Role of peptidoglycan subtypes in the pathogenesis of bacterial cell wall arthritis

E Šimelyte, M Rimpiläinen, X Zhang, P Toivanen

Background: Bacterial cell wall (CW) arthritis develops in susceptible strains of rats after a single intraperitoneal injection of the CW from certain bacterial species, both pathogenic and non-pathogenic. For the development of chronic bacterial CW arthritis, the structure of the bacterial peptidoglycan (PG) has been found to be decisive.

Objective: To define the role of PG subtypes in the pathogenesis of chronic bacterial CW arthritis.

Method: Arthritis was induced with CWs of Lactobacillus plantarum, L casei B, L casei C, and L fermentum. Gas chromatography-mass spectrometry was used to measure the presence of CW derived muramic acid in the liver and to determine PG subtypes. CWs were also tested for their resistance to lysozyme in vitro.

Results: These results and those published previously indicate that PGs of CWs which induce chronic arthritis, no matter whether they were derived from strains of Streptococcus, Bifidobacterium, Collinsella, or Lactobacillus, all have lysine as the third amino acid of the PG stem peptide, representing PG subtypes A3α and A4α. Those strains which induce only transient acute arthritis or no arthritis at all do not have lysine in this position, resulting in different PG subtypes.

Conclusions: In vivo degradation of only those PGs with the subtypes A3α and A4α leads to the occurrence of large CW fragments, which persist in tissue and have good proinflammatory ability. CWs with other PG subtypes, even if they are lysozyme resistant, do not cause chronic arthritis, because the released fragments are not phlogistic. It is emphasised that a variety of microbial components not causing inflammation have been found in animal and human synovial tissue.

The cell wall (CW) of Gram positive bacteria has a peptidoglycan (PG) as a major component. PG polymer is composed of alternating N-acetylmuramic acid and N-acetylglucosamine sugar chains with cross-linking peptide chains containing d- and l-amino acids. In the CW, polysaccharides (PS) and teichoic acids are covalently bound to PG. These structures in the CW complex, or alone, are biologically active, with immunoadjuvant or slow wave sleep promoting activity.1 During Gram positive infections, PG can activate complement2 and granulocytes,3 and up regulate expression of adhesion molecules on the endothelial cells.4 In vitro, PG is known to induce production of the proinflammatory cytokines interleukin 1, interleukin 6, tumour necrosis factor α (TNFα), and monocyte chemoattractant protein-1 (MCP-1) by monocytes.5,6 Bacterial CWs are digested by a number of PG degrading hydrolytic enzymes, including lysozyme, and the amounts of active CW fragments released depend on the activity of these PG hydrolases.7,8

It has been suggested that PG plays a part in the pathogenesis of chronic inflammation such as rheumatoid arthritis. This hypothesis is based on experimental results obtained after systemic injection of high molecular weight PG-PS complexes, resulting in chronic synovial inflammation. CW components isolated from a variety of Gram positive bacterial species are disease inducing.9 A single injection of bacterial CW causes chronic erosive arthritis10–13 and granulomatous enteroctilosis14 in susceptible rat strains. On the other hand, purified PG-PS derived from normal human enteric flora can or non-arthritogenic. Therefore we aimed at determining the factors decisive for the arthritogenicity of the bacterial CW.13 14 21 26

The differences in arthritogenicity between different CWs seem to depend on the chemical structure of PG.25 21 26 Variations of the PG peptide moiety occur in the mode of cross linkage, the interpeptide bridge, and the peptide subunit. Based on the anchorage point of the cross linkage to the peptide subunit, the primary structure of PG is divided into type A (cross linkage between position 3 and 4) and type B (cross linkage between position 2 and 4); these are further classified into different subtypes depending on the type/presence of the connecting interpeptide bridges and the amino acid in the third position of the PG peptide subunit.

In this study we aimed at elucidating the critical role of PG subtypes in the pathogenesis of chronic bacterial CW arthritis. For this purpose, arthritogenicity and concentrations in the liver of the CWs of four Lactobacillus strains were studied in relation to the PG subtypes, and the results obtained were compared with those already published. Additionally, we also evaluated the lysozyme resistance of different PGs.

