SHORT ARTICLE

Influence of infection of cells with bacteria associated with reactive arthritis on the peptide repertoire presented by HLA-B27

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Reactive arthritis (ReA) after infections with various gram-negative bacteria is strongly associated with the MHC class I molecule HLA-B27. It is supposed that the B27 molecule itself plays a role in the pathogenesis of ReA by presenting antigenic peptides to cytotoxic T lymphocytes. The peptide repertoires presented by *Salmonella*-, *Shigella*- and non-infected cells were compared to identify such peptides. From the peptides isolated from the B27 molecules of these cells, profiles were generated by reversed-phase chromatography and peaks present in the profiles from infected cells but not in profiles from non-infected cells were studied for their peptide compositions. Some sequences with identity to those in human histone H3, human ribosomal protein S17 and the heavy chain of HLA-B27 itself were detected only in profiles from infected cells. All peptides identified from infected cells contained the B*2705 peptide-binding motif. The data suggest that HLA-B27-positive cells infected with ReA-inducing bacteria show an increased presentation of certain self-peptides. There was no evidence for altered peptide-binding specificity of B27 after infection. However, the interpretations were hampered by the variation in peptide presentation between different experiments.

Introduction

Reactive arthritis (ReA) occurring after gastrointestinal infection with *Salmonella*, *Shigella*, *Campylobacter* or *Yersinia* spp., or after urogenital infection with *Chlamydia trachomatis*, is strongly associated with HLA-B27. Therefore, it is supposed that the B27 molecule itself plays a role in the pathogenesis of this disease [1]. HLA-B27, like other classical MHC class I molecules, presents antigenic peptides to cytotoxic T-lymphocytes (CTL) or natural killer cells. Originally it was thought that MHC class I molecules present only autologous and viral peptides mainly of endogenous origin. However, it is now clear that intracellular bacteria and even extracellular antigens can also be processed for presentation by MHC class I molecules [2]. A general feature of the bacteria inducing ReA is

that they are able to enter non-professional phagocytic cells, in which they remain viable [3–5].

The ‘arthritogenic peptide’ hypothesis of ReA supposes that B27 molecules present peptides derived from ReA-inducing bacteria to CTL, which cross-react with self peptides presented by HLA-B27 in the joints [6]. Little is known about which bacteria-derived peptides are presented by MHC class I molecules or about the effect of the presence of intracellular bacteria on the peptide repertoire presented by MHC class I molecules. Recently, major differences were observed between HPLC profiles from B27-presented peptides isolated from non-infected mouse fibroblasts and cells infected with *Shigella flexneri* [7]. Two sequences were obtained showing partial homology to bacterial proteins, but lacking the B27 peptide-binding motif. It was suggested that peptide presentation by HLA-B27 was altered during infection due to *Sh. flexneri* [7]. Differences between B27-presented peptide profiles induced by infection of HeLa cells with *Salmonella enterica* serotype Typhimurium have also been observed [8], but these differences were not characterised. If there is a common arthritogenic determinant present

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among the various ReA-associated bacteria, changes in the B27-presented peptide repertoire induced by different ReA associated bacteria will be independent of the bacterial species. The present study determined the peptide repertoire presented by HLA-B*2705 on non-infected cells and cells infected with *S. Typhimurium* or *Sh. flexneri*.

Materials and methods

Bacteria

Sh. flexneri M90-T [9] and a *S. Typhimurium* strain were used. E. Hermann (Mainz) provided the latter, which had been isolated from the stool of a patient with ReA. Bacteria grown in Luria broth (LB) overnight at 37°C were added to RPMI 1640 (Gibco, Eggenstein, Germany) and grown to exponential phase (OD₆₀₀ *c.* 0.6).

Cell culture

The HLA-A and HLA-B deletion mutant of the human-human hybridoma LICR-LON-HMy2 [10] transfected with HLA-B*2705 (C1R-B*2705) was provided by Dr F.G.C.M. Uytdehaag. The cells were cultured in RPMI 1640 (Gibco) supplemented with fetal calf serum (FCS; Boehringer, Mannheim, Germany) 10% at 37°C. The cells were divided into two equal portions of $(1.5-4) \times 10^{10}$ cells. One portion was then infected with bacteria and the other was used as non-infected control.

