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Identification of the *Yersinia enterocolitica* Urease β Subunit as a Target Antigen for Human Synovial T Lymphocytes in Reactive Arthritis

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The local T-cell response to bacterial antigens is involved in the pathogenesis of reactive arthritis (ReA). Here, we have identified a 19-kDa antigen of *Yersinia enterocolitica* O:9 recognized by *Yersinia*-specific synovial fluid CD4$^+$ T cells in two patients with *Yersinia*-induced ReA. N-terminal amino acid sequencing of this protein revealed that it was identical to the 19-kDa urease β subunit of *Y. enterocolitica* O:9. This protein has previously been shown to be arthritogenic in preimmunized rats after intra-articular injection. Analysis of the T-cell response to this protein showed that it contains several T-cell epitopes, one of which cross-reacts with other enterobacteria not able to induce ReA. This indicates that the arthritogenicity of the 19-kDa antigen is not a property of the 19-kDa protein alone but is dependent on its expression in bacteria able to induce ReA.

Reactive arthritis (ReA) is a sterile and usually self-limited inflammatory disease following gastrointestinal or urogenital infection with certain gram-negative bacteria. Among the microorganisms involved in the antecedent extra-articular disease, *Yersinia enterocolitica* is relatively common (14). The pathogenetic mechanisms leading to arthritis are still unclear. Attempts that have been made to culture *Y. enterocolitica* from the affected joints have been unsuccessful (15). However, immunoreactive avital enterobacterial material has been demonstrated to be present in the synovium and in synovial-fluid (SF) phagocytes (4, 5) and it seems to be clear now that development of ReA depends on the dissemination of microbial antigens to the joint.

There is strong evidence that cellular immune responses to antigens of the causing organisms play a major role in the pathogenesis of ReA (6, 8). We and others have recently studied the specificities of CD4$^+$ T cells at the site of inflammation (1–3, 7, 9, 16). In *Yersinia*-induced arthritis, T cells with specificity for *Yersinia* antigens are enriched within the affected joint (6, 9). T-cell clones specific for *Y. enterocolitica* have been propagated from the SF of patients with postenteritic ReA, thus confirming the presence of T cells with specificity for the inciting antigen on the clonal level (7, 16). These clones that express exclusively the T_h1 "inflammatory" phenotype (12) recognize several distinct bacterial antigens, indicating a multiclonal T-cell response against *Y. enterocolitica* (11). This finding argues against the idea that *Y. enterocolitica* triggers an autoimmune reaction against self-antigens but rather suggests that the inflammatory local T-cell response is directed against bacterial constituents that have in a still unclear way reached the joint. One of the key questions has been whether particular immunodominant proteins or protein epitopes drive the cellular immune response in ReA. Although the clonal T-cell responses revealed individually different patterns, we could identify and partially purify two proteins of 14 and 19 kDa from *Y. enterocolitica* O:9 that apparently are major target antigens for the synovial T-cell response in different patients (11). The aim of the present study was to characterize the 19-kDa antigen.

CD4$^+$ T-cell lines were initiated from the SF by stimulating SF mononuclear cells with *Y. enterocolitica* O:9 disintegrated cells (DIC). After 2 weeks, T-cell clones were derived from these multiclonal lines by limiting dilution (11). The proliferative responses of these T-cell lines and clones were used to detect important T-cell antigens during purification. The 19-kDa antigen was purified from lysates of *Y. enterocolitica* O:9 by $(NH_4)_2SO_4$ precipitation, gel filtration, and reverse-phase high-performance liquid chromatography as described previously (11). This protein was identified as an important target antigen for T-cell responses by its ability to stimulate proliferation of T cells in different ReA patients (Table 1 and reference 11). Figure 1 shows the reverse-phase high-performance liquid chromatography profile and the sodium dodecyl sulfate (SDS)–12.5% polyacrylamide gel electrophoresis (PAGE) analysis of highly purified 19-kDa protein. The N-terminal amino acid sequence determined from this preparation of the 19-kDa protein showed strong sequence homology to the small urease β subunit of *Y. enterocolitica* O:3 (Table 2) that has recently been published (13). Remarkably, this protein had been previously described as a putative arthritogenic cationic 19-kDa protein of *Y. enterocolitica* O:3 capable of inducing arthritis with immunologic tissue injury in Wistar rats after intra-articular challenge in preimmunized animals (10). Thus, we have now identified this protein that is arthritogenic in an experimental system as an important target antigen for T cells in ReA in humans.