MATERIALS AND METHODS

Bacterial strains

The following strains of Lactobacillus were used: L plantarum ATCC 4008, L casei C ATCC 25302, L casei B ATCC 11578, and L fermentum ATCC 14931. These strains were purchased from the American Type Culture Collection, Rockville, MD. All bacteria

Abbreviations: CW, cell wall; GC-MS, gas chromatography-mass spectrometry; IP, intraperitoneally; MCP, monocyte chemoattractant protein; PBS, phosphate buffered saline; PG, peptidoglycan; PS polysaccharides; TNFα, tumour necrosis factor α.
were grown by the bioproduction unit in the Department of Biochemistry and Food Chemistry, Turku University.

**CW preparations**

Bacterial CWs were isolated and purified as described previously. To separate 10P (pellet) and 10S (supernatant) fractions, the CW preparations were centrifuged at 10 000 g at 4°C for 30 minutes. The protein content determined by the Lowry method in the 10S fractions was 8.0%, 11.6%, 13.1%, and 7.9% per dry weight for *L. plantarum*, *L. casei*, *L. fermentum*, and *L. aerofaciens*, respectively. The total carbohydrate amount determined by the phenol-sulphuric acid method in these preparations was 28.9%, 20.5%, 30.9%, and 18.5% per dry weight, respectively. To exclude endotoxin contamination, the 10S preparations were tested by the E-TOXATE Limulus amebocyte lysate test (Sigma Chemical Co, MO); all tests were negative, with endotoxin concentrations <0.12 EU/ml. The sensitivity of the test was 0.05–0.1 EU/ml. For sterility, the fractions were tested by bacterial culture on agar. The 10S fractions were tested by bacterial culture on agar. The 10S fractions were used in all experiments.

**Chemical structure of *L. plantarum* CW**

To determine the chemical composition, PG was isolated from *L. plantarum* according to a previously described protocol. Briefly, the lyophilised 10S fraction of CW was extracted with 10% trichloroacetic acid at 60°C for 4 hours. The suspension was centrifuged at 38 800 ×g for 10 minutes. The pellets were resuspended in 1 ml PBS, heat treated at 100°C for 10 minutes, and centrifuged again. The carbohydrate content of the 10S fractions was 8.0%, 11.6%, 13.1%, and 7.9% per dry weight for *L. plantarum*, *L. casei*, *L. fermentum*, and *L. aerofaciens*, respectively. The total carbohydrate amount determined by the phenol-sulphuric acid method in these preparations was 28.9%, 20.5%, 30.9%, and 18.5% per dry weight, respectively. To exclude endotoxin contamination, the 10S preparations were tested by the E-TOXATE Limulus amebocyte lysate test (Sigma Chemical Co, MO); all tests were negative, with endotoxin concentrations <0.12 EU/ml. The sensitivity of the test was 0.05–0.1 EU/ml. For sterility, the fractions were tested by bacterial culture on agar. The 10S fractions were used in all experiments.

<table>
<thead>
<tr>
<th>Origin of CW</th>
<th>Dose (mg)</th>
<th>Muramic acid (mg)†</th>
<th>Total carbohydrate (mg)†</th>
<th>Content of</th>
<th>Chronic arthritis Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. plantarum</em> ATCC 4008</td>
<td>38.7</td>
<td>ND</td>
<td>6.0</td>
<td>Muramic acid†</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>24.0</td>
<td>1.9</td>
<td>6.9</td>
<td>Total carbohydrate†</td>
<td>28.9</td>
</tr>
<tr>
<td></td>
<td>42.9</td>
<td>ND</td>
<td>6.0</td>
<td></td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>24.4</td>
<td>1.0</td>
<td>5.0</td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>24.0</td>
<td>1.0</td>
<td>5.0</td>
<td></td>
<td>20.5</td>
</tr>
<tr>
<td><em>L. casei</em> C ATCC 25302</td>
<td>25.0</td>
<td>ND</td>
<td>6.0</td>
<td>Muramic acid†</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>16.2</td>
<td>0.9</td>
<td>5.0</td>
<td>Total carbohydrate†</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>0.4</td>
<td>2.5</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td><em>L. fermentum</em> ATCC 14931</td>
<td>21.0</td>
<td>ND</td>
<td>6.0</td>
<td>Muramic acid†</td>
<td>28.6</td>
</tr>
<tr>
<td></td>
<td>27.0</td>
<td>1.6</td>
<td>5.0</td>
<td>Total carbohydrate†</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>0.55</td>
<td>1.5</td>
<td></td>
<td>Nd</td>
</tr>
<tr>
<td><em>L. aerofaciens</em> ATCC 25986</td>
<td>16.7</td>
<td>2.5</td>
<td>ND</td>
<td>Muramic acid†</td>
<td>18.5</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>0.4</td>
<td>11.8</td>
<td>Total carbohydrate†</td>
<td>1.9</td>
</tr>
</tbody>
</table>

