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Yersinia enterocolitica Serotype O:3 Alters the Expression of Serologic HLA-B27 Epitopes on Human Monocytes

MAARIT WUORELA,* SIRPA JALKANEN, JUHA KIRVESKARI, PÄIVI LAITIO, AND KAISA GRANFORS

Department in Turku, National Public Health Institute, and Department of Medical Microbiology, University of Turku, Turku, Finland

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The expression of serologic HLA-B27 epitopes on leukocytes of patients with reactive arthritis or ankylosing spondylitis has been shown to be modified in the course of the disease. The purpose of this work was to study whether phagocytosis of arthritis-triggering microbes in vitro alters the expression of HLA-B27 molecules on human antigen-presenting cells and to characterize the underlying mechanisms. Human monocytes and HLA-B27- or HLA-A2-transfected human U-937 cells were exposed to *Yersinia enterocolitica* serotype O:3. The expression of different epitopes of HLA-B27 was monitored by using immunofluorescence, and their synthesis was determined by quantitative immunoprecipitation. Our results show that phagocytosis of *Y. enterocolitica* serotype O:3 changed the expression of serological HLA-B27 epitopes. This was due to the reduced synthesis of HLA-B27 molecules. The expression of especially the epitopes which depend on the presence of peptides in the antigen-binding groove was changed. The expression of the ME1 epitope, which has been shown to be important for T-cell recognition in patients with reactive arthritis, was decreased. Down-regulation of epitopes important for the T-cell recognition may impair the elimination of arthritis-triggering microbes and lead to persistent infection. In addition, *Y. enterocolitica* serotype O:3 seemed to alter the repertoire of peptides presented by the HLA-B27 molecules on human monocytes. This may have a role in the pathogenesis of reactive arthritis via an autoimmune mechanism.

Reactive arthritis is often self-limiting polyarthritis, but in certain patients the disease develops into a chronic inflammatory arthritis or sacroiliitis (24). The association of reactive arthritis with HLA-B27 suggests that the pathogenetic mechanisms underlying this disease may be similar to those of other HLA-B27-associated spondylarthropathies (1). The basis for HLA-B27 association in seronegative spondylarthropathies is not clear.

Microbes that trigger reactive arthritis are facultative or obligate intracellular pathogens, and thus the major histocompatibility complex (MHC) class I-mediated pathway of antigen presentation is probably significant in clearing the arthritis-triggering primary infections and in initiating the arthritic process (15). Persistence of microbial structures has been suggested to be important for the development of arthritis (10). CD8-positive and B27-restricted *Yersinia*-specific, *Salmonella*-specific, or autoreactive cytotoxic lymphocytes have been cloned from the synovial fluid of patients with *Yersinia*-triggered and *Salmonella*-triggered reactive arthritis (15). This emphasizes the role of T cells in the development of the disease. Microbes associated with the development of seronegative spondylarthropathies may also directly interact with HLA-B27 (8, 19, 48).

Earlier studies have shown that certain HLA-B27 epitopes are modified in patients with reactive arthritis and ankylosing spondylitis (2, 20, 29). In the present study, we wanted to characterize the mechanisms behind this modification. Altered expression of epitopes that are critical for T-cell recognition on antigen-presenting cells may have a role in the pathogenesis of HLA-B27-associated diseases via modified antigen presentation. Down-regulation of epitopes important for antigen presentation may also favor the survival of arthritis-triggering microbes in the body.

* Corresponding author. Mailing address: National Public Health Institute, Department in Turku, Kiinamyllynkatu 13, FIN-20520 Turku, Finland. Phone: 358-21-2519255. Fax: 358-21-2519254. E-mail: maarit.wuorela@utu.fi.

MATERIALS AND METHODS

Bacteria. The strain of *Yersinia enterocolitica* serotype O:3 used (4147/83) was a stool isolate from a patient developing reactive arthritis as a result of infection. The strain contains a virulence-associated 72-kb plasmid. Other arthritis-triggering bacteria used in this study were *Y. enterocolitica* serotype O:9 (strain EF-8239 from Culture Collection of University of Gothenburg [CCUG], Gothenburg, Sweden), *Yersinia pseudotuberculosis* serotype I (strain EF-5885 from CCUG), *Y. pseudotuberculosis* serotype III (strain EF-244 from CCUG), and *Salmonella enteritidis* (strain 8822/88, isolated from a patient who developed reactive arthritis after gastrointestinal infection). In addition, a plasmid-cured derivative of *Y. enterocolitica* serotype O:3 obtained after the bacteria were cultured on magnesium-oxalate agar at 37°C was used (9). The presence or absence of the virulence plasmid of *Yersinia* bacteria was verified by autoagglutination (23). As control bacteria, we used *Streptococcus pyogenes* (strain 8184, American Type Culture Collection [ATCC], Rockville, Md.) and enteroinvasive *Escherichia coli* (strain RHE-3459; Central Public Health Laboratory, Colindale, London, United Kingdom). *S. pyogenes* was chosen as a control bacterium because it can cause postinfectious joint complications (rheumatic fever) which are not linked to HLA-B27. Also, *S. pyogenes* does not contain lipopolysaccharide. *E. coli* has lipopolysaccharide but does not induce reactive arthritis. Stock cultures were maintained at −40°C in 20% (vol/vol) glycerol-Trypticase soy broth. The *Yersinia* bacteria were grown in RPMI 1640 medium mimicking the extracellular conditions of the body or in Luria-Bertani broth (LB). The resulting bacterial cultures were suspended in saline, harvested by centrifugation, and washed three times in saline. The bacteria were killed with heat (1 h, 100°C) and stored in phosphate-buffered saline (PBS) at 4°C. *E. coli* was grown in LB and killed with heat like *Y. enterocolitica* serotype O:3. *S. pyogenes* was grown on blood agar plates for 2 days. The resulting bacteria were harvested, killed with heat, and suspended in saline. *S. enteritidis* was grown in LB, washed once with NaCl, and stored in glycerol-Trypticase soy broth at −40°C. Live bacteria were washed twice with PBS before use.

