The Evolutionarily Conserved Ribosomal Protein L23 and the Cationic Urease β-Subunit of *Yersinia enterocolitica* O:3 Belong to the Immunodominant Antigens in *Yersinia*-Triggered Reactive Arthritis: Implications for Autoimmunity

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ABSTRACT

Background: Reactive arthritis (ReA) is a T cell mediated inflammatory process. The immune response is primarily directed against a triggering organism, although autoimmunity has been invoked in long-lasting, antibiotic-resistant disease. Although a variety of different species are known to trigger Reactive arthritis, the clinical manifestations are strikingly similar as well as closely associated to the HLA-B27 (70%).
Materials and Methods: Various antigenic fractions and single antigens of *Yersinia enterocolitica* were prepared, and their immunological activity was assessed by proliferation of synovial fluid mononuclear cells from 10 Reactive arthritis patients. The gene encoding one hitherto unknown antigen has been sequenced. Nonapeptides deduced from sequences of the target antigens were tested in an assembly assay.
Results: Two immunodominant proteins of *Yersinia enterocolitica* were found, one being the urease β-subunit and the other the 50 S ribosomal protein L23. The latter has been sequenced and belongs to the evolutionarily conserved ribosomal proteins with homology to procaryotes and eucaryotes. One nonapeptide derived from the urease β-subunit was identified as a possible epitope for HLA-B27–restricted cytotoxic T cells by its high affinity. This epitope is also highly conserved.
Conclusion: Sharing of conserved immunodominant proteins between different disease triggering microorganisms could provide an explanation of the shared clinical picture in Reactive arthritis. Moreover, autoimmunity in Reactive arthritis might be mediated by antigen mimicry between evolutionarily conserved epitopes of ribosomal proteins and their host analogs.

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INTRODUCTION

Reactive arthritis (ReA) is a sterile inflammatory joint disease that follows a preceding gastrointestinal or urogenital infection. Major microorganisms known to trigger ReA are *Chlamydia trachomatis* and *Yersinia enterocolitica* (1,2). Although the disease as a group is etiologically dissimilar, the association with HLA-B27 is a consistent feature (3).

This study focuses on yersinial ReA. *Yersinia* cannot be cultured from the site of inflammation, but bacterial products are present in synovial fluid (SF), phagocytes (4), and in the synovium (5,6) as judged by immunofluorescence. There is strong evidence that *Yersinia*-triggered ReA is mediated by T cells (7). The immune response is directed against *Yersinia* antigens (8) and seems to be driven locally (9,10) by TH2-type cells present in the synovial membrane (11), a type of cell which might interfere with elimination of these intracellular bacteria.

The similar clinical manifestations produced by bacteria as diverse as *Yersinia* and *Chlamydia* pose the question of whether conserved immunodominant proteins may be involved. Furthermore, in chronic courses of the disease, an autoimmune response might be triggered by antigen mimicry between self and such conserved bacterial proteins (12).

The bacterial antigens that elicit T cell response are largely unknown (13). The present study, which aims to identify immunodominant proteins, was guided by an earlier study (14), which identified a cationic urease β-subunit as arthritogenic in the rat. We found two major target antigens for the cellular immune system of the 10 patients tested, one being the urease β-subunit and the other a novel 13 kD ribosomal protein. Sequence determination of the gene encoding the latter revealed it to be the highly conserved 50 S ribosomal protein L23, which has strong homology to genes present in other procaryotes and a weaker homology to the human homolog. Furthermore, we describe possible epitopes for HLA-B27–restricted $CD8^+$ cytotoxic T cells (CTL) using synthetic peptides, deduced from the primary amino acid sequence of these antigens, and tested in an in vitro HLA-assembly assay. One of these urease β-subunit epitopes—a high affinity binding peptide—is also evolutionarily conserved.

MATERIALS AND METHODS

Patients

Synovial fluid mononuclear cells (SFMC) were obtained from 10 patients; 6 presented with undifferentiated oligoarthritis (15) (UOA) (2 female and 4 male patients; mean age 51 years; range 40–67) and 4 with reactive arthritis (3 female and 1 male; mean age 17; range 9–35). UOA was defined as primarily the lower limb affecting oligoarthritis with fewer than five joints involved, which did not meet the criteria for diagnosis of any other distinct rheumatic disease. ReA is an oligoarthritis with a clear history of antecedent diarrhea in the preceding 4 weeks. All patients showed a specific proliferation of SFMC to *Yersinia enterocolitica*, with specificity being defined as a stimulation index (SI) to Yersinia ≥ 5 and at least twice the values of SI to any other bacteria. Four out of 10 patients bore the HLA-B27 allele.