ND, not determined.

†Dry weight, per 100 g of rat body weight; †percentage of CW dry weight; ‡determined on the basis of the present study; §determined on the basis of our previous study; ¶formerly Eubacterium aerofaciens.

**Arthritis induction and clinical evaluation**

Two separate experiments were done: *L. plantarum* and *L. casei* were used for the first experiment, and *L. casei* with *L. fermentum* for the second. On day 0, each of the four groups of rats (n = 30–32) was injected intraperitoneally (IP) with sterile phosphate buffered saline (PBS) suspensions of CW preparations (3 ml for each rat). The doses used were chosen on the basis of previously published results (table 1). In the first experiment, with *L. plantarum* and *L. casei* the injection dose was 24 mg of CW dry weight per 100 g of rat body weight, whereas in the second experiment with *L. casei* and *L. fermentum* the dose was 8 mg. Such doses, based on the total carbohydrate concentrations determined in each CW, correspond with a carbohydrate content of 7.3 mg, 5.2 mg, 2.6 mg, and 1.6 mg per rat for *L. plantarum*, *L. casei*, *L. casei*, and *L. fermentum*, respectively.

Control rats (n = 15) were injected IP with 3 ml of PBS alone. To monitor the development of arthritis, the front and hind paws were scored with a naked eye inspection daily for the first 10 days, and later three times a week. The arthritic symptoms were graded from 0 to 4, based on the degree of erythema, oedema, and functional disorder of the ankle and metatarsal joints (wrist and metacarpal joints), by two independent observers as described previously. The mice injected with *L. plantarum* or *L. casei* C the development of arthritis was also monitored by caliper (resolution 0.05 mm; Mitutoyo Asia Pacific Pte Ltd, Japan) measurement of paw swelling; the results were found to agree with those obtained by visual scoring. Rats were killed at different times by cardiac puncture bleeding under methoxyflurane (Metofane; Pitman-Moore, Inc, Washington Crossing, NJ) anaesthesia.

**Liver samples**

Four to six rats of each group were killed 1, 3, 7, 14, and 28 days after the CW injection. On each occasion, livers from these and from three control rats were collected. The organs were weighed and stored at −20°C until used. To prepare the liver samples, 5 ml of sterile water was added to each sample before homogenisation with an Ultra Turrax T25 tissue homogeniser (Janke and Kunkel, IKA, Labortechnik, Staufen, Denmark); all handling was performed in a laminar-flow hood. The rats were given autoclaved standard diet and water freely. Before the experiments the animals were allowed to adapt to the local environment for one week. The animal experiments were performed in compliance with national and international laws and policies, and were approved by the Institutional Committee for Animal Research.
Germany). The final volume was measured, and the suspensions were analysed by gas chromatography-mass spectrometry (GC-MS) for the muramic acid content. When necessary, liver suspensions were diluted with distilled water to 1:3, 1:5, 1:10, or 1:30. The presence of muramic acid was determined in the whole liver for each rat.

