Aetiological role of bacteria associated with reactive arthritis in pauciarticular juvenile chronic arthritis

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Abstract

Background The cause of juvenile chronic arthritis (JCA) is unknown. Pauciarticular JCA is the most common subtype and can be subdivided into early (type I) and late onset (type II) forms, the latter clinically resembling reactive arthritis.

Methods The cellular immune responses to bacteria associated with reactive arthritis in blood and synovial fluid from 39 children with pauciarticular JCA, three children with classical reactive arthritis, and two children with psoriatic arthritis were examined. Specific titres of antibodies to bacteria in serum samples were measured in all patients.

Results A bacteria specific synovial cellular immune response was found in two of three (67%) patients with reactive arthritis and 14 of 28 (50%) patients with pauciarticular JCA type II but only in one of 11 (9%) patients with pauciarticular JCA type I and none in patients with psoriatic arthritis. Six patients responded specifically to *Chlamydia trachomatis* and 11 to *Yersinia enterocolitica*. Antigen specific lymphocyte proliferation correlated poorly with the specific antibody response.

Conclusions These findings suggest that bacteria with associated reactive arthritis may have a causative role in pauciarticular JCA type II but not in JCA type I.

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Juvenile chronic arthritis (JCA) is defined as arthritis beginning before the age of 16 and lasting at least three months.¹ It is divided into pauciarticular, polyarticular, and systemic forms of which the most common, accounting for 50% of patients, is pauciarticular JCA.² This is further subdivided into early onset pauciarticular JCA (type I), which primarily affects young girls and is associated with antinuclear antibodies and chronic iridocyclitis, and late onset pauciarticular JCA (type II) which occurs mainly in older HLA-B27 positive boys who may develop spondyloarthropathy.³ These subtypes are heterogeneous, however, overlap between them and spondyloarthropathies is common. Spondyloarthropathies account for up to 10% of the incidence of arthritis in children^{3–5} and, in addition to psoriatic arthritis, reactive arthritis, ankylosing spondylitis, and enteropathic arthritis, include atypical spondyloarthropathy, which is often classified as pauciarticular JCA. There is also a clinical similarity between reactive arthritis and certain cases of pauciarticular JCA type II, which are

often self limiting, affect mainly the legs and may include features of spondyloarthropathy.

The aetiopathogenesis of JCA⁶ is unknown. In support of a general pathogenetic role for T cells, synovial T cells in patients with JCA are activated and enriched for γ - δ T cells.^{7,8} However, the various subgroups appear to have distinct aetiologies because they are not only clinically but also immunologically^{2,9} and genetically¹⁰ distinct.

The idea that infection may play a part in the aetiology of JCA is not new. Many viruses, notably rubella and parvovirus, cause a transient arthritis. Whether viruses are involved in JCA is less clear,¹¹ though there is evidence for rubella¹² and influenza A.¹³ Less attention has been paid to bacteria, except in studies of reactive arthritis. Antibodies to peptidoglycan, a bacterial cell wall constituent, have been reported in juvenile ankylosing spondylitis and pauciarticular JCA.¹⁴ Some work¹⁵ has shown that T cells from patients with JCA proliferate to mycobacterial 65 kilodalton heat shock protein (hsp) and to the homologous human hsp60. Although this reactivity may be perpetuated by abnormal expression of human hsp60 in the joint, acting as an autoantigen, it could be initiated by infection as all bacteria have proteins from this conserved family.

Owing to its clearly defined cause, there has been considerable interest in the pathogenesis of reactive arthritis.¹⁶ In adults *Chlamydia trachomatis*, *Yersinia enterocolitica*, salmonella, shigella, and campylobacter are common causes.¹⁷ In children, reactive arthritis accounts for 5–10% of the cases of arthritis.^{4,18} Common triggers include *Y enterocolitica* and salmonella¹⁹ but cases due to *C trachomatis* have also been described.^{20,21} *Borrelia burgdorferi* may induce reactive arthritis but may also cause arthritis by other means such as direct infection.²²