Proliferation Assays

Mononuclear cells (MC) were obtained by arthrocentesis, which was necessary for diagnostic or therapeutic reasons. Separation from the synovial fluid and resuspension in tissue culture medium was performed as previously described (16). Cells were cultured for 6 days in 96-well plates at 2×10^5 cells/well in a carbon dioxide incubator at 37°C. Triplicate wells were stimulated with antigens (see antigen preparation) at optimal protein concentration between 3 and 10 μg/ml of the following agents: tissue culture medium alone (background proliferation); *Yersinia enterocolitica* O:3 and O:9 (3 μg/ml), *Chlamydia trachomatis* serovar L1 (5 μg/ml), *Salmonella enteritidis* (5 μg/ml); *Shigella flexneri* (5 μg/ml) and *Campylobacter jejuni*; *Borrelia burgdorferi* (5 μg/ml); pokeweed mitogen (1 μg/ml, Sigma, Poole, U.K.). All bacteria were prepared as described (15,17). Wells were pulsed with [^{3}H]-thymidine (7.4 kBq/well) for the last 18 hr of culture, and uptake was measured at day 6.

Chloroquine Inhibition Experiments

T cells and non-T cells were separated using adherence to a plastic petri dish. Five thousand adherent cells (APC) were incubated with antigen in tissue culture medium supplemented with 50 μM chloroquine for 2 hr. After washing, 50,000 nonadherent cells (mainly T cells) were added and incubated for 6 days in the presence of 5 μM chloroquine.