Prompted by this result, we characterized the response to this protein in more detail. We used SF-derived T cells from two patients with *Yersinia*-induced ReA. Patient 1 had an HLA-DR6 phenotype, and patient 2 had an HLA-DR2,4 phenotype. As shown in Table 2, we found T cells specific for only the 19-kDa protein of *Y. enterocolitica*, whereas other T cells cross-reacted with other enterobacteria, e.g., *Klebsiella pneumoniae*. The T cells described in Table 2 were all HLA-DR restricted but showed different patterns of HLA restriction. The T-cell line 1-DIC from patient 1 was

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TABLE 1. Specificities of T-cell lines and clones[a]

Stimulating antigen	Stimulation index in response to antigen responder cells[b]			
	1-DIC	2-DIC	2-D3	2-D15
Y. enterocolitica	177	24	20	12
19-kDa protein	143	16	23	10
Salmonella enteritidis	1	12	10	2
K. pneumoniae	2	14	31	3
Escherichia coli	1	3	5	1

[a] The specificities of the T-cell lines and clones were determined by testing their proliferation in response to bacterial antigen preparations (the 19-kDa protein at 2 μg/ml and the other bacterial antigens at 80 μg/ml) in 96-well round-bottom microtiter plates (Nunc) presented by autologous (patient 2) or HLA-DR-matched monocyte-enriched and B-cell-enriched peripheral blood mononuclear (patient 1) cells as described previously (11). The T-cell line 1-DIC was derived from patient 1, and the T cell-line 2-DIC and the T-cell clones 2-D3 and 2-D15 were derived from patient 2. Cells were cultured for 2 days and pulsed with 0.02 μCi of [^{3}H]thymidine (Amersham) for the last 18 h.

[b] Stimulation index = counts per minute incorporated with antigen/counts per minute incorporated without antigen.

TABLE 2. Homology of the N-terminal amino acid sequence of the 19-kDa antigen from *Y. enterocolitica* O:9 with the *Y. enterocolitica* O:3 urease β subunit[a]

Protein	N-terminal amino acid sequence	Position in sequence
19-kDa antigen	STKTNSTKATSQKTDSLKTNAGTK	1–24
	:::::::::::: ::::::::: :::	
Urease β subunit	STKTNSTKATSEKTDSLKTNRGTK	1–24

[a] The N-terminal 24-amino-acid sequence of the 19-kDa T-cell antigen from *Y. enterocolitica* O:9 was determined by Edman degradation. The sequence of the 19-kDa *Y. enterocolitica* O:3 urease β subunit was received after a homology search in the EMBL data library. The sequence of the urease β subunit is part of the urease operon of *Y. enterocolitica* (13).

restricted by HLA-DR6, while the T-cell line 2-DIC of patient 2 responded to antigen on HLA-DR2$^+$ and -DR4$^+$ cells (data not shown). Clone 2-D3 was restricted by HLA-DR2, as shown by the fact that allogeneic DR2$^+$ cells could present the 19-kDa protein. In contrast, clone 2-D15 recognized the 19-kDa protein only on autologous cells but not on several HLA-DR2- or HLA-DR4-matched antigen-presenting cells (Table 3). These results indicate that the 19-kDa protein contains at least three different T-cell epitopes, two specific for the *Y. enterocolitica*-derived protein and recognized in the context of HLA-DR6 or autologous HLA-DR2,4 cells, respectively, and a third epitope shared by other bacteria that is recognized by clone 2-D3 and the line 2-DIC in the context of HLA-DR2. Because the response of clone 2-D3 to *K. pneumoniae* had the same HLA-DR2 restriction as that to *Y. enterocolitica* (data not shown), it is very unlikely that 2-D3 recognizes a cross-reacting peptide from a completely different protein, but this result argues that in fact the 19-kDa protein of *K. pneumoniae* is recognized.