There is considerable evidence that the initiating infection is often subclinical^{16,17} and it is therefore likely that many cases of reactive arthritis in children, as in adults,^{23,24} are not diagnosed as such. The advantages and disadvantages of the various diagnostic methods have been discussed in detail elsewhere and will not be considered in depth here.^{16,23,25} The triggering organisms cannot be cultured from the joint in reactive arthritis^{26,27} and urethral swabs and stool cultures are often negative by the time the arthritis develops.¹⁷ Antibody titres are also unreliable, giving false negative and false positive results.^{28–30} It has been shown that, in reactive arthritis, synovial T cells respond specifically to the triggering bacterium.^{29,31,32} Synovial lymphocyte prolifera-

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tion to bacterial antigens can thus be used to indicate the identity of the triggering organism.¹⁶

We have studied the proliferative response of synovial mononuclear cells from patients with JCA to a range of bacterial antigens. Our study provides unique evidence of synovial T cell reactivity to bacteria which trigger reactive arthritis in a subset of patients with pauciarticular JCA type II, which is associated with HLA-B27 and bears considerable resemblance to adult undifferentiated oligoarthritis; such specific reactivity is found only rarely in patients with pauciarticular JCA type I.

Patients and methods

PATIENTS

Forty two consecutive children (18 girls, 24 boys; mean age 11, range 3–19 years) attending a general paediatric rheumatology clinic were investigated. Patients were included if they met the criteria for pauciarticular JCA or classical reactive arthritis, as defined in the following, and presented with a knee effusion. Two patients with psoriatic arthritis, diagnosed by the presence of typical skin lesions or nail changes, and knee effusions were also studied as controls. Table 1 gives the characteristics of these patients. Paired peripheral blood and synovial fluid samples were obtained from each patient during diagnostic or therapeutic aspiration and the blood or synovial fluid was taken into sterile containers with 10 U/ml preservative free heparin (Braun, Melsungen, Germany). Blood samples were also obtained from nine normal control subjects from the same area (four girls, five boys; mean age 8.6, range 3–16 years).

Classical reactive arthritis was defined as an oligoarthritis preceded by a clear history of urethritis or gastroenteritis in the previous four weeks.¹⁷ Juvenile chronic arthritis was defined as arthritis affecting one or more joints, beginning before the age of 16 years, and lasting more than three months, other causes of arthritis having been excluded;¹ it was further divided into systemic JCA (fever and rash at onset), polyarticular JCA (five or more joints affected in the first six months of the disease) and pauciarticular JCA (four or less joints affected in the first six months of the disease). Children with polyarticular and systemic forms of the disease were excluded from the study. Pauciarticular JCA was subdivided³ into type I (presence of antinuclear antibodies or a history of chronic iridocyclitis, or both) and type II (absence of these features).

The study patients were subdivided prospec-

tively into three major groups: (a) classical reactive arthritis, three children; (b) pauciarticular JCA type I, 11 children; and (c) pauciarticular JCA type II, 28 children. All children with JCA type I were HLA-B27 negative; two in the reactive arthritis group were HLA-B27 positive. From the children with JCA type II, 14 were HLA-B27 positive and 14 were HLA-B27 negative.

Atypical or undifferentiated spondyloarthropathy³³ was diagnosed when European spondyloarthropathy study group criteria were met³⁴ but not the full criteria for ankylosing spondylitis, reactive arthritis or psoriatic arthritis; similar criteria have been proposed for children.⁵

