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# The relationship between colonization and haemagglutination inhibiting and B cell epitopes of *Porphyromonas gingivalis*

C. G. KELLY, V. BOOTH*, H. KENDAL, J. M. SLANEY†, M. A. CURTIS† & T. LEHNER *Departments of Immunology and *Periodontology and Preventive Dentistry, UMDS at Guy's Hospital, and †MRC Molecular Pathogenesis Group, Department of Oral Microbiology, London Hospital Medical College, London, UK*

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## SUMMARY

Passive immunization with the monoclonal antibody 61BG1.3 selectively prevents colonization by *Porphyromonas gingivalis* in humans (Booth V, Ashley FP, Lehner T. *Infect Immun* 1996; **64**:422–7). The protective MoAb recognizes the $\beta$ component of the RI protease of *P. gingivalis* which is formed by proteolytic processing of a polyprotein precursor termed PrpRI. This subunit is both a haemagglutinin and an antigen which is recognized by sera from patients with periodontitis. In this study the relationship was investigated between a colonization epitope which is recognized by the MoAb 61BG1.3, a haemagglutinating and B cell epitope which are recognized by sera from patients with periodontitis. B cell epitopes were mapped by Western blotting with a series of truncated recombinant polypeptides spanning the adhesion domain within residues 784–1130 of PrpRI and by ELISA using a panel of synthetic peptides spanning the same sequence. The epitope which is recognized by the protective MoAb was mapped within residues 907–931 of PrpRI, while serum responses of patients were directed predominantly to the adjacent carboxy-terminal sequence within residues 934–1042. The haemagglutinating epitope was mapped to residues 1073–1112. In view of our previous findings that the MoAb 61BG1.3 prevents colonization of *P. gingivalis in vivo* and inhibits haemagglutination, these two epitopes may be in proximity in the native protein. Active or passive immunization strategies which target the protective or haemagglutinating epitopes of the adhesion domain of PrpRI may provide a means of preventing infection with *P. gingivalis*.

**Keywords** periodontitis *Porphyromonas gingivalis* bacterial adhesion colonization epitope haemagglutinin

## INTRODUCTION

*Porphyromonas gingivalis* has been implicated in the pathogenesis of human periodontitis [1], which is the main cause of tooth loss. In animal models *P. gingivalis* causes destructive disease and bone loss [2,3]. The bacterium elaborates numerous potential virulence determinants, including proteases, which may provoke or deregulate the inflammatory response in the host tissues, which in turn can lead to destructive disease [4]. In addition, *in vitro* studies suggest that proteases may contribute directly to destruction of host tissue [5]. The gene encoding an extracellular arginine-specific protease of *P. gingivalis* has been cloned and sequenced [6,7]. The deduced amino acid sequence is that of a polyprotein precursor, termed PrpRI, which undergoes proteolytic processing. Processing produces an $\alpha$ proteolytic domain and a $\beta$ adhesion domain which associate non-covalently to form the mature protease termed RI. The $\beta$ domain mediates haemagglutination, although recent studies indicate that the primary function may be to bind to proteins of the extracellular matrix and suggest a role in adhesion of the micro-organism [8]. A mutant of *P. gingivalis* which was deficient in RI showed reduced binding to extracellular matrix proteins, human oral epithelial cells and Gram-positive bacteria, which is also consistent with a role in adhesion [9].

The specific immune response may play an important role in the control of the microbial insult to the host, either by affecting the pattern of colonization by periodontal organisms or by neutralizing products released from the biofilm. Higher titres of antibodies were reported against putative periodontal pathogens in patients with periodontitis compared with clinically healthy controls [10–14]. Immunization with whole cells or purified components of *P. gingivalis* reduced levels of the bacterium in experimental animal models and resulted in less bone loss compared with sham-immunized controls [15,16], although in a separate study increased inflammation was observed following immunization [17].

Correspondence: Charles G. Kelly, Department of Immunology, Floor 28 Guy's Tower, Guy's Hospital, London Bridge, London SE1 9RT, UK.