Peptide extract. *Y. enterocolitica* serotype O:3 was grown, killed with heat, and suspended in saline as described above. The suspension was sonicated with a Soniprep 150 Ultrasonic Disintegrator (MSE Scientific Instruments, Sussex, England) for 60 s and treated thereafter with proteinase K (100 μg/ml) for 2 h at 37°C and then for 2 h at room temperature (Boehringer GmbH, Mannheim, Germany) and DNase, grade II (100 μg/ml) (Boehringer). The sonicate was centrifuged at 13,000 × *g* for 10 min, and the peptides were collected. The peptide extract was diluted in saline (1 mg/ml) and boiled at 100°C for 5 min to inactivate proteinase K.

Monocyte isolation. Monocytes from healthy blood donors (Finnish Red Cross, Turku, Finland) or healthy laboratory personnel were isolated as described elsewhere (52). Briefly, human peripheral blood mononuclear cells were isolated by Ficoll-Paque gradient centrifugation (Pharmacia LKB Biotechnology AB, Uppsala, Sweden), and monocytes were allowed to adhere to plastic tissue culture chambers for 1 h. Thereafter, nonadherent cells were washed off. The purity of monocyte populations was ≥85% as analyzed by using morphological characteristics and in some samples also by using immunofluorescence staining of the monocyte-specific CD14. In addition, monocytes of three patients with ankylosing spondylitis fulfilling the New York criteria (the international diagnostic criteria for ankylosing spondylitis) (two males, one female) and of three individuals with a history of reactive arthritis (one male, two females) were isolated.

HLA-B27 and HLA-A2 transfectants. Human genomic HLA-B27 DNA (6-kb fragment) in pUC19 vector (43) and human genomic HLA-A2 DNA (5.1-kb fragment) in pUC9 vector (21), both gifts from J. D. Taurog, Dallas, Tex., were used to transfect human histiocytic cell line U-937 (40) from ATCC by electroporation. The HLA type of U-937 cells is A3, W19; B5, 18; CW1, W3 (40). Stable transfectants were generated by continuous selection with geneticin (Sigma, St. Louis, Mo.) (500 μg/ml). The expression of HLA-B27 or HLA-A2 was confirmed by using monoclonal antibodies (MAbs) ME1 (4) and BB7.2, respectively (32, 37, 38). Two days before the experiments, the transfectants were removed from the selection medium and the cells were maintained in culture medium (RPMI 1640 medium with gentamicin [G-mycin; Orion, Espoo, Finland], supplemented with L-glutamine and 10% fetal calf serum). Maturation of the cell line was induced by using phorbol 12-myristate 13-acetate (PMA) (Sigma). The cells were incubated in medium with 10 ng of PMA per ml for 30 min, washed twice with Hanks' balanced salt solution (HBSS), and incubated in culture medium at 37°C overnight.

Incubation with bacteria and peptide extract. Monocytes and transfected U-937 cells were allowed to phagocytose the bacteria in RPMI 1640 medium supplemented with 10% AB serum without antibiotics for 1 h. Then the plates were washed three times with HBSS without antibiotics to wash away the bacteria which were not phagocytosed by the cells. Thereafter, the cells were allowed to process the bacteria in culture medium for different periods as described previously (52) (Table 1). Usually the incubation times for monocytes of one individual were 1 h, 1 or 2 days, and 7 days, but several other incubation times between these were also tested. Control monocytes were incubated in the same way but without any contact with bacteria. Peptide extract (10 μg/ml) was incubated with the monocytes, like the bacteria. Then the cells were detached by using 5 mM EDTA in Ca^{2+}-Mg^{2+}-free HBSS and scraping with a rubber policeman.

Incubation of monocytes with cycloheximide, brefeldin A, or chloroquine. Monocytes were exposed to bacteria in medium containing 10 μg of cycloheximide {3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide; Sigma} per ml. The incubation was continued for an additional 1 h, 4 h, or 1 day after feeding in the same medium. Thereafter, the cells were detached and stained with MAbs against HLA-B27. For brefeldin A experiments, monocytes were isolated and incubated in RPMI 1640 medium with 10% AB serum and brefeldin A (Sigma) (10 μg/ml) for 1 h. Then the monocytes were exposed to the bacteria or treated with the peptide extract and incubated for 1 h after washes in the continuous presence of brefeldin A (3.3 μg/ml). To block the activity of the lysosomal compartment of the cells, monocytes were incubated in RPMI 1640 medium with 10% AB serum and chloroquine (Sigma) (100 μM) for 1 h. Then the monocytes were exposed to the bacteria and incubated in the presence of chloroquine (100 μM) for 1 h.