**Sample preparation for GC-MS**

Muramic acid content in the liver samples was analysed as the trifluoroacetylated methylglycoside derivative by GC-MS, using the chemical ionisation mode with negative ion detection as previously described. To obtain the muramic acid standard for the negative chemical ionisation, *E. limosum* (ATCC 8486, American Type Culture Collection, Rockville, MD) CW suspension, isolated as described, was used. The muramic acid concentration of the *E. limosum* CW was determined by GC-MS using the electron impact ionisation as described previously. The standard curve was linear with muramic acid (Sigma Chemical Co, St Louis, MO) concentrations from 10 to 130 ng (injected amount).

For the negative chemical ionisation, samples from liver suspensions and *E limosum* CW suspension were evaporated to dryness under a stream of nitrogen at 40°C. The samples were then methanolysed under a nitrogen atmosphere at 85°C for 24 hours in 2 ml of 4 M methanolic hydrochloric acid. Hexane (SupraSolv purity, Merck and Co, Inc, NJ) extraction (3 ml) was performed for the methanolysate. The internal standard *N*-methyl-α-glucamine (Sigma Chemical Co, St Louis, MO), dissolved in methanol (SupraSolv purity, Merck and Co, Inc, NJ), was dried under nitrogen atmosphere at 40°C and further processed in the same way as the samples. After derivatisation, samples were diluted with 400 µl of toluene (SupraSolv purity, Merck KGaA, Darmstadt, Germany), 50 µl aliquots were put into the sample vials and diluted with derivatised internal standard (50 µl), and 1 µl was injected into the GC-MS system.

Glassware was used first treated with DECON 90 (Decon Laboratories Ltd, Sussex, UK), and 10% DECONEX 11 UNIVERSAL (Borer Chemie Ab, Zuchwil, Switzerland), rinsed with water (2–6 times) between the steps, heated at 170°C for two hours, before autoclaving for 20 minutes.

**GC-MS of muramic acid in the liver**

The GC-MS analyses were performed with a gas chromatograph (model 6890, Hewlett-Packard, Little Falls, DE) coupled to a mass selective detector (model 5973, Hewlett-Packard, Palo Alto, CA). The GC-MS apparatus was equipped with an HP-5MS capillary column (30 m length, 0.25 mm diameter, Agilent Technologies, Palo Alto, CA). The GC-MS analyses were performed with a gas chromatography (GC)-MS system.

The GC-MS system was equipped with an electron impact ionisation as described previously. The Assays were performed in triplicate. A CW was considered lysozyme resistant when degradation of the 10S preparation was <30%.

**Statistics**

The differences between study groups were compared by Mann-Whitney U test for unpaired data and Wilcoxon matched pairs test for paired data. Values of *p*<0.05 were considered significant.

**RESULTS**

**PG subtypes**

Here we determined the PG subtype of *L plantarum* ATCC 4008, which was previously unknown. For this purpose the content of carbohydrates and amino acids in the *L plantarum* CW preparation used was determined. In accordance with the other strains of *L plantarum* studied, the results obtained show that the *L plantarum* CW used has dianminopimelic acid, alanine and glutamine as PG amino acids, indicating the PG subtype A1γ (table 2; fig 1). The structures of the three other PGs used here are also already known, they are presented in fig 1, with subtype A4α for the other arthritogenic *L casei* strains used and the subtype A4β for the non-arthritogenic *L fermentum* strain. It should also be noted that *L plantarum* does not contain rhamnose (table 2), even though the presence of rhamnose has not been found to be related to the arthritogenicity or non-arthritogenicity of bacterial CWs, in contrast with a previous suggestion.

