup> monocytes and interleukin-1<sup>19</sup> suggests an active role for these cells in the local pathogenetic mechanisms in Reiter's syndrome, too.

T lymphocyte is the predominant mononuclear cell in joint effusions, not only in rheumatoid arthritis but also in ankylosing spondylitis, juvenile arthritis, and psoriatic arthropathy.<sup>20</sup> Our study shows conclusively that this is also the case in Reiter's syndrome: sheep red blood cell receptor positive T lymphocytes clearly outnumbered B lymphocytes in synovial fluid as defined by anti-T11 and pan-B monoclonal antibodies applied in an immunoperoxidase staining procedure.

Some monocytes and B lymphocytes display Ia, which is not present on resting T lymphocytes but is acquired by these cells upon activation.<sup>21</sup> Ia is therefore a useful activation marker for T lymphocytes. The added proportion of monocytes and B lymphocytes was clearly lower than the total proportion of Ia<sup>+</sup> synovial fluid mononuclear cells, and it can be concluded that many of the synovial fluid T cells are activated in Reiter's syndrome. Furthermore, suppressor/cytotoxic T cells displaying T8 differentiation marker predominate in the synovial fluid in most effusions from chronic inflammatory arthritis. Surprisingly, in Reiter's syndrome inducer/

helper T cells displaying T4 differentiation marker outnumbered T8<sup>+</sup> cells. This contrasts with the findings observed in chronic RA, where T8<sup>+</sup> cells predominate in the joint effusion. High T4:T8 ratios in synovial fluid have been described in arthritis of less than one month's duration, whereas in chronic arthritis the T4:T8 ratio in synovial fluid was 1.1.<sup>22</sup> However, the high T4:T8 ratio in our patients with Reiter's syndrome is hardly due to the acute nature of their synovitis, because the mean duration of the disease at the time of synovial fluid analysis was 11.6 months and within the Reiter's syndrome the duration of the disease did not correlate with the T4:T8 ratio. Instead, our findings suggest an active role for T4<sup>+</sup> inducer/helper cells in Reiter's syndrome and differences in the local pathogenetic mechanisms between rheumatoid arthritis and Reiter's syndrome.

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