MAbs, immunofluorescence staining, and flow cytometry. Monocytes were stained by a double-immunofluorescence technique as described previously (52). MAbs and their specificities are listed in Table 2. Cells which appeared to be weakly positive in flow cytometry analysis after staining with MAb HLA-ABC-m3 were not HLA-B27 positive but had some of the HLA-B27-cross-reacting epitopes, usually HLA-B7 (Table 2). ME1 is a conformational epitope, and amino acid substitutions at positions 67 or 69 to 71 disrupt the binding of ME1 to HLA-B27 (5, 6, 27, 42). The B27M2 epitope is not disrupted by a Tyr67 mutation like the ME1 epitope (5). It has been shown previously that the reactivity of B27M1 and B27M2 is partly dependent on the peptide in the HLA-B27 complex (17, 49). The reactivity of ME1 is enhanced when empty HLA-B27 molecules are incubated with peptides, but ME1 can also react slightly with empty HLA-B27 molecules alone (49, 50). MAb FD705 is specific for HLA-B27 and does not show any cross-reactivities (33). The HLA-B7-specific MAb BB7.1 seems to bind to the HLA-B7 molecule from the top across the peptide-binding groove, or it may recognize an HLA α_2 configuration that depends on the α_1 α-helix or the bound peptide (26).

Analyses were performed with a FACScan flow cytometer (Becton Dickinson & Co., Mountain View, Calif.). Monocytes were gated according to their size and granularity. This correlated well with their expression of monocyte-specific CD14. Routinely, 10,000 cells were analyzed per sample. A 5% or greater change in the positive cell population or a shift of 100 U in the mean fluorescence intensity was considered to be significant. The expression of tissue antigens on

TABLE 1. Experimental design[a]

Antigen	No. of subjects studied whose monocytes were exposed to the following bacteria:									
	Y. enterocolitica serotype O:3	Live/killed *Y. enterocolitica* serotype O:3[b]	*E. coli*	*S. pyogenes*	Live *S. enteritidis*	Plasmid-cured *Y. enterocolitica* serotype O:3	*Y. enterocolitica* serotype O:9	*Y. pseudotuberculosis* serotype I	*Y. pseudotuberculosis* serotype III	Peptide extract (*Y. enterocolitica* serotype O:3)
HLA-B27	31	5	19	12	7	5	5	2	2	12
HLA-B7	24	3	15	3	7	6	6	4	4	9
HLA-A2	30		18	10						

[a] Heat-killed bacteria were used unless indicated otherwise. Monocytes from one individual were usually divided, and some of the cells were exposed to *Y. enterocolitica* serotype O:3, some were left as nonstimulated control monocytes, and other were exposed to some of the control bacteria or peptide extract of *Y. enterocolitica* serotype O:3.

[b] Monocytes from individuals were divided, and a portion of the cells was exposed to live *Y. enterocolitica* serotype O:3 organisms and another portion was exposed to heat-killed organisms.

TABLE 2. Antibodies used in this study

Designation	Class	Specificity	Amino acid residue(s) critical for binding[a]	Reference(s) or source
HLA-ABC-m3	IgG2a	HLA-B27, HLA-B7		45; Serotec[b]
ME1	IgG1	HLA-B27, HLA-B7, HLA-Bw22, HLA-Bw42	67–71, 41, 43	4, 27, 30; ATCC
B27M1	IgG2a	HLA-B27, HLA-B7, HLA-Bw42	67–71, 41, 43, 32	11, 27; ATCC
B27M2	IgM	HLA-B27, HLA-Bw47	77–81	12; ATCC
FD705	IgG2b	HLA-B27		33; One Lambda INC[c]
BB7.1	IgG1	HLA-B7, HLA-Bw42	67, 166, 169	5, 26, 30, 44; ATCC
BB7.2	IgG2b	HLA-A2, HLA-Aw69	107, 161	32, 37, 38; ATCC
MA2.2		HLA-A2, HLA-Aw69	107	38; Incstar Corp.[d]
W6/32	IgG2a	Monomorphic class I HLA		31; ATCC
4D12	IgG1	HLA-B5		14; ATCC
GAP A3	IgG2a	HLA-A3		3; ATCC
3C10	IgG2b	CD14		ATCC
3G6	IgG1	Chicken T cells (negative control)		Our laboratory
1B2	IgG1	Vascular adhesion protein 1 (negative control)		36; our laboratory
10D3	IgM	Chicken lymphocytes (negative control)		Our laboratory

[a] Amino acid residues 67 to 71 and 77 to 81 are in the α_1 α-helix, and residues 166 to 169 are in the α_2 α-helix. Residues 41 and 43 belong to the solvent-accessible connecting loop, and amino acid residue 32 is at the bottom of the peptide-binding groove.
[b] Oxford, United Kingdom.
[c] Canoga Park, Calif.
[d] Stillwater, Minn.

monocytes exposed to the bacteria was always compared to that on monocytes which were incubated in the same way but without any exogenous stimuli.