**Arthritogenicity in vivo**

To determine the arthritogenicity of the four *Lactobacillus* CWs used, two separate experiments were carried out. In the first experiment with *L plantarum* and *L casei* strains, rats were injected IP with 24 mg of CW dry weight per 100 g of body weight. This dose of *L casei* CW has previously been found to be arthritogenic, and for *L plantarum* CW, even higher doses have been reported to be non-arthritogenic. On day 2 after CW injection 5/32 rats had died in the *L casei* C group, and 1/31 in the *L plantarum* group. Therefore for the second experiment

<table>
<thead>
<tr>
<th>Component</th>
<th>105 fraction of the CW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates, total</td>
<td>16.5*</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>0</td>
</tr>
<tr>
<td>Galactose</td>
<td>+1</td>
</tr>
<tr>
<td>Mannose</td>
<td>+</td>
</tr>
<tr>
<td>Fucose</td>
<td>+</td>
</tr>
<tr>
<td>N-acetylmuramic acid</td>
<td>8.1</td>
</tr>
<tr>
<td>N-acetylglucosamine</td>
<td>8.0</td>
</tr>
<tr>
<td>Amino acids, total</td>
<td>79.5</td>
</tr>
<tr>
<td>Diaminopimelic acid</td>
<td>24.8</td>
</tr>
<tr>
<td>Alanine</td>
<td>19.8</td>
</tr>
<tr>
<td>Glutamic acid/glutamine</td>
<td>12.2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.3</td>
</tr>
<tr>
<td>Lysine</td>
<td>3.6</td>
</tr>
<tr>
<td>Aspartic acid/asparagine</td>
<td>3.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.1</td>
</tr>
<tr>
<td>Valine</td>
<td>2.7</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.2</td>
</tr>
<tr>
<td>Proline</td>
<td>2.0</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.4</td>
</tr>
<tr>
<td>Total</td>
<td>96.0</td>
</tr>
</tbody>
</table>

*Values are expressed as percentage of PG dry weight; †detected, not measured.*
with *L* casei B and *L* fermentum, a lower dose of 8 mg of CW dry weight per 100 g of body weight was chosen. In the preliminary experiments this dose of *L* casei B CW was found to be arthritogenic, and for *L* fermentum CW considerably higher doses were non-arthritogenic (<13 19 table 1). It should also be noted that the amount of muramic acid injected into each rat equalled that previously used to induce chronic CW arthritis with *Collinsella aerofaciens* 21 (table 1). In contrast with the previous findings indicating the non-arthritogenicity of *L* plantarum CW, 19 we observed arthritis which lasted for at least 28 days after a single IP injection of *L* plantarum CW (fig 2). Injection of CWs from *L* casei C and *L* casei B also resulted in development of arthritis lasting for at least 28 days, whereas *L* fermentum induced only a mild acute arthritis, which completely subsided in 10 days; these findings were as expected. Rats injected with PBS alone did not develop any signs of arthritis.

**Muramic acid in the liver**

The presence of CW in the liver was studied on days 1, 3, 7, 14, and 28 by measuring the muramic acid content. This amino sugar is a component of the PG backbone and is not synthesised by mammalian cells. The detection limit of our method was 1 pg (the amount injected into the chromatograph column). Because muramic acid concentrations in the liver are known to reflect those found in the spleen, 81 only liver samples were here collected and analysed.

CWs of both *L* casei (C and B) accumulated in the liver similarly, persisting on day 28 with 20% of the injected muramic acid remaining in the liver, in contrast with the non-arthritogenic *L* fermentum, which accumulated in the liver only to a small extent and finally cleared away, with <5% of the total dose injected detectable on day 28 (fig 3). Deposition of *L* plantarum CW was highest on days 3 and 7, and reflected the occurrence of acute arthritis, whereas on days 14 and 28 it was

**Figure 1** PG structures in the *Lactobacillus* strains used. The *L* plantarum belongs to the α-glutamine-m-Diaminopimelic acid-d-alanine subtype (A1γ), the two *L* casei share the L-lysine-o-saragagine subtype (A4α), whereas *L* fermentum has the subtype L-ornithine-o-saragagine (A4β). The enzymes degrading PG cleave specific bonds: lysozyme (β-N-acetylmuramidase) and N-acetylmuramyl-l-alanine-amidase.