CELL SEPARATION AND CULTURE

Mononuclear cells were separated as described previously²⁹ from paired samples of peripheral blood and synovial fluid by density gradient centrifugation (Lymphoprep, Nycomedas, Norway) and resuspended at 2×10^6 cells/ml in tissue culture medium consisting of RPMI 1640 (Gibco, Paisley, UK) with 10% fetal calf serum (Gibco), penicillin/streptomycin (100 U/100 µg/ml; Biochrom, Berlin, Germany) and glutamine (2 mM/ml; Biochrom). Cells were aliquoted into 96 well plates at 2×10^5 cells/well and cultured for six days in a 5% carbon dioxide incubator. Triplicate wells were stimulated with some or all of the following agents: tissue culture medium alone (background proliferation); *Chlamydia trachomatis* serovar L1 (5 µg/ml), grown and purified as described previously;³⁵ *Yersinia enterocolitica* (0.3 and 0.9 (3 µg/ml), grown in trypticase soya bouillon over 48 hours and washed in phosphate buffered saline; *Salmonella enteritidis* (5 µg/ml), *Shigella flexneri* (5 µg/ml), *Campylobacter jejuni* (5 µg/ml), grown in broth, boiled for one hour to inactivate them, then washed and tested for sterility by culturing a sample; *Borrelia burgdorferi* (5 µg/ml), isolation B29 from a Berlin tick, grown in Kelly's medium and washed in phosphate buffered saline;³⁶ tetanus toxoid (Behring, Marburg, Germany, 1 µg/ml); or pokeweed mitogen (Sigma, Poole, UK, 1 µg/ml).

Wells were pulsed with [³H]thymidine (7.4 kBq/well) for the last 18 hours of culture and incorporation measured at day 6 as described previously.²⁹ The optimum dose and time for proliferation assays were investigated in preliminary experiments (data not shown) and stimulation was carried out with whole bacteria. *C. trachomatis*, *Y. enterocolitica*, and *B. burgdorferi* were tested in all patients as *C. trachomatis* and *Y. enterocolitica* are the most common causes of

Table 1 Characteristics of patients with juvenile arthritis in different subgroups

	Girls/boys	Mean (range) current age (years)	Mean (range) age at onset (years)	No of patients positive for antinuclear antibodies	No of patients with irido- cyclitis	No of patients HLA-B27 positive
Reactive arthritis (n=3)	0/3	13.3 (8–17)	11.7 (7–15)	0	0	2
JCA type I (n=11)*	9/2	10.5 (6–14)	4.9 (4–9)	9	6	0
JCA type II, B27 positive (n=14)*	3/11	11.4 (6–19)	9.3 (2–14)	0	0	14
JCA type II, B27 negative (n=14)*	6/8	11.1 (3–17)	8.6 (2–13)	0	0	0
Psoriatic arthritis (n=2)	1/1	8 (7–9)	6.5 (6–7)	0	0	0
All patients (n=44)	19/25	11.0 (3–19)	8.1 (2–15)	9	6	16

*JCA=juvenile chronic arthritis.

reactive arthritis in Berlin (J. Sieper, unpublished data); salmonella, campylobacter, and shigella were tested when cells were available.

Results are expressed as [^3H]thymidine incorporation in counts/min (cpm) or in stimulation indices (SI). Stimulation indices, defined as the proliferation induced by an antigen divided by the background proliferation, were also used to define the positivity and specificity of responses as follows: SI greater than or equal to 5 were considered positive. If responses to two or more antigens were positive, the highest SI had to be double the value of the next highest to be considered specific, otherwise this was regarded as a non-specific response. Subjects with SI less than 5 for all pathogens were considered to be non-responders.

To exclude the possibility that a higher response in synovial fluid compared with peripheral blood was due simply to recruitment of memory or activated T cells into inflammatory lesions by mechanisms unrelated to the triggering antigens,¹⁶ synovial fluid and peripheral blood from all patients were tested with the recall antigen tetanus toxoid to which the German population is routinely immunised.

MEASUREMENT OF ANTIBODY TITRES

IgG, IgM, and IgA antibodies to chlamydia were measured by standardised microimmunofluorescence as described previously.³⁷ The following serotypes were used as antigens: *Chlamydia trachomatis* (serotypes A-C, D-K, L1-L3), *Chlamydia pneumoniae* (TWAR; isolates TWI 183 and IOL-207), and a pool of *Chlamydia psittaci* isolates (human 33/L and IOL-395, cat 457-F, sheep abortion A-22, pigeon SDP-247). Titres were considered to be positive when $\geq 1/64$ for IgG and $\geq 1/16$ for IgA and IgM.