Infection of cells with bacteria

Infection experiments were performed as described previously [3]. Briefly, cells were incubated with *S. Typhimurium* at a multiplicity of infection (MOI) of between 50 and 100 cfu/cell and *Sh. flexneri* at a MOI of 10 cfu/cell, at 37°C for 1 h. Non-adherent bacteria were removed and cells were treated with gentamicin [3]. Cells were harvested 4 h after infection and stored at -70°C until lysis for isolation of HLA-B27-bound peptides. Control batches of non-infected cells were prepared in the same way as the infected batch of cells, except that no bacteria were added. Also at 4 h after infection, the number of intracellular bacteria was determined as described previously [3].

Isolation of HLA-B27 molecules and peptides

The HLA-B27 molecules with bound peptides were isolated as described by Verreck *et al.* [11]. Briefly, frozen cell pellets of C1R-B*2705 cells $(1.5-4 \times 10^{10})$ were dissolved in lysis buffer containing detergent NP40 0.5% and protease inhibitors. An affinity column of CNBR-activated Sepharose (Pharmacia, Uppsala, Sweden) containing the monoclonal antibody W6/32, which recognises the trimolecular structure of HLA

class I heavy chain, β_2 -microglobulin and peptide, was used to isolate the B27-peptide complexes.

Peptides were eluted from the MHC molecules with trifluoroacetic acid (TFA) 0.1% in distilled water. Peptides were separated from high mol. wt material with a Centricon (Amicon, Beverly, USA) device with a 3-kDa cut-off. The peptide pool was fractionated by reversed-phase chromatography (RPC) on a SMART-system (Pharmacia) equipped with a μ RPC C2/C18 SC 2.1/10 column (Pharmacia) and with an acetonitrile gradient of 0-45% (TFA 0.1%, with increases in acetonitrile of 0.25%/min, flow rate 80 μ l/min).

Peptide characterisation

Peptides were characterised by Edman degradation by means of a Procise 494 sequencer (Perkin Elmer, Applied Biosystems, Foster City, USA) with a sequence detection limit of *c.* 100 fmol. Peptide sequences were run against the SwissProt protein sequence databank for identification of the protein source of the peptides.

Results

Infection of C1R-B*2705 cells

C1R-B*2705 cells exposed to *S. Typhimurium* or *Sh. flexneri* for 60 min and treated with gentamicin for 4 h to remove extracellular bacteria contained *c.* 10^6 cfu of intracellular bacteria/ 10^6 cells. This number of intracellular bacteria was similar to that found in earlier experiments [3].

Peptide isolation and characterisation

In four individual experiments with *S. Typhimurium* and one with *Sh. flexneri*, UV absorption profiles were obtained by RPC fractionation of B*2705-associated peptides. The profiles from infected C1R-B*2705 cells and non-infected cells were similar but not identical. Two representative profiles from *S. Typhimurium*-infected cells, one from *Sh. flexneri*-infected cells and corresponding profiles from non-infected control cells are shown in Fig. 1a, b and c, respectively. In each experiment, two-to-eight distinct differences between the peptide profiles from infected and non-infected cells were noted (marked with arrows in Fig. 1a, b and c). In profiles from infected cells, some peaks were higher than in profiles from non-infected cells, or peaks were present that were absent from profiles from non-infected cells. Fractions containing the material representing such peaks were subjected to Edman degradation sequence analysis.

In all analysed fractions, only nonamers with arginine (Arg) at position 2 were present. For cells infected with *S. Typhimurium*, only sequences of peptides in fractions corresponding to three different peaks (1Ai,

Fig. 1. Profiles showing UV absorption (AU₂₁₅ nm) versus RPC elution time (min) of HLA-B27-associated peptides from infected (upper traces) and non-infected (lower traces) C1R-B*2705 cells. Representative profiles from two experiments (a) and (b) with *S. Typhimurium*-infected and non-infected cells, and (c) from *Sh. flexneri*-infected and non-infected cells are shown. Arrows mark the positions of analysed peaks that are different between non-infected and infected cells at the same positions. Labelled peaks are referred to in the text.

Table 1. Sequences of peptides corresponding to peaks present only in profiles from infected cells

Experiment	Infection	Peak	Sequence	Homology with human proteins
1	<i>S. Typhimurium</i>	1Ai	RRYQKSTEL	Histone H3.3
2	<i>S. Typhimurium</i>	2Ei	ARFELAFXX	Not found
3	<i>S. Typhimurium</i>	3Ai	GRVRTKTVK	S17 ribosomal protein
5	<i>Sh. flexneri</i>	5Ai	RRKSSGGKG	B27 heavy chain
5	<i>Sh. flexneri</i>	5Ai	XRVRTKTVK	S17 ribosomal protein

X, could not be determined.

2Ei and 3Ai) could be determined, as the other fractions contained mixtures of peptides, or the amount of peptides was less than the detection limit of the sequencer used (Table 1).