We have demonstrated a role for specific antibody in the prevention of colonization with *P. gingivalis* by topical application of MoAb 61BG1.3 [18] in patients with periodontitis [19]. Passive immunization prevented recolonization of *P. gingivalis* for 9 months and may provide an effective procedure to prevent potentially damaging host response. The MoAb 61BG1.3 recognizes a protein determinant within the $\beta$ adhesion domain of the protease RI and inhibited haemagglutination of erythrocytes mediated by intact cells of *P. gingivalis* or by a preparation of RI protease [20], which might account for its *in vivo* effect.

Sera from patients with periodontal disease may be distinguished from control sera on the basis of antibody recognition of the RI protease complex [21]. Furthermore, several MoAbs which inhibit binding of human serum IgG antibodies to *P. gingivalis* recognize the $\beta$ adhesion domain of RI, suggesting that part of the human IgG antibody response may be directed against the adhesion rather than the proteolytic component of RI [22,23]. The $\beta$ adhesion domain may be an abundant antigen, since at least three further gene loci which encode homologous sequences have been identified in *P. gingivalis* [23].

In the present study, we aimed to map the epitope within RI which is recognized by MoAb 61BG1.3, to investigate the relationship of that binding site to the regions of the molecule involved in adhesion, and finally to map B cell epitopes of the $\beta$ adhesion domain of RI which are recognized by patients with chronic adult periodontitis.

## PATIENTS AND METHODS

*Materials*

Fmoc amino acids, benzotriazole-l-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) and Rink Amide MBHA resin were purchased from Calbiochem-Novabiochem (Nottingham, UK). Dimethylformamide, trifluoroacetic acid, diethyl ether and piperidene were purchased from Romil Chemicals Ltd (Loughborough, UK). Di-isopropylethylamine was from Aldrich Chemical Co. (Poole, UK).

*Patients and controls*

Patients ($n=14$), aged 26–55 years, were selected from those referred for periodontal treatment to Guy's Dental Hospital, in whom *P. gingivalis* was detectable and constituted at least 1% of the microbial flora in plaque samples by an immunofluorescent assay [19]. Patients had at least 20 standing teeth and had been given oral hygiene instruction and scaling but still had at least two probing pocket depths of 5 mm or more which bled after probing. Periodontally healthy control subjects ($n=9$), aged 30–53 years, were selected from dental personnel at UMDS. The mean age of the patients group was 36·0 years (s.d. 12·7) and that of controls was 40·1 years (s.d. 8·4).

*Antibodies*

MoAb 61BG1.3 (IgG1 class) was kindly provided by Dr R. Gmür (Department of Oral Microbiology and Immunology, Dental Institute, University of Zürich, Switzerland). An isotype-matched control MoAb raised against human papilloma virus (HPV) was kindly provided by Dr P. Shepherd (Department of Immunology, UMDS). The anti-glutathione S-transferase (anti-GST) MoAb (also IgG1) was from Autogen Bioclear UK Ltd. (Potterne, UK).

*Bacteria and culture conditions*

*Porphyromonas gingivalis* W50 was maintained by subculture on blood agar at 37°C in an atmosphere of 80% $N_2$, 10% $CO_2$ and 10% $H_2$ [19]. *Escherichia coli* XL-1 Blue was grown on Luria–Bertani medium supplemented with 20 $\mu$g/ml tetracycline and 50 $\mu$g/ml ampicillin.

*Recombinant protein expression*

The portion of *prpR1* which encodes residues 784–1130 was cloned into pGEX-3X (Pharmacia Biotech, St Albans, UK) and truncated derivatives were produced following digestion with *Kpn*I, *Age*I, *Ngo*MI or *Eag*I, as described previously [23]. Expression of recombinant polypeptides in *E. coli* XL-1 Blue was induced by isopropyl $\beta$-D-thiogalactoside (1 mM). *Escherichia coli* cells were harvested by centrifugation and lysed in 1:10 of the original culture volume in 3% SDS. Recombinant polypeptides were expressed as fusion proteins with GST. For purification of GST-fusion proteins, cells were harvested, resuspended in 50 mM Tris–HCl pH 8·0 containing 10 mM EDTA, and lysed by sonication. Sonicates were centrifuged (18 000 *g* at 4°C for 30 min) and supernatants were applied to a column of glutathione-Sepharose (Pharmacia Biotech). Non-retarded material was removed by washing in PBS pH 7·2 and bound material was eluted with 20 mM glutathione in 50 mM Tris–HCl pH 8·0. Glutathione was removed by dialysis against 50 mM Tris–HCl pH 8·0. To purify recombinant proteins which remained in the insoluble fraction after sonication and centrifugation, pellets were resuspended in 6 M urea, which was subsequently diluted to 1·5 M before purification as above.