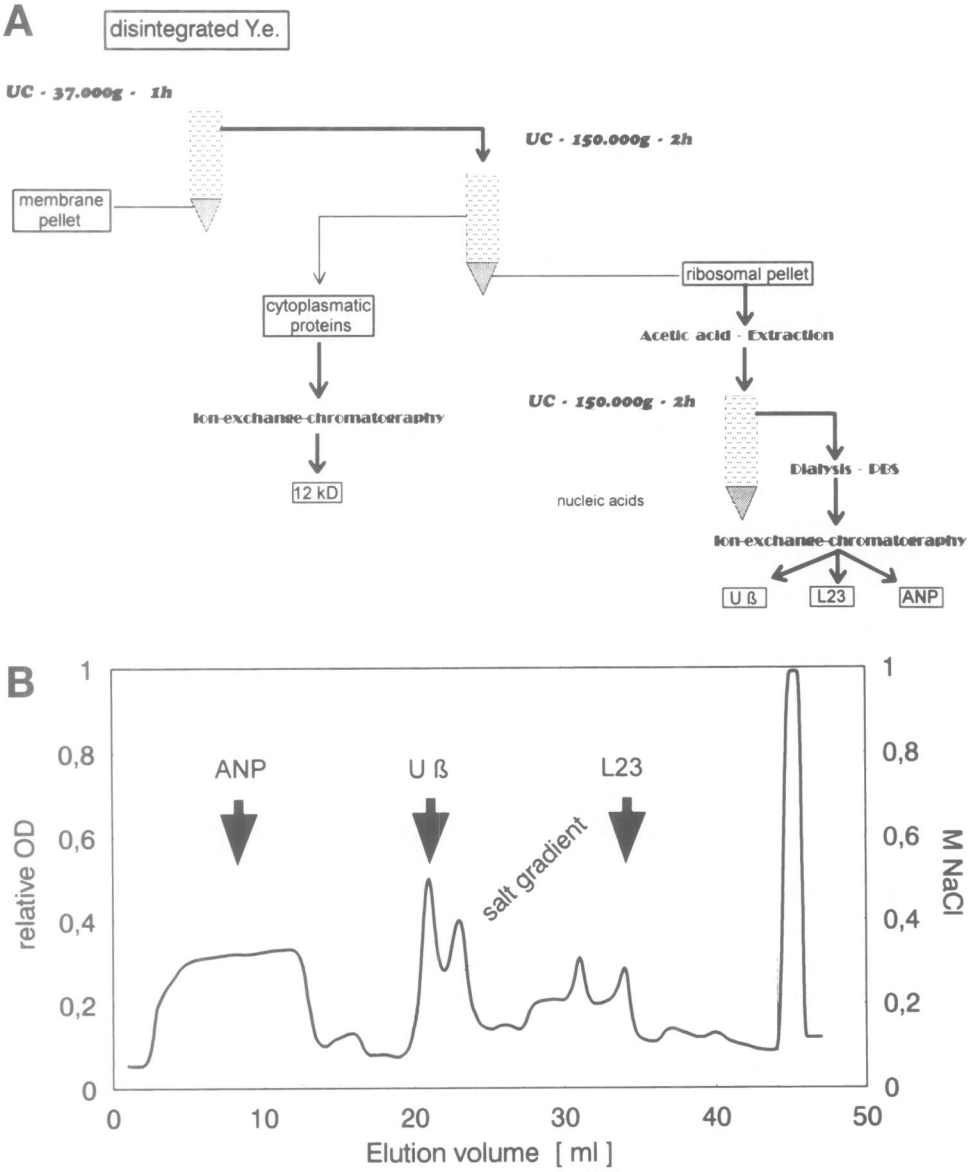

FIG. 1. Antigen fractions and proteins

(A) Schematic illustration of the preparation of Yersinia antigenic fractions and single proteins. Using differential centrifugation, three major fractions were obtained from disintegrated Yersinia: membrane fraction, cytoplasmatic proteins, and ribosomal pellet. Acetic acid extraction of the latter, followed by cation exchange chromatography, resulted in purification of the urease β-subunit, the ribosomal protein L23, and a fraction, composed of anionic and neutral proteins (ANP). UC: ultracentrifugation. (B) Elution profile of ribosomal pellet proteins from cation exchange chromatography. A protein fraction without affinity elutes at first and is composed of anionic and neutral proteins (ANP). The urease β-subunit (Uβ) and ribosomal protein L23 are strongly cationic and elute under high molarity conditions. (C) SDS–PAGE under reducing conditions showing the protein content of the ribosomal pellet after acid extraction and the protein peaks obtained by cation exchange chromatography as illustrated in Fig. 1B.

Preparation of Antigen

A *Y.e.* serotype O:3 strain isolated from a case of enterocolitis (Freiburg strain 10543) was used as a source for purifying antigen fractions and proteins. The procedure was described earlier (14) and is illustrated in Fig. 1A. Briefly, bacteria were grown in brain heart infusion medium (Merck, Darmstadt, Germany) at 30°C. Fifty grams of washed cells were disrupted in a french press in the presence of a proteinase inhibitor cocktail. After DNAase treatment, differential centrifugation was applied resulting successively in a membrane pellet, ribosomal pellet, and a cytoplasmatic fraction. The ribosomal pellet was acid extracted and the soluble fraction was dialyzed to neutrality. Further purification was performed on a fast protein liquid chromatography system (Pharmacia-LKB, Freiburg, Germany) using a Mono-S™-cation exchange column and a sodium chloride salt gradient (0.07–1 M). The 19 kD urease β-subunit eluted at 0.3 M NaCl and the 13 kD at 0.65 M NaCl. The material in the void volume, exhibiting no apparent affinity to the ion exchanger, was judged to be composed of anionic and neutral proteins and termed ANP. A 12 kD protein, dominant in the cytoplasmatic fraction, could be isolated by anion exchange chromatography.

SDS–PAGE and Western Blot Procedure

The SDS–PAGE was essentially done as described by Laemmli (18) under reducing and nonreducing conditions, without boiling (gel concentration $T = 12.6\%$, $C = 2.7\%$). For molecular mass determination a standard protein mixture (range 14–94 kD; Pharmacia) was used. The gels were stained with Coomassie Blue R 250.

Transfer of proteins after electophoresis was achieved by electroblotting (19) to Immobilon™-PVDF membranes (Millipore Corp., Bedford, MA, U.S.A.). Membrane strips were incubated overnight with patients sera diluted 1:100, followed by incubation with 1:5,000 diluted peroxidase-labeled goat antihuman IgG (Dianova, Hamburg, Germany) for 1 hr. Finally, the strips were developed with diaminobenzidine (Sigma). For amino-terminal sequencing, strips were shortly stained with amido black.

Amino-Terminal Sequence Determination of the *Yersinia enterocolitica* O:3 13 kD Antigen

Thirteen kiloDalton bands were sliced out of the Immobilon™-PVDF membrane and sequencing was performed in a gas-phase sequencer (Applied Bio-Systems Instruments, model 477A, Foster City, CA, U.S.A.) with on-line identification of the amino acid derivatives (model 120A) according to the manufacturer's recommendations.