Metabolic labelling and quantitative immunoprecipitation. HLA-B27- and HLA-A2-transfected U-937 cells were stimulated, and the adherent population was exposed to the bacteria or incubated without any stimulus (controls). Then the cells were washed three times with PBS and incubated in methionine-cysteine-free Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% dialyzed fetal calf serum, 4 mM L-glutamine, and 10 mM HEPES for 30 min. The U-937 cells were labelled with [^{35}S]methionine/[^{35}S]cysteine (Translabel; ICN Pharmaceuticals Inc., Irvine, Calif.) (0.5 mCi/30 × 10^6 cells) for 3 h. After being labelled, the cells were detached with a rubber policeman and washed three times with HBSS. Then the cells were lysed with lysis buffer (0.1 M NaCl, 0.01 M sodium phosphate, 0.5% deoxycholate, 1% Nonidet P-40, and 1 mM phenylmethylsulfonyl fluoride [pH 7.4]), and the lysate was cleared by centrifugation at 10,000 × *g* for 15 min. The lysate was precleared three times with protein A-Sepharose 4B beads (Pharmacia LKB) conjugated with rabbit anti-mouse immunoglobulin (DAKO Immunoglobulins, Copenhagen, Denmark). For quantitative immunoprecipitation, the activity of the lysates was determined with a 1217 Rackbeta liquid scintillation counter (Wallac LKB, Turku, Finland), and aliquots with exactly the same activity were subjected to immunoprecipitation. Immunoprecipitation was carried out with protein A-Sepharose 4B beads conjugated with MAbs ME1 for HLA-B27, MAb BB7.2 for HLA-A2, or the control antibody 1B2 (36). The beads were washed six times in lysis buffer, the radiolabelled antigens were eluted by boiling in 30 μl of Laemmli's sample buffer under mild reducing conditions (0.1% β-mercaptoethanol) (22), and the immunoprecipitate was analyzed by vertical sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a stacking gel of 5% acrylamide and a resolving gradient gel of 5 to 12.5% acrylamide. Standards of known molecular weight were included in each gel run (Rainbow protein molecular weight markers; Amersham, Buckinghamshire, England). After electrophoresis, the gels were fixed, soaked in Enlightening (DuPont, Boston, Mass.) for 30 min, dried, and subjected to autoradiography at −70°C. Two independent immunoprecipitation experiments were done. Autoradiographs of the gels were scanned on an Image analyzer (Microcomputer Imaging Device; Imaging Research Inc., St. Catharine's, Ontario, Canada) allowing assessment of band intensity by optical density.

Statistical analysis. Statistically significant differences between the groups were determined by Fisher's exact test. The differences between the mean fluorescence intensities of monocytes exposed to the bacteria and control monocytes were determined by paired *t* test (41).

RESULTS

Arthritis-triggering bacteria and expression of MHC class I molecules. We found that *Y. enterocolitica* serotype O:3 was able to reduce the expression of all HLA-B27 epitopes except B27M2 on human monocytes and on the HLA-B27-transfected U-937 cells. Both the percentage of positive monocytes and the mean fluorescence intensities decreased. The initial level of expression of B27M2 on monocytic cells is already very low. Examples of the decreases of expression are shown in Fig. 1, and an example of the expression of the B27M2 epitope is shown in Fig. 2. The decrease occurred without a concomitant change in the overall expression of MHC class I complexes in monocytes from 21 of 31 individuals which were studied after short incubation periods (from 1 h to 1 day) (Table 3). The effect of *Y. enterocolitica* serotype O:3 on the ME1 epitope was observed only if the epitope was in a certain background (HLA-B27) because the expression of HLA-B27-cross-reacting epitopes on HLA-B7-positive monocytes was not reduced. The expression of the peptide-dependent B27M2 epitope was increased, especially after 1 day of incubation (Fig. 2). The most significant changes were observed in patients with ankylosing spondylitis or a history of reactive arthritis. Certain individuals were tested up to 10 times and no variation between experiments was observed. *Y. enterocolitica* serotype O:9, *Y. pseudotuberculosis* serotypes I and III, a plasmid-cured derivative of *Y. enterocolitica* serotype O:3, and *S. enteritidis* decreased the expression of ME1, B27M1, and HLA-ABC-m3 epitopes, like the virulent *Y. enterocolitica* serotype O:3. When monocytes of three individuals were exposed to both live and heat-killed *Yersinia* bacteria in three separate experiments, live bacteria induced more-significant changes than the heat-killed bacteria. Live *S. enteritidis* also had a more significant effect than the heat-killed *Salmonella* bacteria. An example of the change induced by live *S. enteritidis* using MAb FD705 is shown in Fig. 3.