**Figure 2** Development of arthritis in rats injected IP with *Lactobacillus* CW. The injection dose was 24 mg of CW dry weight/100 g of rat body weight for *L* plantarum ATCC 4008 or *L* casei C ATCC 25302, and 8 mg for *L* casei B ATCC 11578 or *L* fermentum ATCC 14931. The arthritis score is calculated as a mean value (SEM) for the number of rats indicated at the top. Rats which died on day 2 are excluded from this figure.
The muramic acid levels are given as the mean value (SEM) for four to six rats. Asterisks indicate significant differences (p<0.05) when the rats injected with L plantarum, L casei C or L casei B CWs were compared with those injected with L fermentum CW. Significance is shown separately for each day of analysis.

Resistance to lysozyme

Incubation for 24 hours with the lysozyme did not produce any degradation of L casei C or L casei B CWs, whereas CWs of L fermentum and L plantarum were lysozyme sensitive, with a 57% and 48% decrease in OD at 560 nm, respectively.

DISCUSSION

Table 3 summarises the ability of different bacterial CWs to induce chronic arthritis in relation to the PG subtypes, based on the present results and those published by us and by others previously. It appears that PGs of bacterial strains inducing chronic arthritis in the rat, no matter whether they are derived from strains of Streptococcus, Bifidobacterium, Collinsella, or Lactobacillus, have lysine as the third amino acid of the PG stem peptide, representing PG subtypes A3α or A4α. On the other hand, all those strains which induce only a transient acute arthritis or no arthritis at all do not have lysine in this position; the position is occupied instead either by ornithine or diaminopimelic acid, resulting in a variety of PG subtypes. Most interesting is a pair of almost identical strains of Collinsella aerofaciens (formerly Eubacterium aerofaciens), one of them causing chronic arthritis with lysine in the critical position of the PG stem peptide, and the other with ornithine in the same position, and causing only a slight acute, transient arthritis. All these findings strongly suggest that the PG structure with lysine as the third amino acid in the stem peptide is required for induction of chronic bacterial CW arthritis. The only potential exception, which does not fit into the above suggestion, is L plantarum CW (table 3). However, the lack of long term tissue accumulation, as seen in the present study, indicates that arthritis caused by this CW cannot be long lasting.

Several previous observations support our suggestion that PG subtypes A3α and A4α with lysine as the third amino acid in the stem peptide are more arthritogenic than other PG subtypes. On the basis of studies on adjuvant arthritis evidence has been presented that structural variations of the chemically defined PG subunits result in drastic changes of the arthritogenic ability, ranging from complete non-arthritogenicity to production of severe arthritis in high frequency. Kohashi et al compared the arthritogenicity of PGs from the CWs of Staphylococcus aureus and L plantarum. They concluded that a PG subunit with a chain length of two disaccharide units was the minimal structure responsible for arthritogenicity. Consequently, muramyl dipeptide was defined as the minimal arthritogenic structure in adjuvant arthritis. Variation of the third amino acid of the stem peptide has been shown to change the biochemical activity of PG fragments. In studies on subarachnoidal inflammation in the rabbit, PG fragments with lysine in position three were found to be highly inflammatory. Also, branched stem peptides with lysine in position three, isolated from Streptococcus

Table 3  Arthritogenicity of bacterial CWs in relation to PG subtype and resistance to lysozyme in vitro