PCR Amplification and Nucleotide Sequence Determination of the Gene Coding for the *Yersinia enterocolitica* O:3 13 kD Ribosomal Protein

Y.e. O:3 strain 6471/76-c (20) was used to isolate genomic DNA for PCR, as control *Y. pseudotuberculosis* strain YPIII (21) was taken. The oligonucleotide primers for PCR and sequencing were constructed using conserved regions on both sides of the *E. coli* (accession number X02613) and *Y. pseudotuberculosis* (accession number X14363) ribosomal protein L23 gene sequences. 13 kD-Pr1 (5′-AGCCT GATCG CCTTC GAC-3′) corresponds to nucleotides 941–958, and 13 kD-Pr2 (5′-TTACG GCCAC CGCTT TTG-3′) corresponds to nucleotides 1458–1441 of the *Y. pseudotuberculosis* sequence. These primers were chosen to encompass the whole coding region of the *Y.e.* 13 kD protein. They amplified a DNA product of 518 bp in PCR. Amplification was carried out in a reaction mixture containing approximately 20 ng of *Y.e.* genomic DNA, 10 pmol of each primer, 100 μM of each dNTP, 0.5 U of *Taq* polymerase (HyTest, Turku, Finland), in 67 mM Tris-HCl, 1.5 mM $MgCl_2$, 16 mM $(NH_4)_2SO_4$, plus 0.01% Tween 20, in a reaction volume of 50 μl at pH 8.3 at 20°C. Amplification for 30 cycles was carried out under the following conditions: denaturation at 94°C for 1 min, annealing at 48°C for 1 min, extension at 72°C for 1 min. DNA sequencing of both strands was performed on purified PCR products using the cyclic sequencing protocol (22). Purification of the PCR products was done from preparative agarose gel using the Millipore MC purification system. Primers for sequencing were the same as those used for PCR. The DNA sequences were handled by the Genetic Computer Group program packages (23). Databank searches for homologous polypeptides were performed by the TFasta program (24).

Peptide Synthesis

Peptides were synthesized on a robot system developed for multiple peptide synthesis (Multi-SynTech, Bochum, Germany). p-Benzyloxyben-

zylalcohol-resin (10 mg) loaded with the first Fmoc-amino acid was filled in separate small filter tubes. Fmoc deprotection was carried out with 50% piperidine in N,N-dimethylformamide (0.2 ml) for 5 min and repeated for 15 min. Couplings were performed with Fmoc-amino acids in 10-fold excess and 1-hydroxybenzotriazole/diisopropylcarbodiimide activation in N,N-dimethylformamide within 1 hr. After coupling and Fmoc deprotection the tubes were washed with N,N-dimethylformamide (0.3 ml) seven times each. The peptides were removed from the resin, and side-chain deprotection was performed with trifluoroacetic acid (0.5 ml) containing thioanisole (25 μl), thiocresol (25 μl), and ethanedithiole (25 μl) within 4 hr. The filtrate was poured into cold ether. The precipitates were washed three times with ether. Amino acid analysis and ion-spray mass spectrometry proved the identity of the peptides.

In Vitro HLA-Assembly Assay

The procedure was done as described elsewhere (25,26). The mutant human B lymphoblastoid cell line LCL 5.2.4 (D. Pious, Department of Pediatrics, University of Washington, Seattle, WA, U.S.A.) used lacks both TAP transporter genes and bears the MHC alleles HLA-A2, B27, and DP4.1. Briefly, 1×10^7 [^{35}S]-methionine-labeled cells were lysed in the presence of either synthetic peptide (100 μM) or PBS. The lysates were incubated overnight and stable class I molecules were then precipitated with conformation-dependent HLA-B27–specific monoclonal antibody ME1 (27). The precipitates were analyzed on 12% polyacrylamide gels, which were visualized by autoradiography and quantitated by densitometry (Howtek Scanmaster 3, Howtec, Inc., Hudson, NH, U.S.A.; pdi software, Huntington Station, NY, U.S.A.).

RESULTS

Identification of Relevant Antigens

Three major protein fractions could be obtained using differential centrifugation (Fig. 1A): membrane pellet, cytoplasmatic protein fraction, and ribosomal pellet. Each fraction was then tested for its capacity to stimulate SFMC from 10 patients with *Y.e.*-triggered arthritis. The strongest proliferation was seen in response to the ribosomal pellet. The proliferative response to the membrane and cytoplasmatic fraction showed large variation from patient to patient, ranging from moderate to high (data not shown). The ribosomal pellet was then further investigated to identify the relevant antigens.