*Synthetic peptides*

Peptide amides (20 mers overlapping by 10 residues) spanning residues 785–1122 were synthesized on Rink amide MBHA resin using the BT7400 manual peptide synthesis block (Biotech Instruments Ltd, Kimpton, UK) with Fmoc chemistry. PyBOP was used as coupling agent and Fmoc amino acids were activated *in situ* by addition of diisopropylethylamine. Following 20 cycles of synthesis, resin was washed with dimethylformamide followed by diethyl ether, and peptides were cleaved with trifluoroacetic acid-ethanedithiol-anisole-phenol-$H_2O$ (82·5:2·5:5:5:5; v/v/v/w/v) as described in the manufacturer's manual. Peptides were precipitated by the addition of 5 volumes ether, recovered by centrifugation and washed three times with ether. Finally, peptides were dissolved in water and lyophilized. The scale of synthesis was 5 $\mu$mol. Selected peptides were analysed by mass spectrometry. Two further peptides spanning residues 907–926 and 912–931 were synthesized in which Cys 913 was substituted with Ala for analysis of MoAb 61BG1.3 recognition.

*ELISA*

Antibody (MoAb 61BG1.3 and human sera) recognition of synthetic peptides was determined by ELISA. Peptides (10 $\mu$g/ml) in PBS were adsorbed to wells of polystyrene microtitre plates (Dynatech Labs Ltd, Billingshurst, UK) for 2 h at room temperature. Plates were washed and wells were treated with 0·5% (w/v) bovine serum albumin (BSA) for 1 h at room temperature to block unbound sites. After washing, bound peptides were incubated with serially diluted sera in duplicates. Bound IgG antibodies were determined by incubation with alkaline phosphate-conjugated goat anti-mouse IgG or goat anti-human IgG (Sigma Chemical Co., Poole, UK) and subsequent reaction with paranitrophenyl

phosphate (Sigma). Plates were read at 410 nm using a Dynatech MR 700 microplate reader. The assay was repeated at least three times with each serum. Intact cells of *P. gingivalis* were included in each assay, as was an irrelevant peptide (HQAAMQIIRDII-NEEAADWD) derived from the sequence of simian immunodeficiency virus (SIV) p27 protein. For human sera, titres were determined as the highest dilution giving an absorbance $\geq 0{\cdot}2$ (and twice background absorbance) and titres $\geq 1{:}20$ were regarded as indicating positive recognition.

*Western blotting (immunoblotting)*

Epitopes which were recognized by the MoAb 61BG1.3 or human sera were also mapped by Western blotting. Lysates of *E. coli* harbouring recombinant plasmid (probed with MoAb 61BG1.3) or purified GST-fusion proteins (probed with human sera) were prepared as above and separated by SDS–PAGE with gels of 10% acrylamide, by using a mini-gel system (Hoeffler Scientific Instruments, San Francisco, CA). Proteins were electrophoretically transferred to nitrocellulose and nitrocellulose strips were blocked with 10% (w/v) non-fat milk powder and 0·1% sodium azide in PBS pH 7·2. Strips were subsequently incubated with MoAb 61BG1.3 or control MoAb specific to GST (diluted 1:100) or with human sera diluted 1:20 in PBS containing 1·0% (w/v) non-fat milk powder, 1·0% (w/v) BSA and 0·05% (v/v) Tween 20. Bound antibody was visualized by using alkaline phosphatase-conjugated second antibody with 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium (Sigma) as substrates. At least three blots were performed with each antibody.

*Haemagglutination-inhibition assay*

Erythrocytes were separated from human blood and haemagglutination assays were carried out in round-bottomed microtitre plates (Dynatech) with PBS containing 0·1% BSA. Washed *P. gingivalis* (W50) cells were suspended at an absorbance optimum for haemagglutination of 0·02 at 540 nm, which represents four times the minimum haemagglutinating dose, and used to agglutinate a 0·3% (packed cell v/v) suspension of erythrocytes. The panel of peptides and an irrelevant control peptide from the cell surface antigen I/II of *Streptococcus mutans* (VIDGKTVLAGSTNYYELTWD) were serially diluted from 50 to 1·5 $\mu$M and tested for their ability to inhibit haemagglutination. The MoAb 61BG1.3 was used as a control inhibitor of haemagglutination.