<table>
<thead>
<tr>
<th>Origin of CW</th>
<th>Chronic arthritis in rat</th>
<th>PG structure</th>
<th>Stem peptide amino acids</th>
<th>Resistance to lysozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/- Reference</td>
<td></td>
<td>1 2 3 4 Subtype Reference</td>
<td>+/- Reference</td>
</tr>
<tr>
<td>Lactobacillus casei B ATCC 25302</td>
<td>+</td>
<td>19</td>
<td>Ala Glu Lys Asp Ala A4α</td>
<td>13, 36 + 13, 19</td>
</tr>
<tr>
<td>- ATCC 11578</td>
<td>+</td>
<td>19, 27, PS</td>
<td>Ala Glu Lys Asp Ala A4α</td>
<td>1, 13 + 13, 27, PS</td>
</tr>
<tr>
<td>- ATCC 4008</td>
<td>+</td>
<td>13, 5</td>
<td>Ala Glu Lys Asp Ala A4α</td>
<td>13, 36, 37 + 13, 19, PS</td>
</tr>
<tr>
<td>Streptococcus pyogenes D58 (group A)</td>
<td>+</td>
<td>11, 16, 35</td>
<td>Ala Glu Lys Thr Ala A3α</td>
<td>1 + 16</td>
</tr>
<tr>
<td>- faecium ATCC 9790 (group D)</td>
<td>+</td>
<td>16</td>
<td>Ala Glu Lys Asp Ala A4α</td>
<td>1 - 16</td>
</tr>
<tr>
<td>- faecium F24 (group D)</td>
<td>+</td>
<td>16</td>
<td>Ala Glu Lys Asp Ala A4α</td>
<td>1 - 16</td>
</tr>
<tr>
<td>Bifidobacterium breve ATCC 15700</td>
<td>+</td>
<td>17</td>
<td>Ala Glu Lys Gly Ala A3α</td>
<td>1 + 17</td>
</tr>
<tr>
<td>- adolescents ATCC 15703</td>
<td>+</td>
<td>14, 17</td>
<td>Ala Glu Lys† Asp Ala A4α</td>
<td>14 + 14</td>
</tr>
<tr>
<td>- adolescents ATCC 15704</td>
<td>+</td>
<td>14</td>
<td>Ala Glu Lys† Asp Ala A4α</td>
<td>14 + 14</td>
</tr>
<tr>
<td>Collinsella aerofaciens†</td>
<td>+</td>
<td>8, 17, 20, 21</td>
<td>Ala Glu Lys Asp Ala A4α</td>
<td>21 +§ 8, 26</td>
</tr>
<tr>
<td>Collinsella aerofaciens† ATCC 35085</td>
<td>+</td>
<td>8, 21</td>
<td>Ala Glu Orn Asp Ala A4β</td>
<td>21 + 8</td>
</tr>
<tr>
<td>Eubacterium limosum ATCC 8486</td>
<td>+</td>
<td>20, 21, 23</td>
<td>Ser Glu Orn Lys Ala B2α</td>
<td>21, 55 - 26</td>
</tr>
<tr>
<td>- alactolyticum ATCC 17927</td>
<td>+</td>
<td>21</td>
<td>Ala Glu Dop Ala A1γ</td>
<td>21, 56 + 25</td>
</tr>
<tr>
<td>Lactobacillus plantarum ATCC 4008</td>
<td>+**</td>
<td>19</td>
<td>Ala Glu Dop Ala A1γ</td>
<td>19, PS</td>
</tr>
<tr>
<td>- plantarum ATCC 8014</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PS, present study; ND, not determined; †, interpeptide bridge.

*Chronic arthritis was not observed by Lehman et al*12 whereas in the present study arthritis lasted for 28 days, with no long term CW accumulation in liver; ††Resistance was reported by Lehman et al*13 but not seen in the present study.
Lysozyme resistant CWs are degraded in vivo considerably less of the arthritogenic strain was a twofold more potent stimula-
ty of a CW to lysozyme would bear no correlation to its ability
lysozyme, in relation to their ability to induce chronic arthritis
without lysine in position three.

Further-more, in our previous study, evidence was obtained to indicate
that in strains of Bifidobacterium adolescentis, with intraspecies
variation from lysine to ornithine in position three of the stem
peptide, a high lysine content is related to the severity of the
chronic arthritis.

What might be the mechanism by which a single amino acid
in a certain position of the PG stem peptide is decisive for the
arthritogenicity of a CW? Recently, a direct comparison of the
immunostimulatory power of PGs with or without lysine
in position three of the stem peptide was carried out using two
strains of Collinsella aerofaciens, one of them being arthritogenic
with lysine in the critical position and the other being
non-arthritogenic with ornithine replacing lysine.14,15

The CW of the arthritogenic strain was a twofold more potent stimula-
tor of the proinflammatory cytokines TNFα and MCP-1 than the
non-arthritogenic CW. After enzyme degradation, the
ability of the arthritogenic PG to stimulate production of
the non-arthritogenic PG was significantly decreased. In other
words, after enzyme degradation the arthritogenic PG with
lysine in position three had a four- to fivefold stronger stimu-
latory ability than the enzyme-treated non-arthritogenic PG
without lysine in position three.