At first the proteins were extracted by acetic acid and subsequently subjected to ion exchange chromatography after careful dialysis to neutral pH. The elution profile is given in Fig. 1B. At low salt conditions, a large "shoulder" was recorded representing proteins without affinity to the cation exchanger. These proteins were thus judged to be anionic or neutral proteins (ANP) (Fig. 1C). Under a linear increasing salt gradient, two sharp single peaks could be eluted at 0.3 M NaCl, consisting of the well known 19 kD protein, i.e., the urease β-subunit described in our earlier study (14,28) and at 0.65 M NaCl, containing a 13 kD protein. We then tested the urease β-subunit and the 13 kD protein as well as the ANP-fraction for their ability to stimulate SFMC. The results are given in Fig. 2. Both the urease β-subunit and the 13 kD protein showed overall strongest proliferation, although substantial variation from patient to patient was observed. In contrast, the proliferative response to ANP—containing the majority of the ribosomal pellet proteins—was moderate or weak. The response to urease β and 13 kD is not due to a nonspecific proliferation of SFMC to cationic proteins, since the very basic calf thymus histone and cytochrome c were not recognized. Both *Yersinia* proteins do not act as superantigens or mitogens, since the proliferative response could be suppressed in the presence of chloroquine by more than 70% (data not shown). A major constituent of the cytoplasmatic protein fraction, a 12 kD protein (pI 8.0, in isoelectric focusing, data not shown), was shown to be irrelevant.

Identification of the *Y.e.* 13 kD Protein as the 50 S Ribosomal Protein L23 and Sequence Determination

Appropriate bands of the 13 kD protein were sliced out of the PVDF membrane, and N-terminal sequence determination was performed. At least 30 amino acids could be accurately identified and comparison with protein sequences in the SWISS PROT database revealed strong homology to the 50 S ribosomal protein L23 of *E. coli* and *Yersinia pseudotuberculosis*. For complete determination of the amino acid sequence, the PCR amplified gene for the 13 kD protein was sequenced using primers selected from the pub-

lished nucleotide sequence of *Yersinia pseudotuberculosis*. The primers specifically amplified a similarly sized product from *Yersinia enterocolitica* 6471/76 and *Yersinia pseudotuberculosis*. This fragment was sequenced directly by the cyclic sequencing protocol, and an internal sequence of 390 bp was obtained. The nucleotide sequence of the *rpl/W* named gene of *Yersinia enterocolitica* O:3 has been submitted to the EMBL, GenBank, and DDBJ nucleotide sequence databases under accession number U11251. The deduced amino acid sequence is given in Fig. 3A.

Database Search for Homology

A homology search in the Gen-EMBL nucleotide database yielded several high scores. All of these turned out to be ribosomal proteins. Substantial homology was not only present among procaryotes (archebacteria and eubacteria), but also eucaryotic organisms including plants and rodents. Even a human homolog could be found showing an overall identity of 32% and similarity of 56% to the L23 protein (Fig. 3B). The homologous proteins of *Chlamydia* and other ReA-inducing enterobacteria have not been sequenced to date.

Identification of HLA-B27 Binding Nonapeptides of the Urease β-Subunit and the L23 Protein

The nonamer peptides presented by the B27 molecule generally have an arginine at their second position and often but not necessarily basic residues at position one and nine (29). This motif was used to search for peptides in the primary sequence of the urease β-subunit and the L23 protein, which might be able to bind to the B27 molecule. The sequence of the urease β-subunit contains 10 nonapeptides fitting this motif (Table 1). They were synthesized and tested in an in vitro assembly assay; four out of these were found to exhibit significant affinity to the B27 molecule (Fig. 4). Among those, one strong binder could be identified (RRAAERGFK). The sequence of the L23 protein contains seven nonapeptides with the B27 consensus motif; three out of seven bound to the B27 molecule (Table 1).

Database Search for Homology

A search in the SWISS PROTEIN database for homology of the strong binding nonapeptide of the urease β to stretches of proteins containing the B27 binding motif showed significant homology to various human proteins including complement C4 precursor, laminin A chain precursor, a human retinal enzyme, as well as ribosomal proteins and the 60 kD chaperonin of mycobacteria and streptomyces (Table 2). Moreover, sequence identities are located within peptide positions proposed to be directly accessible by the T cell receptor (30). The homologous parts of the L23 and the eucaryotic L23a proteins do not bear the B27 consensus motif.