The fraction corresponding to peak 1Ai from cells infected with *S. Typhimurium* (Fig. 1a) contained a peptide with the sequence RRYQKSTEL. Database analysis revealed that this peptide was identical to a stretch of nine amino acids from the human histone H3 protein. The fraction corresponding to peak 2Ei (Fig. 1b) contained the peptide sequence ARFELAFXX (X designates residues that could not be determined). This sequence did not match with protein sequences in the SwissProt protein sequence database. The fraction corresponding to peak 3Ai from cells infected with *S. Typhimurium* contained the peptide sequence GRVRTKTVK, which is identical to a stretch of nine amino acids of the human ribosomal protein S17.

Two peaks present in the profile obtained from *Sh. flexneri*-infected cells were very large in comparison to the peaks in the profile from non-infected cells (Fig. 1c). The fraction corresponding to peak 5Ai contained the sequences RRKSSGGKG and XRVRTKTVK. These sequences were not detected in the corresponding fraction from non-infected cells. The first of these two sequences is identical to amino-acid residues 330–338 from the HLA-B27 heavy-chain, and the second sequence is identical to a fragment of the human ribosomal protein S17. This latter sequence is the same as the sequence present in peak 3Ai from *S. Typhimurium*-infected cells.

Reproducibility of the experiments

When peptide profiles derived from the non-infected cells in the different experiments were compared, it appeared that these profiles were different. Likewise, the peptide profiles from cells infected by *S. Typhimurium* were compared and found to be different.

Discussion

ReA after gastrointestinal infection with certain gram-negative bacteria and urogenital infection with *C. trachomatis* occurs especially in persons expressing the MHC class I molecule HLA-B27 [1]. A study of

the influence of infection with these different bacteria on the peptide repertoire presented by HLA-B27 might provide insight into the nature of the peptides postulated in the 'arthritogenic peptide' hypothesis [6]. As both *S. Typhimurium* and *Sh. flexneri* are able to induce ReA, the present study attempted to identify common changes in the peptide repertoire presented by B*2705 induced by these organisms.

As in the study of Maksymowych *et al.* [8], differences were found between peptide profiles from *S. Typhimurium*-infected and non-infected cells. In contrast to their work, the present study was able to identify the amino acid sequence of a few peptides present in the peaks of profiles obtained from infected cells that were not detectable in the profiles from non-infected cells. In the present study, sequences detected only in profiles from infected cells showed identity with those of human histone H3, the heavy chain of HLA-B27 itself and human ribosomal protein S17. The latter peptide was isolated from *S. Typhimurium*-infected and from *Sh. flexneri*-infected cells. It is reasonable to suggest that the peptides were derived from the host cells. RT-PCR showed that mRNA levels for the histone H3, ribosomal protein S17 or HLA-B27 heavy-chain were not increased after infection (data not shown). The higher recovery of peptides derived from these proteins was not likely to have been caused by an increased synthesis of these proteins. The increase in the presentation of peptides derived from HLA-B27 by B27 molecules on C1R B-cells infected with *Sh. flexneri* may be explained by selective uptake of MHC class I molecules during macropinocytosis of bacteria, resulting in enhanced access of these molecules to the MHC class I presentation pathways [2, 12–14].

There was no evidence for the ability of B27 to present unusual peptides after bacterial infection. The peptides isolated from infected C1R cells were never longer than nine amino acids and all contained Arg at position 2, which is in agreement with the known peptide binding motif for HLA-B*2705 [15]. This finding contrasts with the results of Boisgrault *et al.* [7], who found that the peptide specificity of B27 was changed after infection with *Shigella*. No bacteria-derived peptides presented by B27 on the infected cells were identified in the present work.

The major drawback of this study was that the peptide

profiles from non-infected cells obtained in five different experiments were not similar. Furthermore, differences between profiles from infected and non-infected cells varied between experiments. These variations were not caused by the chromatographic procedure used, as this technique generated fully reproducible profiles (data not shown). Maksymowych *et al.* [8] also found that the profiles from their untreated control cells differed extensively from each other. The findings of both these studies indicate extensive variability between different experiments which cannot be attributed to infection of the cells.

The present study showed that the methods used to characterise differences between peptide repertoires presented by infected cells *versus* non-infected cells have to be improved to determine which peptides, if any, may play a pathogenic role in ReA. In particular, the lack of reproducibility and the problem of sequencing complex peptide mixtures have to be solved. New techniques in mass spectrometric identification of peptides may provide the ability to solve the latter problem in the near future.

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