Titres of IgA and IgG antibodies against yersinia were estimated by immunoblotting;³⁰ only IgA $\geq 1/200$ or IgG $\geq 1/200$ were considered positive. Antibodies to *B burgdorferi* were measured by indirect immunofluorescence (positive $\geq 1/128$) and, for samples positive by immunofluorescence, by enzyme linked immunosorbent assay (ELISA) to differentiate between IgG and IgM antibodies.³⁸ Antibodies to salmonella were measured by ELISA and to campylobacter by immunoblotting. Antibodies to shigella were not measured.

Rheumatoid factor was measured by the standard Rose-Waaler test. Antinuclear antibodies were estimated by indirect immunofluorescence using Hep 2 cells as substrate and a titre $\geq 1/160$ was considered positive.

HLA TYPING

Limited HLA typing for HLA-B27 was performed using the standard microlymphocytotoxicity test.³⁹

STATISTICAL METHODS

The Fisher 2 $\times$ 2 test was used to analyse the differences between the groups.

Results

SYNOVIAL FLUID AND PERIPHERAL BLOOD RESPONSES TO TETANUS TOXOID

To exclude the possibility that antigen responses are always non-specifically increased in synovial fluid with respect to peripheral blood we compared synovial fluid and peripheral blood mononuclear cell responses to tetanus toxoid, an antigen not thought to play any part in arthritis. Table 2 gives the results. Twenty one (50%) of these patients responded to tetanus toxoid in their peripheral blood and 24 of 42 (57%) in the synovial fluid with an SI ≥ 5 . Fourteen (33%) showed a synovial fluid response in the absence of a response in peripheral blood and 10 (24%) showed a peripheral blood response in the absence of a synovial fluid response. Furthermore, in the whole group the synovial fluid response to tetanus toxoid was greater than that in peripheral blood in 18/42 (43%), whereas the peripheral blood response was greater in 17 of 42 (40%). In seven patients the SI was < 5 in peripheral blood and synovial fluid. Taken together these data do not support the proposal of a general increase in antigen responses in synovial fluid compared with peripheral blood.

Table 2 Lymphocyte proliferation to tetanus toxoid (TT) in patients with reactive arthritis and juvenile chronic arthritis (JCA). Results expressed as [^3H]thymidine incorporation ($\text{cpm} \times 10^{-3}$)

Patient No	Synovial fluid		Peripheral blood	
	Medium	TT	Medium	TT
<i>Reactive arthritis</i>				
1	0.2	0.1	2.3	5.8
2	0.4	9.4	0.1	13.2
3	0.3	10.5	0.2	5.5
<i>JCA type I</i>				
4	3.4	16.3	6.3	7.8
5	4.3	14.3	2.1	13.5
6	1.7	9.2	3.6	6.4
7	0.3	19.1	3.1	9.9
8	0.3	6.2	2.6	7.5
9	0.5	23.5	4.7	14.6
10	1.2	7.9	0.5	11.9
11	1.9	5.7	0.8	6.8
12	0.8	7.9	1.3	4.3
13	1.5	17.6	4.4	10.6
14	2.2	32.6	5.9	16.8
<i>JCA type II, HLA-B27 positive</i>				
15	2.4	4.9	2.2	11.9
16	1.3	4.6	1.0	5.0
17	0.8	3.9	0.4	4.0
18	1.2	11.9	1.3	15.7
19	1.7	3.0	3.8	3.8
20	1.5	12.6	5.7	13.9
21	1.9	11.8	4.2	4.4
22	1.1	1.5	0.4	6.1
23	1.2	17.1	2.1	8.7
24	1.1	1.4	2.6	4.1
25	0.4	14.5	0.2	14.9
26	0.4	8.5	1.1	4.0
27	1.1	2.3	0.9	3.3
28	2.0	5.7	2.1	3.2
<i>JCA type II, HLA-B27 negative</i>				
29	1.2	5.0	0.5	11.9
30	1.7	13.4	0.9	14.6
31	1.8	6.8	3.1	7.4
32	0.2	0.6	0.4	2.3
33	0.5	14.9	2.6	23.5
34	1.9	3.7	0.8	6.5
35	0.5	17.3	0.9	14.5
36	1.5	22.6	4.4	25.7
37	0.8	2.3	13.3	19.9
38	0.2	0.7	0.6	4.5
39	0.8	10.8	0.3	4.1
40	0.6	2.1	0.9	16.3
41	1.6	9.3	0.3	14.2
42	1.9	25.4	5.9	18.7