Table 3 indicates the resistance of different bacterial CWs to
lysosome, in relation to their ability to induce chronic arthritis
in the rat. At first sight, it seems that resistance or susceptibil-
ity of a CW to lysosome would bear no correlation to its ability
to induce chronic arthritis, even though all CWs causing
chronic arthritis, with the exception of Streptococcus faecium
have been reported to be lysozyme resistant (table 3).
However, it must be emphasised that the arthritis caused by
the CW of Streptococcus pyogenes is not as long lasting as that
induced by the CW of Streptococcus faecium, the former subsid-
ing at two months and the latter being still active at four
months after the initial CW injection.16 On the other hand, it
also appears that lysozyme resistance is seen among the CWs
not causing chronic arthritis (table 3). These seeming
inconsistencies can be fully explained, if it is assumed that
lysozyme resistant CWs causing longstanding, chronic arthritis
accumulate in large quantities in the liver and spleen, known
as the major reservoirs after an IP CW injection. This
assumption is compatible with the following observations: (a)
Lysozyme resistant CWs are degraded in vivo considerably less
than the lysosome susceptible ones, leading to the occurrence
of large CW fragments and their longer persistence in the
tissues.22–24 The degree of lysosome resistance has been found
to correlate directly with the amount of CW derived muramic
acid found in the tissues,25–27 which is also apparent in the
present study (fig 3). (b) The liver and spleen act as reservoirs,
releasing CW fragments to the circulation and joint tissues.
28–30 Moreover, in the pathogenesis of chronic bacterial
arthritides the deposition and persistence of CW degradation
products in the synovial tissues is crucial.14 22 47 However,
not all CWs deposited are arthritogenic; a decisive factor is
whether the CW fragments released are proinflammatory.

Ample evidence exists both from animal31–46 and human37–45
studies to indicate that a variety of microbial components are
found in the synovial tissues which do not cause inflamma-

On the basis discussed above it is not difficult to accept the
possibility that even lysozyme resistant bacterial CWs may be
unable to induce chronic arthritis; they represent PG subtypes
other than A30 or A46, and their degradation products are
simply not sufficiently proinflammatory, even though they
may end up in the joint tissues. In the pathogenesis of chronic
bacterial CW arthritis, a PG of an appropriate subtype is
required, degradation of which leads to the presence and per-

REFERENCES


2. Martin SA, Kornovsky ML, Krueger JM, Pappenheimer JR, Biemann K, Pedrini A. Peptidoglycan a promoter of slow-wave sleep. I. Structure of the


8. Zhang X, Rimpliainen M, Simelyte E, Toivanen P. Enzyme degradation and proinflammatory activity in arthritogenic and non-arthritogenic

9. Majcherzyck PA, Langen H, Heumann D, Fountoulakis M, Gauler MP, Moreillon P. Digestion of Streptococcus pneumoniae cell wall with its

10. Schobbs BH. Phlogistic properties of peptidoglycan-poly saccharide polymers from cell walls of pathogenic and nonpathogenic bacteria which


12. Hazenberg MP, Klaas IS, Kool J, Ruseler-van-Embden JG, Severijnen AJ. Are intestinal bacteria involved in the etiology of rheumatoid arthritis?


17. Severijnen AJ, van Kleef R, Hazenberg MP, van der Meers WP. Cell wall fragments from major resident of the human intestinal flora induce


Role of peptidoglycan subtypes in the pathogenesis of bacterial cell wall arthritis

E Simelyte, M Rimpiläinen, X Zhang and P Toivanen

doi: 10.1136/ard.62.10.976

Updated information and services can be found at:
http://ard.bmj.com/content/62/10/976

These include:

References
This article cites 55 articles, 25 of which you can access for free at:
http://ard.bmj.com/content/62/10/976#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

Degenerative joint disease (4641)
Musculoskeletal syndromes (4951)
Immunology (including allergy) (5144)
Inflammation (1251)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/