Cycloheximide, brefeldin A, and chloroquine were used to characterize the mechanisms behind the changes in cell surface expression. Cycloheximede is a protein synthesis inhibitor; brefeldin A blocks the traffic of newly synthesized molecules from the endoplasmic reticulum through the Golgi complex to the cell surface and leads to mixing of the trans-Golgi network with the recycling endosomal system; and chloroquine inhibits lysosomal degradation and the recycling of molecules from the plasma membrane through acidic endosomes. Cycloheximide did not modify the effect induced by *Y. enterocolitica* serotype O:3, although the basal level of the MHC class I expression reduced gradually after the monocytes were treated with cycloheximide (not shown). Brefeldin A abrogated the effect of *Y. enterocolitica* serotype O:3 on the ME1 and FD705 epitopes

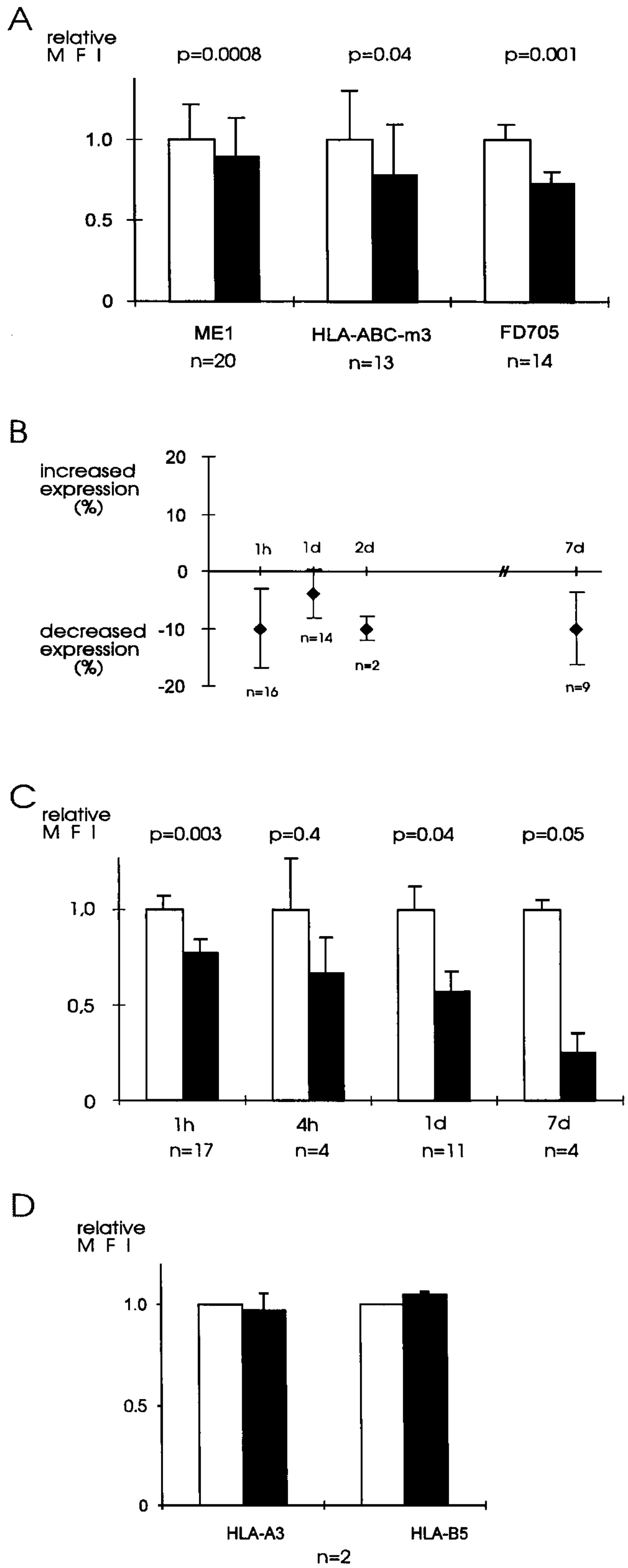

FIG. 1. (A) *Y. enterocolitica* serotype O:3 down-regulates the mean fluorescence intensity (MFI) of HLA-B27 on monocytes exposed to *Y. enterocolitica*. The MFI of control monocytes stained with MAbs against different HLA-B27 epitopes is 1. Results are presented as relative MFI ratios. The incubation time is 1 h, and the numbers of individuals studied are indicated. Results for all subjects studied with these MAbs are included in the figure regardless of the fact that in most of the individuals studied there was a decrease and in certain individuals there was no change in expression level. Values are means ± standard errors of the means. The relative MFI values for control monocytes stained with the indicated MAbs (open bars) and for monocytes exposed to the bacteria (closed bars) are shown. (B) *Y. enterocolitica* serotype O:3 decreases the percentage of HLA-B27-positive monocytes (experimental values were subtracted from the control value so that the zero line represents the level of positive monocytes of the controls; values are means ± standard deviations). Results for monocytes of all individuals studied with MAb HLA-ABC-m3 have been included in the figure regardless of the fact that there was a decrease in the expression of HLA-B27 in most subjects and no change in the level of expression in certain individuals. The incubation times and numbers of individuals studied are indicated. d, day. (C) *Y. enterocolitica* serotype O:3 down-regulates the MFI of the HLA-B27M1 epitope on monocytes exposed to *Y. enterocolitica*. The MFI of control monocytes stained with MAb B27M1 is 1. Results are presented as relative MFI ratios. The incubation times and numbers of individuals studied are indicated. The values after 4 h of incubation do not reach statistical significance because of the small number of individuals studied. Results for all subjects studied with this antibody are included in the figure regardless of the fact that in most of the individuals studied there was a decrease and in certain individuals there was no change in the level of expression. Results are means ± standard errors of the means. Relative MFI values for control monocytes stained with the indicated MAbs (open bars) and for monocytes exposed to the bacteria (closed bars) are shown. (D) *S. enteritidis* does not down-regulate the expression of HLA-B5 and HLA-A3 epitopes on HLA-B27-transfected U-937 cells. The MFI of control monocytes stained with MAbs against different HLA-B27 epitopes is 1. Results are presented as relative MFI ratios (means ± standard deviations). The incubation time is 1 h, and the results are from two independent experiments. Relative MFI values for control monocytes stained with the indicated MAbs (open bars) and for monocytes exposed to the bacteria (closed bars) are shown.