SYNOVIAL CELLULAR IMMUNE RESPONSE TO BACTERIAL ANTIGENS

Two of three (67%) patients with reactive arthritis had a specific immune response to *C. trachomatis* or *Y. enterocolitica*; the remaining patient had a non-specific immune response (table 3). The two patients with psoriatic arthritis had a negative response (SI<4) to all antigens tested. In 39 children with pauciarticular JCA, 15 (38%) had a specific response to *Y. enterocolitica* or *C. trachomatis* and 16 (41%) showed a non-specific response to several antigens. Only eight (21%) showed no response to any antigen (table 3).

The most interesting results were obtained when patients with JCA were subdivided into types I and II. In JCA type I only one child (9%) showed an antigen specific immune response to *Y. enterocolitica*; 10 of 11 (91%) had a non-specific response. In contrast, 14 (50%) of

the children with pauciarticular JCA type II showed a specific synovial lymphocyte proliferation, nine to *Y. enterocolitica* and five to *C. trachomatis*. Table 3 gives detailed results for these patients. No specific proliferation was found to any of the other antigens. The percentage of patients with specific proliferation was significantly different in patients with pauciarticular JCA type II (50%) from those with pauciarticular type I (9%) ($p=0.01$) (figure).

CELLULAR IMMUNE RESPONSE TO BACTERIAL ANTIGENS IN PERIPHERAL BLOOD

Peripheral blood mononuclear cell responses to bacterial antigens in the children with arthritis did not show a significant difference compared with normal controls (table 4). The SI was always <5 except in patients Nos 21 (SI=20 to *Y. enterocolitica*), 39 (SI=9.5 to *B. burgdorferi*), and 10 (10.0 to *C. trachomatis* and 8.0 to *Y. enterocolitica*). As can be seen from table 3, however, in patients 21 and 39 no significant proliferation was found in synovial fluid to the triggering bacterium and in patient 10 there was a non-specific synovial response to *C. trachomatis* and *Y. enterocolitica*. Thus no evidence of a systemic immune response to bacterial antigens was seen in any of the specific responders in synovial fluid.

HLA-B27 AND FEATURES OF SPONDYLOARTHROPATHY

The HLA-B27 antigen was determined in the 44 patients with pauciarticular JCA, psoriatic arthritis, and reactive arthritis and was positive in 16 (38%). Two (66%) of the patients with reactive arthritis and 14 (50%) of the patients

Table 3 Synovial lymphocyte response to yersinia (YE), chlamydia (CT), borrelia (BB), salmonella (SA), shigella (SH), and campylobacter (Cf) in patients with reactive arthritis and juvenile chronic arthritis (JCA). Results are expressed as [³H]thymidine incorporation (cpm × 10⁻³); specific responses are in bold type