It is remarkable that the third epitope of the 19-kDa antigen is also expressed in *K. pneumoniae*, a bacterium that does not induce ReA. This finding suggests that the arthritogenicity is not a property of the 19-kDa protein alone but of the producing microorganism *Y. enterocolitica*. Thus, it can be concluded that *Y. enterocolitica* and other bacteria inducing ReA are provided with factors that favor the transport of putative arthritogenic antigens to the joint. One possibility is that *Y. enterocolitica* has a specific tropism for joints or that the facultatively intracellular bacterium *Y. enterocolitica* persists in phagocytes and is transported to the joints by such cells (17).

In summary, we have identified the 19-kDa urease β subunit of *Y. enterocolitica* as a target antigen for T cells in

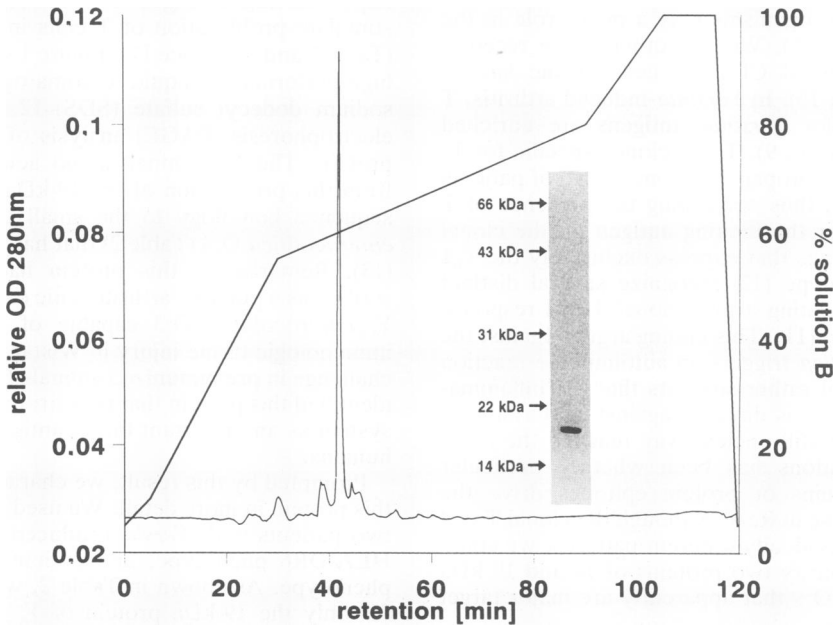

FIG. 1. Purification of the 19-kDa antigen from *Y. enterocolitica* O:9. The rerun of the pooled 19-kDa antigen reverse-phase high-performance liquid chromatography fractions and the SDS–12.5% PAGE of the highly purified 19-kDa antigen after the rerun are shown. Pooled 19-kDa high-performance liquid chromatography fractions were injected into a Delta-Pak C_4 (Millipore Waters) as described elsewhere (11). Solution A, 0.1% trifluoroacetic acid in water; solution B, 0.085% trifluoroacetic acid–80% acetonitril in water.

TABLE 3. HLA-DR restriction of T-cell clones from patient 2[a]

T-cell clone	Stimulation index in response to *Y. enterocolitica*[b]							
	Autologous HLA-DR		Allogeneic HLA-DR					
	2,4	+L243 2,4	2,4	1,4	1,4	2,3	2,8	6
2-D3	32	2	23	1	1	27	16	1
2-D15	123	1	3	2	2	2	4	2

[a] The 19-kDa specific T-cell clones 2-D3 and 2-D15 were tested for their proliferative response to *Y. enterocolitica* antigens presented by monocytes and B cells with the HLA-DR types indicated. The response on autologous cells was blocked by the addition of the HLA-DR-specific monoclonal antibody L243 (5 μg/ml; American Type Culture Collection).

[b] Stimulation index = counts per minute incorporated with antigen/counts per minute incorporated without antigen.

Yersinia-induced ReA in humans. This protein has—by a completely different approach—been identified as arthritogenic in Wistar rats after intra-articular injection. Our results suggest that the potential to induce ReA is not a property of this protein by itself but is dependent on the producing bacteria. It is likely, however, that the physicochemical characteristics of the 19-kDa protein contribute to the development of ReA. Because of its highly cationic nature, the 19-kDa antigen may have a particularly high affinity for the negatively charged structures of the cartilage and may therefore possibly persist in the joints longer than other bacterial antigens. This makes the urease β subunit an important target antigen for T cells that trigger ReA.

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