on monocytes, and chloroquine abolished the effect of *Y. enterocolitica* serotype O:3 on the B27M2 epitope (not shown). These results suggest that the *Y. enterocolitica* effect needs normal intracellular traffic to occur. Monocytes were exposed to peptide extract of *Y. enterocolitica* serotype O:3 to rule out the possibility that free peptides in the solution would attach to MHC molecules and thus change the HLA-B27 epitopes. The peptide extract did not modify the expression of HLA-B27 (not shown).

Control bacteria and expression of MHC class I molecules. Monocytes were exposed to *S. pyogenes* and *E. coli* to analyze whether the effects of *Y. enterocolitica* serotype O:3 were specific to reactive-arthritis-triggering microbes. *S. pyogenes* reduced specifically the expression of ME1 or HLA-ABC-m3 epitopes in only 2 of 14 individuals during the first day of incubation (Table 3), but it did not considerably change the overall expression of the peptide-dependent B27M1 and B27M2 epitopes (not shown). The expression of HLA-B27 epitopes on monocytes exposed to *S. pyogenes* also stayed at the level of control cells during the 1-week follow-up time after a small initial fall in a few individuals. *E. coli* did not decrease the expression of HLA-B27 epitopes as often as the arthritis-triggering bacteria (Table 3). The expression of HLA-A2 was not decreased specifically after an incubation with *S. pyogenes* or *E. coli* (Table 3; Fig. 4).

Synthesis of HLA molecules. In metabolic labelling experiments, the synthesis of HLA-B27 decreased 26% after the cells were fed with *Y. enterocolitica* serotype O:3, while the synthesis of HLA-A2 increased up to threefold compared to that of the control cells (Fig. 5A). During the same 3-h incubation period, the expression of HLA-B27 on the surface of U-937 cells exposed to *Y. enterocolitica* decreased (mean fluorescence intensity on a linear scale decreased from 48 to 23). Despite the increase in HLA-A2 synthesis, no significant increase in the expression of HLA-A2 was seen (mean fluorescence intensity changed from 30 to 28) (Fig. 5B).

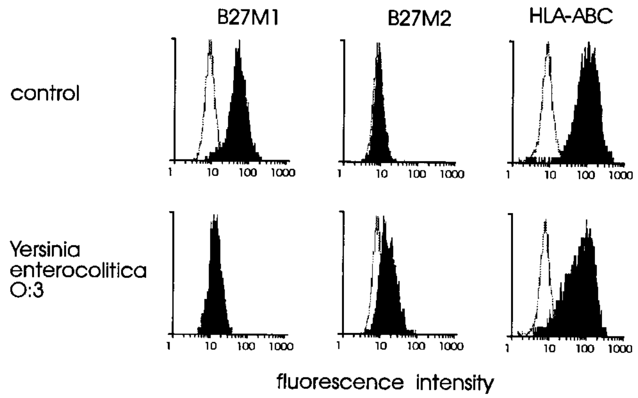

FIG. 2. *Y. enterocolitica* serotype O:3 down-regulates the expression of B27M1 and increases the expression of B27M2 on HLA-B27-transfected U-937 cells after 1 day of incubation. *x* axis, fluorescence intensity on a log scale; *y* axis, relative number of cells. The cells have been exposed to heat-killed *Y. enterocolitica* serotype O:3 or incubated without bacteria (control) and stained with MAbs against HLA-B27 or a monomorphic human MHC class I epitope (black histograms) or with the negative-control MAb (white histograms).

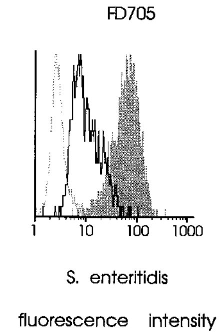

FIG. 3. Live *S. enteritidis* organisms reduce the expression of HLA-B27. *x* axis, fluorescence intensity on a log scale; *y* axis, relative number of cells. Monocytes have been exposed to bacteria (black-outlined lined histogram) or incubated without bacteria (black-filled histogram) and stained with MAb FD705. Results for monocytes exposed to bacteria stained with the negative-control MAb are also shown (white histogram). The incubation time is 1 h.