Patient No	Medium	YE	CT	BB	SA	SH	Cf
Reactive arthritis							
1	0.2	6.2	0.6	0.5	1.6	2.0	0.8
2	0.4	8.5	19.0	6.2	ND	ND	ND
3	0.5	3.0	3.1	2.0	ND	ND	ND
JCA type I							
4	3.4	17.0	21.0	8.4	ND	ND	ND
5	4.3	20.0	15.0	10.0	8.0	9.0	8.0
6	1.7	36.0	24.0	7.1	10.0	12.0	7.5
7	0.3	14.0	25.0	13.0	ND	ND	ND
8	0.4	41.0	23.0	14.0	29.0	22.0	3.9
9	0.5	25.0	17.0	8.4	ND	ND	ND
10	1.2	13.0	14.0	8.2	1.7	2.9	3.4
11	2.0	12.0	17.0	4.7	24.0	2.6	2.6
12	0.8	13.0	1.3	4.5	5.7	4.0	2.1
13	1.5	11.0	1.8	8.0	ND	ND	ND
14	2.2	23.0	11.0	8.3	14.0	22.0	8.0
JCA type II, HLA-B27 positive							
15	2.4	23.0	3.1	4.1	10.0	11.0	
16	0.8	5.2	2.3	1.8	2.2	1.9	3.4
17	0.3	3.4	4.7	2.9	2.2	4.2	1.8
18	1.2	7.0	3.2	ND	2.9	3.4	4.4
19	1.7	29.0	18.0	4.7	ND	ND	3.1
20	1.5	16.0	12.0	5.8	ND	ND	ND
21	1.9	3.2	1.8	1.3	3.6	2.9	ND
22	1.1	2.8	5.8	ND	ND	ND	2.4
23	1.2	5.1	15.0	3.6	ND	ND	ND
24	1.1	4.1	5.9	1.4	ND	ND	ND
25	0.4	3.0	9.3	1.8	2.2	1.5	ND
26	0.4	6.2	0.9	2.5	2.0	2.2	1.2
27	1.1	4.1	2.4	5.1	3.0	2.2	1.8
28	2.1	4.1	2.8	7.7	4.3	6.3	3.5
							6.6
JCA type II, HLA-B27 negative							
29	1.2	13.0	7.0	3.8	4.2	5.3	2.0
30	1.7	14.0	2.0	2.8	ND	ND	ND
31	1.8	11.0	6.2	4.3	ND	ND	ND
32	0.2	8.8	2.0	1.2	1.3	2.1	1.0
33	0.5	17.0	9.2	11.0	6.4	3.9	5.1
34	2.0	15.2	21.2	8.3	ND	ND	ND
35	0.5	0.5	1.8	0.4	0.6	0.9	0.4
36	1.5	4.4	2.1	1.2	3.6	3.3	2.4
37	0.2	0.4	1.0	0.2	0.8	0.8	0.5
38	0.2	0.6	0.3	0.1	0.3	0.3	0.3
39	0.8	0.2	0.2	0.2	ND	ND	ND
40	0.6	3.9	9.0	1.4	ND	ND	ND
41	1.6	3.4	7.6	3.6	ND	ND	ND
42	1.9	12.0	4.8	2.8	1.6	2.0	0.7

Percentage of specific responders (as defined under Patients and methods) to bacterial antigens in synovial fluid in juvenile chronic arthritis type I (JCA I), juvenile chronic arthritis type II (JCA II), and reactive arthritis (ReA). The hatched area represents the proportion of specific responders; the open area represents the non-specific responders or non-responders. n=number of patients.

Table 4 Peripheral blood lymphocyte response to yersinia (YE), chlamydia (CT), borrelia (BB), salmonella (SA), shigella (SH) and campylobacter (Cf) in patients with reactive arthritis (ReA) and juvenile chronic arthritis (JCA). Results given as mean (SD) stimulation index

Patient group	YE	CT	BB	SA	SH	Cf
Reactive arthritis	2.1 (1.5)	0.8 (0.4)	1.9 (1.5)	0.4	0.3	0.4
JCA type I	1.7 (2)	1.8 (2.7)	1.7 (1.3)	1.6 (0.4)	1.2 (0.4)	1.1 (0.4)
JCA type II HLA-B27 positive	3.3 (5)	1.8 (2.1)	2.6 (3.4)	2.2 (1.9)	2.3 (1.8)	1.9 (1.7)
JCA type II HLA-B27 negative	1.4 (0.7)	1.3 (0.7)	1.8 (0.8)	1.1 (0.4)	1.2 (0.5)	1.1 (0.4)
Healthy controls	3.2 (2.7)	1.8 (1.5)	3.5 (2.8)	2.6 (2.9)	1.3 (1.2)	0.9 (0.8)

with JCA type II but none of the patients with psoriasis or JCA type I were positive (table 1). In the 28 patients with JCA type II a similar proportion of HLA-B27 positive patients (seven of 14) and HLA-B27 negative patients (seven of 14) were specific responders (figure, table 3). The presence of clinical features suggestive of spondyloarthropathy (in patients 15, 23, and 24 of the JCA type II HLA-B27 positive patients and in patients 32 and 34 of the HLA-B27 negative patients) also did not correlate with a specific response. Three such patients were non-specific responders or non-responders and two were specific responders.