Western Blot Analysis of Patients Sera

Nine patients were tested for serum antibodies to the urease β-subunit of *Yersinia enterocolitica* in Western blots and were found to be positive in eight cases (88%), thus identifying the β-subunit

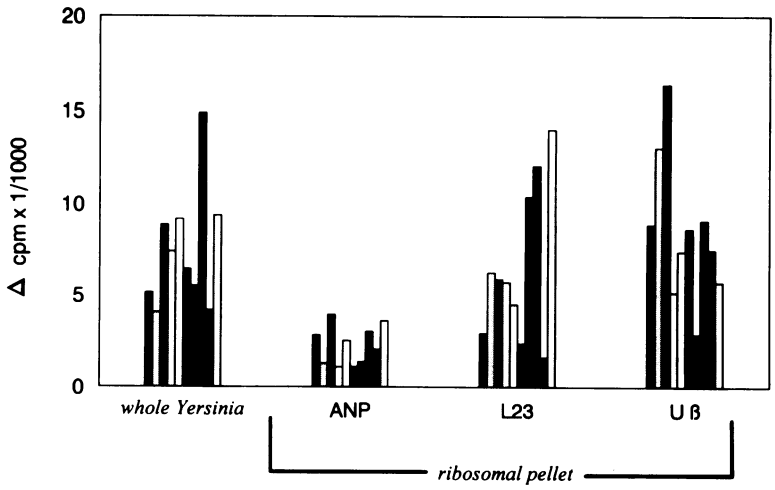

FIG. 2. Proliferative response of synovial fluid mononuclear cells of all 10 reactive arthritis patients to whole *Yersinia* and purified ribosomal pellet proteins and protein fractions

The highest proliferation is seen in response to the urease β-subunit (Uβ) and to a slightly less degree in response to the ribosomal protein L23. The fraction of anionic and neutral proteins (ANP), although containing a large array of proteins, induces weak proliferation.

FIG. 3. The deduced amino acid sequence

(A) Homology between the deduced complete amino acid sequences of the 50 S ribosomal protein L23 of *Yersinia enterocolitica* O:3 (accession number for the rp/W: U11251), *E. coli* and *Yersinia pseudotuberculosis* and the archebacterium *Halobacterium marismortui* (Hma), respectively. Vertical lines indicate identical amino acids, (:) indicate conservative substitutions. Strong homology is present between the proteins of the three enterobacteria, and substantial homology is found to the archaebacterial analog. (B) Homology between the 50 S ribosomal protein L23 of *Yersinia enterocolitica* and its eucaryotic analogs, i.e., rat 60 S ribosomal protein L23a and human analog. Partial sequence alignments of the deduced amino acid sequence was performed using the TFASTA (24) program and the GENEMBL database. Vertical lines indicate identical amino acids, (:) indicate conservative substitutions. An overall identity of 36% and a similarity of 56% was found.

of the urease as an immunodominant protein both on the cellular and on the humoral level.

DISCUSSION

In this study we describe two cationic proteins of *Yersinia enterocolitica* that are immunodominant for the local T cell response in reactive arthritis: the 19 kD β-subunit of the urease and the 13 kD 50 S ribosomal protein L23. The identification of the latter as a member of the highly conserved group of ribosomal proteins (31,32) could explain not only why ReA can be induced by diverse bacteria but also how autoimmunity might result from homology between the bacterial and human proteins.

SFMC from 10 different arthritis patients giving a specific response to *Yersinia* antigen were used. Differential centrifugation of the disrupted bacterium yielded a membrane pellet, a cytoplasmatic protein fraction, and a ribosomal pellet. The latter induced the highest proliferative response and was therefore further fractionated by cation exchange chromatography. Among the products, the 19 kD urease β-subunit and the L23 protein were always dominant, although substantial variation among patients was observed. Most of the proteins in the ribosomal pellet did not bind to the strong cation exchanger and did not stimulate proliferation. The cytoplasmatic fraction and the membrane pellet also induced a proliferative response. This might be partly explained by the fact that traces of the urease β-subunit and the L23 protein were also found in the cytoplasmatic fraction and membrane pellet (data not shown).