DISCUSSION

In the present study, we investigated whether phagocytosis and processing of reactive-arthritis-triggering bacteria in vitro would change the expression of HLA-B27 molecules on human antigen-presenting cells. We found that arthritis-triggering microbes decreased the expression of epitopes which have been shown to be important for T-cell recognition in reactive arthritis (15, 46). Arthritis-triggering microbes also changed the three-dimensional structure consisting of the HLA-B27 molecule and the bound peptide which is recognized by antibodies or cytotoxic T cells. Interestingly, the changes in expression of HLA-B27 were most significant in patients with ankylosing spondylitis and in individuals with a previous history of reactive arthritis (unpublished data). This suggests that this phenomenon has a role in the pathogenesis of reactive arthritis.

The effects of bacteria were found to be specific for certain epitopes if the epitopes were in a particular background. The explanation for this may be the HLA-B27-specific cysteine at position 67. This residue forms part of the peptide-binding B-pocket in HLA-B27 which binds the second amino acid residue relative to the amino terminus of the peptide that associates with the MHC class I molecule (13, 25). Cysteine 67 in HLA-B27 has been shown to carry an unusually reactive thiol group (51). This group may be modified by low-molecular-weight compounds such as nitric oxide and superoxide ions, which are produced by activated phagocytes. Such a modification most probably alters the specificity of the cleft, so that wrong self-peptides will be presented to T cells (51). In addition, the cysteine at position 67 has been shown to be oxidized by homocysteine originating from arthritis-triggering bacteria. This modification leads to a modified T-cell response (7).

Metabolic labelling and immunoprecipitation experiments showed that the regulation of the synthesis of HLA-B27 and HLA-A2 was different after phagocytosis of *Y. enterocolitica* serotype O:3. This is likely to be responsible for the down-regulation of HLA-B27 expression seen in our experiments after prolonged incubation. The expression of HLA-A2 did not increase, although the synthesis of HLA-A2 was greatly enhanced after phagocytosis of *Y. enterocolitica* serotype O:3. These results suggest that in addition to inducing changes in the synthesis of HLA molecules, phagocytosis of *Y. enterocolitica* serotype O:3 modifies the posttranslational modification of MHC class I molecules or the traffic to the cell surface so

TABLE 3. Down-regulation of HLA epitopes on human peripheral blood monocytes

Bacterium	Result for the indicated epitope[a]		
	HLA-B27	HLA-A2	HLA-B7
Y. enterocolitica	21/31[b]	11/30	4/13
S. pyogenes	2/14	3/10	
E. coli	8/19	5/17	4/13

[a] Values are numbers of individuals in whom the expression of the given MHC class I allele decreased without a concomitant change in the overall expression of MHC class I molecules after 1 h to 1 day of incubation divided by the total number of individuals studied. HLA-B27 epitopes are the ones recognized by MAbs ME1, B27M1, HLA-ABC-m3, and FD705; the HLA-B7 epitope is recognized by MAb BB7.1 (HLA-B7-specific epitope); and HLA-A2 epitopes are recognized by MAbs BB7.2 and MA2.2.

[b] P is <0.05 when the specific decrease in the expression of HLA-B27 induced by *Y. enterocolitica* serotype O:3 is compared to that of control alleles or *S. pyogenes*. P is <0.08 when the specific decrease of HLA-B27 caused by *Y. enterocolitica* serotype O:3 is compared to the specific decrease induced by *E. coli.*

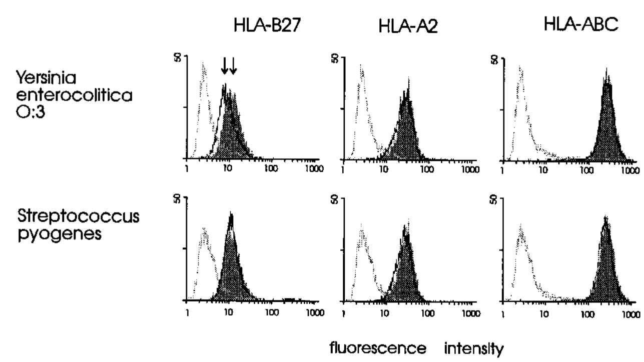

FIG. 4. *Y. enterocolitica* serotype O:3 down-regulates the expression of the ME1 epitope. The individual whose results are shown was both HLA-B27 and HLA-A2 positive, and although the decrease of HLA-B27 was not very significant, the expression of HLA-A2 or MHC class I molecules did not change at all. *x* axis, fluorescence intensity on a log scale; *y* axis, relative number of cells. Monocytes have been fed with bacteria (*Y. enterocolitica* O:3 or *S. pyogenes* [black-outlined histograms]) or incubated without bacteria (black-filled histograms). The cells have been stained with MAb against HLA-B27 (ME1) or HLA-A2 (BB7.2) or against a monomorphic epitope of MHC class I heavy chain (HLA-ABC). Results for bacterium-fed monocytes stained with the negative control MAb are also shown (white histograms). The incubation time is 1 h.