HUMORAL IMMUNE RESPONSES

In none of the patients with psoriatic arthritis and JCA type I were antibodies against the bacteria tested found. In reactive arthritis and JCA type II one and five patients had antibodies to *Y enterocolitica* and one patient in each of these groups had antibodies to *C trachomatis*. Four of nine patients (56%) with a *Y enterocolitica* specific synovial cellular immune response had increased antibodies to *Y enterocolitica* (patient 1 in the group with reactive arthritis). No patient with a *C trachomatis* specific response had antibodies against *C trachomatis*. This was also true for patient 2 with reactive arthritis who had a symptomatic preceding urethritis. There was no significant difference between the antibody titres in synovial fluid and peripheral blood (data not shown). Of the nine normal controls, none had antibodies against *C trachomatis* and only two against *Y enterocolitica*. Thus there was no significant difference in antibody titres among the various groups.

Discussion

There are cogent reasons why organisms associated with reactive arthritis might play an important part in JCA. Paramount among these is the clinical similarity between reactive arthritis and pauciarticular JCA, particularly of the late onset type II variety. In this paper we present evidence that not only childhood reactive arthritis but also some cases of pauciarticular JCA might be due to pathogens such as *Y enterocolitica* and *C trachomatis* which have been shown to play an aetiological part in adult reactive arthritis and undifferentiated oligoarthritis.^{26 29 32}

Seventeen of the 44 children (39%) with oligoarthritis examined showed a *C trachomatis* or *Y enterocolitica* specific response in synovial lymphocyte proliferation assays. Two of the three patients with classical reactive arthritis responded to bacteria (one to *Y enterocolitica* and one to *C trachomatis*) whereas two control patients with psoriatic arthritis did not. Most importantly, an antigen specific response was also found in patients with pauciarticular JCA; 14 of the 28 type II children (50%) responded specifically to *C trachomatis* or *Y enterocolitica* but only one of the 11 children (9%) with type I JCA showed a specific response. We did not study patients with systemic or polyarticular disease. The results of the study, which are in

good agreement with our study of oligoarthritis in adults,²³ support the idea that a proportion of children with JCA type II have a forme fruste of reactive arthritis similar to adult undifferentiated oligoarthritis. The limited number of positive control patients with classical reactive arthritis reflects the relative rarity of clearly defined cases in children, especially with large knee effusions. The negative controls (patients with psoriatic arthritis) are also few but, again, children with large effusions are uncommon. The specific nature of the responses we found is, however, supported by the rarity of a specific response in the patients with pauciarticular JCA type I.

One difficulty in interpreting the lymphocyte proliferation test is that a considerable proportion of patients show non-specific reactivity, that is an equivalent response to several organisms. To distinguish between non-specific reactivity and a specific response to a putative aetiological agent, we defined stringent criteria for a specific response which are described in detail under Patients and methods. There are several explanations for the demonstration of non-specific reactivity (reviewed by Kingsley and Panayi¹⁶) including cross reactive antigens between enterobacteriaceae³² or between human and bacterial antigens,¹⁵ high monocyte numbers in synovial fluid⁴⁰ and non-specific recruitment into the joint of memory lymphocytes sensitised in previous infections.⁴¹ Sixty five per cent of the children with pauciarticular arthritis who did not respond specifically to a single antigen showed such non-specific reactivity. Apart from the mechanisms described here, such a generalised response could also arise as a result of increased reabsorption of bacterial antigens due to enhanced gut permeability. The demonstration of asymptomatic chronic gut inflammation in most patients with pauciarticular JCA⁴² provides some support for this concept.