The cellular response in ReA is primarily directed against the triggering pathogen (8–10), components of which are present in inflamed joints (4–6,33). Immunoblotting has identified *Yersinia* antigens ranging between 20 kD and 12 kD that are recognized by T cells (13). A 19 kD *Yersinia* urease β-subunit evokes experimental rat arthritis (14,28) and elicits antibodies in *Yersinia enteritis* patients. Lahesmaa et al. (34) found two T cell lines from one patient and one clone from another patient reactive with this antigen. Meanwhile, using a completely different approach, Probst et al. (35) also found a 19 kD protein target antigen in two *Yersinia* arthritis

TABLE 1. Peptides from urease β-subunit and L23 protein and binding affinity to HLA-B27

Position Within Protein	Sequence[a]	Binding Affinity[b]
U β-[21–29]	N **R** G T K S S A G	−
U β-[60–68]	V **R** N T G D R P I	+
U β-[65–73]	D **R** P I Q V G S H	−
U β-[80–88]	N **R** A L E F D R A	−
U β-[86–94]	D **R** A A A Y G K R	−
U β-[93–101]	K **R** L N I S S T T	+
U β-[103–111]	I **R** F E P G D E T	+
U β-[144–152]	E **R** P D K L E A I	−
U β-[152–160]	I **R** R A A E R G F	−
U β-[153–161]	R **R** A A E R G F K	++
L23-[2–10]	I **R** E E R L L K V	−
L23-[5–13]	E **R** L L K V L R A	−
L23-[11–19]	L **R** A P H V S E K	+
L23-[67–75]	K **R** H G Q R V G R	+
L23-[71–79]	Q **R** V G R R S D W	−
L23-[74–82]	G **R** R S D W K K A	−
L23-[75–83]	R **R** S D W K K A Y	+

[a]Single letter code is used; R at P2 as dominant anchor is given in bold and underlined.

[b]Relative binding affinities were determined as examplified in Fig. 4: (−) nonbinders; optical band density/mm² greater than ×2 the band density of untreated control. (+), low to intermediate binders; band density greater than ×2, greater than ×4 the untreated control. (++) strong binder; band density greater than ×4 the untreated control.

patients and in a subsequent study identified it as the urease β-subunit (36).

The response to the urease β-subunit may be of advantage to the host, since the urease seems to be important for pathogenicity. Urease activity may facilitate survival in host cells by raising the intracellular pH (37). A related mechanism was found in mouse immunity to *Listeria monocytogenes* (38), where the immune response is directed against listeriolysin as a virulence factor. However, the urease is not present in other ReA-triggering bacteria, such as urease-negative Salmonella or Shigella or Chlamydia, although it is found in Klebsiella, a candidate triggering organism in the related disease of ankylosing spondylitis (39).

A different situation is found with the second immunodominant antigen reported here. This protein with a size of nearly 13 kD is also cationic, requiring 0.65 M NaCl for elution from the cation exchanger. N-terminal amino acid sequence determination revealed strong homology to the 50 S ribosomal protein L23 of *E. coli* and Yersinia pseudotuberculosis. PCR amplification and direct sequencing of its gene confirmed this, showing a single amino acid substitution at position 13 relative to *Yersinia pseudotuberculosis* and only seven substitutions relative to the *E. coli* homolog. No homologous protein in the other ReA-triggering enterobacteria has yet been described, although such are likely to be found in the future. A systemic DNA database search revealed evolutionary conservation not only among other procaryotic but also among eucaryotic organisms. It is found in archaebacteria and even in plants, yeasts, rodents (60 S ribosomal protein L23a), and man (Fig. 3B).

The in vitro proliferation assay using SFMC does not reflect the complete response in vivo. Antigens able to stimulate $CD8^+$ T cells probably

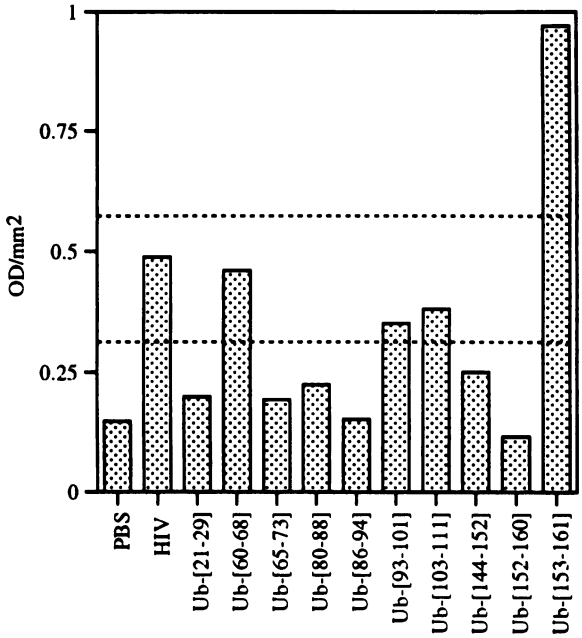

FIG. 4. Binding of the urease β-subunit (Uβ) derived peptides to HLA-B27, measured by densitometry of the [^{35}S]-methionine-labeled B27 heavy chain