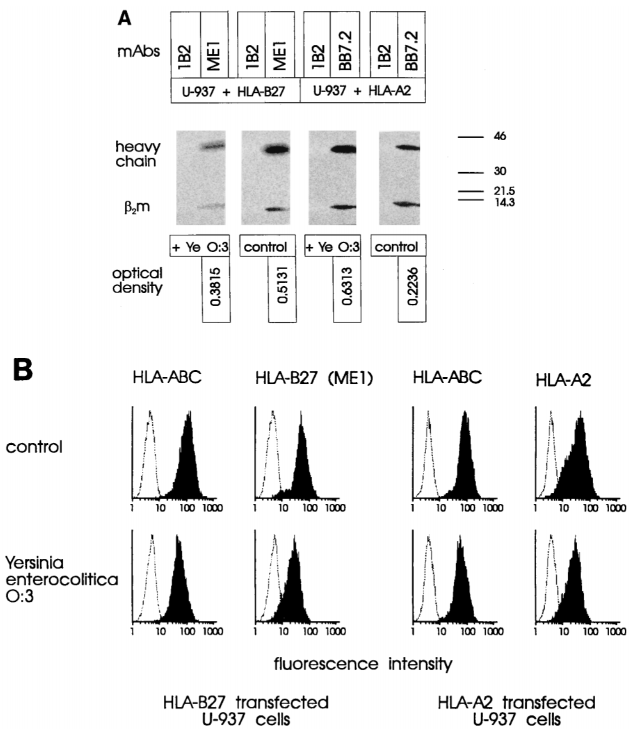

FIG. 5. (A) Phagocytosis and processing of *Y. enterocolitica* serotype O:3 decrease the synthesis of HLA-B27 in HLA-B27-transfected U-937 cells but increase the synthesis of HLA-A2 in HLA-A2-transfected U-937 cells. The optical densities of the heavy chain bands are indicated. Aliquots with exactly the same activity were subjected to immunoprecipitation. Molecular masses (in kilodaltons) are shown on the right. (B) Expression of HLA-B27 decreased, together with the synthesis. The expression of HLA-A2 was not increased despite the threefold increase in protein synthesis. *x* axis, fluorescence intensity on a log scale; *y* axis, relative number of cells. Transfected U-937 cells have been exposed to *Y. enterocolitica* serotype O:3 or incubated without any stimulus and stained with MAbs ME1 and BB7.2 (black histograms) or with the negative-control MAb (white histograms). The incubation time is 3 h.

that the newly synthesized MHC molecules do not reach the plasma membrane. Certain DNA viruses have been shown to prevent the expression of MHC class I molecules via mechanisms interfering with posttranslational processing of these glycoproteins as early as 2 h after infection (16). The effect of glycosylation on the expression of MHC class I molecules has been shown to be allele specific (28). In addition, bacteria have been shown to modify the glycosylation of the host cell proteins (47). Experiments conducted in the presence of brefeldin A suggest that *Yersinia* bacteria may interfere also with the intracellular traffic of HLA-B27 molecules. Brefeldin A, which blocks the transport of molecules from the endoplasmic reticulum through the Golgi complex to the cell surface, reduced slightly the effect of *Y. enterocolitica* serotype O:3 on all epitopes studied.

Brefeldin A also leads to mixing of the trans-Golgi network with the recycling endosomal system, impairing the traffic between endosomes and lysosomes. MHC class I antigens are continuously endocytosed via coated pits and recycled back to the plasma membrane through acidic endosomes (34). The acidic pH has recently been shown to lead to considerable exchange of peptides in MHC class I molecules (39). The MHC class I recycling is inhibited by chloroquine, as was the effect of *Y. enterocolitica* serotype O:3 on the B27M2 epitope (34). The recyling half-life of MHC class I molecules on the cell surface has been shown to be only 41 min (34). The rapid onset of the modification and the sensitivity induced by *Y. enterocolitica* serotype O:3 to brefeldin A and chloroquine suggest that *Y. enterocolitica* serotype O:3 may affect the recycling process. Peptides responsible for the B27M2 reactivity may be especially susceptible to the low pH in the acidic organelles.

Phagocytosis and processing of *Y. enterocolitica* serotype O:3 seem to increase the availability or binding affinity of peptides which are important for the creation of the serological B27M2 epitope and decrease the binding of peptides needed for B27M1 epitope. Arthritis-triggering bacteria have been shown to change the peptide repertoire presented by transfected cells (35). Our studies of the effect of peptide extract of *Y. enterocolitica* serotype O:3 on the expression of HLA-B27 ruled out the possibility that the changes of expression would be due merely to the attachment of peptides to the HLA molecules on the cell surface (18).

Our results show that reactive-arthritis-triggering bacteria can alter the expression of certain HLA-B27 epitopes on human antigen-presenting cells. Down-regulation of epitopes shown to be important for T-cell recognition in patients with reactive arthritis may impair the elimination of arthritis-triggering microbes. An altered repertoire of peptides in the HLA-B27 molecules may lead to the pathogenesis of reactive arthritis via an autoimmune mechanism.

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