There have previously been few similar studies in children, perhaps because of the difficulty of obtaining synovial fluid. Southwood *et al*⁴³ also showed synovial T cell responses to infective agents in over 50% of children with unselected JCA. They found responses to *Y enterocolitica*, salmonella, and to influenza A but not to *C trachomatis*. Unlike our study they did not differentiate between specific and non-specific reactivity nor among the various JCA subgroups so that the interpretation of the results from an aetiological perspective is difficult. In another investigation reactivity to human hsp60 was found in 60% of children with JCA, mainly in HLA-B27 negative, antinuclear antibody positive children.⁴⁴ This may delineate a different aetiological group from the bacterial responders in our study.

On clinical grounds it has long been thought that some of the children with pauciarticular type II might have unrecognised reactive arthritis as they often have self limiting disease, sometimes with features of spondyloarthropathy such as enthesitis or dactylitis. Our study provides some evidence to support this concept. It is also clear that some children with type II JCA go on to develop ankylosing spondylitis or other defined spondyloarthropathy which have

an entirely different prognosis. The synovial cellular immune response may help to differentiate those with cryptic reactive arthritis from other patients. Somewhat unexpectedly, we did not find that specific lymphocyte proliferation correlated with the possession of the HLA-B27 antigen. Southwood *et al*⁴⁵ did find that HLA-B27 positive patients responded better to bacterial antigens. This difference may be explained in that, unlike our study, they did not separate specific and non-specific responses to bacteria. The presence of symptoms suggestive of spondyloarthropathy also did not correlate with a specific lymphocyte response in our study, possibly because such clinical features are also present in other spondyloarthropathy such as ankylosing spondylitis and psoriatic arthritis where bacteria associated with reactive arthritis are probably not relevant.²⁹

In our study the most common pathogen was *Y enterocolitica* (11 specific responders) whereas six patients responded specifically to *C trachomatis*. The demonstration of a specific response to *Y enterocolitica* is less surprising than the finding of a *C trachomatis* specific response. Reactive arthritis induced by enteric infection is well recognised in children and *Y enterocolitica* and salmonella are important pathogens, though other organisms may play a part.^{4 18 19}

As *C trachomatis* is normally transmitted by sexual intercourse, *C trachomatis* induced reactive arthritis would be expected to be rare in childhood; however, a few cases have been reported.^{20 21} A possible explanation for our results is that *Chlamydia pneumoniae* rather than *Chlamydia trachomatis* is the causative agent as there is cross reactivity in T cell responses of the different species. *Chlamydia pneumoniae* is a common cause of respiratory tract infections in adults and children⁴⁶ and a role for it in reactive arthritis has been shown.⁴⁷ With respect to this, we tested our patients for antibodies against a wide array of chlamydial subtypes as they are serologically distinct, but none was positive. Chlamydia could also be transmitted at birth from mother to infant via the vagina⁴⁸ or sexually, either through voluntary sexual contact in older children or sexual abuse.^{49 50} Transmission of *C trachomatis*, probably by hand, from the urogenital tract of infected parents to their children has also been reported.²¹ Our data suggest that chlamydia might play a pathogenetic part in JCA, though the site of the initiating infection is not clear.

The absence of antibodies to chlamydia and the low occurrence of antibodies to *Y enterocolitica* (found in 57% of patients with a *Y enterocolitica* antigen specific cellular response) confirms in children what has already been shown in adults, namely that patients with active reactive arthritis may not have a humoral response.^{28 29}

In summary, our results emphasise the possible role of infectious triggers, in this instance bacteria, in childhood arthritis and suggest that pauciarticular JCA type II, which clinically resembles reactive arthritis, may often be an occult form of that disease. The most important pathogens in this cryptic form of reactive arthritis appear, as in adults in Germany,

to be yersinia and chlamydia.²³ Such information is becoming increasingly important in view of the various studies supporting the use of long term antibiotic treatment in patients with arthritis precipitated by bacterial agents.⁵¹ Our study also serves to substantiate the idea that JCA is T cell mediated and that each type may have a distinct aetiology.

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