Column 2 represents the relative binding affinity of the control peptide HIV gag 265–276, which has been defined as an intermediate binder (26). Dotted lines are drawn at optical densities/mm² × 2 and × 4 higher in the peptide treated lysates than in lysates treated with PBS instead of peptide.

TABLE 2. Sequence similarities between U β-[153-161] and other proteins

Sequence	Source
R R A A E R G F K	*Yersinia enterocolitica* urease β-subunit [153–161]
R R V R T R G F S	*Chlamydia trachomatis* 50 S ribosomal protein L15
H R A K E R G F H	*Helicobacter pylori* urease α-subunit
R R A L E R G V N	*Streptomyces albus* 60 kD Chaperonin 1 (GROEL homolog)
R R G L E R G L N	*Mycobacterium leprae* 60 kD Chaperonin
N R A T E R I F K	Human laminin A chain precursor
R R A L E R G L Q	Human complement C4 precursor
R R Q A E R L K P	Human leukocyte antigen related protein precursor
I R A L E R G Y R	Human HCK protein tyrosine kinase
S R A R E R G A S	Human protein lysine 6-oxidase precursor
R R T G E R K R K	Human 40 S ribosomal protein S6
R R Q A E R M S Q	Human CD4 molecule precursor
R R A Q E R R E L	Human retinal guanylyl cyclase precursor
R R H L E R G R V	Human epithelial-cadherin precursor
E R F G E R G F F	Human aldehyde dehydrogenase
G R K G E R G F V	chicken collagen alpha 3 (VI) chain

Results of a protein database search. Similar sequences from human proteins, bacterial and fungal proteins are shown. Each peptide bears the HLA-B27 binding motif at least at position two (arginine). Identical amino acids are shaded, conserved substitutions are underlined. A standard search in SWISS PROTEIN database was performed using the TFASTA program (24).

have escaped detection. *Yersinia* is a facultative intracellular bacterium (40,41) and should therefore present antigens also via MHC class I molecules to cytotoxic T cells (42). After all, the association with HLA-B27 implicates $CD8^+$ cytotoxic T cells and B27-restricted CTL have recently been demonstrated (43). However, linked class I and class II restricted epitopes produce the best $CD8^+$ response (44,45), so the test we used here could help the search for CTL epitopes.

Having identified the urease β-subunit and the L23 protein as immunodominant antigens, the amino acid sequences of both proteins were screened for the binding motif of the B27 molecule. All of the nonapeptides so identified were subsequently synthesized and tested for their affinity to B27. Four peptides from the urease β-subunit were scored as positive, including one strong binder, potentially able to serve as a $CD8^+$ T cell epitope (46,47). This nonapeptide has sim-

ilar sequences in various proteins from other sources, including the 60 kD chaperonin 1 (GROEL homologue 1) from *Mycobacterium leprae* and *Streptomyces albus* and ribosomal proteins from *Chlamydia trachomatis*. Striking homology is also found with human complement C4 precursor, leukocyte antigen-related protein precursor, CD4 precursor, and P59 tyrosine kinase. The homology applies to amino acid residues accessible to the T cell receptor (30). A minimal requirement for induction of autoimmunity via molecular mimicry has recently been established in a mouse model of autoimmune oophoritis (48); sharing of four amino acids within a nonapeptide including three in a row is sufficient for disease induction, a requirement that is fulfilled in the present case. Conserved epitopes are also thought to play an important role in the immune regulatory network in the so-called immunological homunculus (49), a concept that could be extended to include self-peptide antagonists (50). Elution studies (29,51) have indeed shown that nuclear and ribonuclear proteins together with heat shock proteins are major self-ligands of human class I molecules. We feel that these findings throw important light on possible mechanisms of autoimmunity in ReA.

The importance of conserved proteins in the pathogenesis of ReA is also underlined by the identification of a cationic 18 kD histone-like protein in *Chlamydia*-induced ReA (52,53).

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