*Statistical analysis*

Differences in the end-point titre of antibodies recognizing peptides of RI and the irrelevant SIV peptide were tested by the Wilcoxon signed-ranks matched-pairs test. Differences in the antibody titres between healthy control subjects and patients to the six most frequently recognized RI peptides and the SIV control peptide were initially investigated by Kruskal Wallis analysis of variance and subsequently for each individual peptide by Mann–Whitney *U*-tests. All tests were accepted as statistically significant if $P < 0{\cdot}05$.

## RESULTS

*Mapping of the epitope recognized by MoAb 61BG*

We have shown previously that the MoAb 61BG1.3 recognizes a recombinant polypeptide which comprises residues 784–1130 within the $\beta$ adhesion domain of the PrpR1 polyprotein [20]. This polypeptide is encoded by the plasmid pJM11 [23]. The insert from pJM11 was subcloned into pGEX-3X and a series of 3′ truncations was produced by digestion with restriction endonucleases as described previously [23]. Lysates of *E. coli* which expressed the recombinant fusion polypeptides were analysed by Western blotting for recognition by MoAb 61BG1.3 (Fig. 1, lanes a–f) or by the anti-GST MoAb (lanes g–l). Polypeptides comprising residues 784–949 and 784–931 (expressed by plasmids which were digested with *Kpn*I and *Age*I, respectively) were recognized strongly by the MoAb 61BG1.3, while the polypeptide corresponding to residues 784–907 (derived from plasmid digested with *Ngo*MI) was recognized only very weakly. The polypeptide corresponding to residues 784–803 (derived from plasmid digested with *Eag*I) or the GST component were not recognized. In addition to the band of the predicted $M_r$, several lower $M_r$ forms were evident in each lane and presumably represent proteolytic products. No recognition of any fusion protein by the isotype-matched MoAb of irrelevant specificity (raised against HPV) was evident (data not shown). These results map the epitope which is recognized by MoAb 61BG1.3 to residues 907–931 of PrpRI (GVSPKVCKDVTVEGSNEFAPVQNLT), although residues N-terminal to this may make some contribution to recognition.

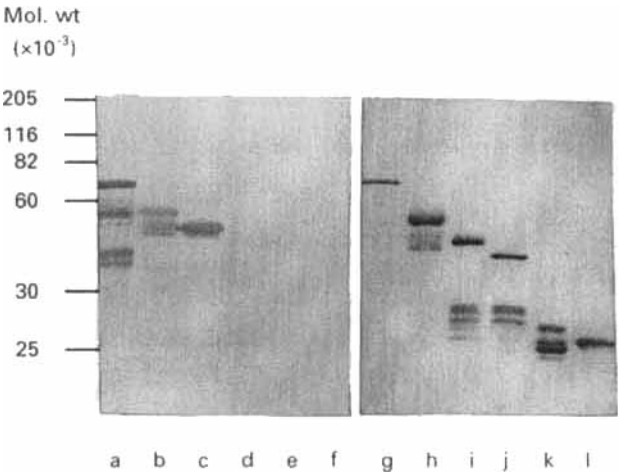



**Fig. 1.** Recognition of $\beta$ adhesion polypeptides by MoAb 61BG1.3. Lysates of *Escherichia coli* expressing recombinant polypeptides of PrpRI from *Porphyromonas gingivalis* comprising residues 784–1130 (lanes a and g), 784–949 (lanes b and h), 784–931 (lanes c and i), 784–907 (lanes d and j) and 784–803 (lanes e and k) were analysed together with lysates from *E. coli* harbouring non-recombinant pGEX-3X (lanes f and l). Blots were probed with MoAb 61BG1.3 (lanes a–f) or anti-glutathione-S-transferase (GST) MoAb (lanes g–l).

The panel of overlapping synthetic peptides, which span the sequence encoded by pJM11, was also analysed for recognition by MoAb 61BG1.3 by ELISA. No recognition of peptides was evident. To exclude the possibility that the lack of recognition was due to the formation of disulphide-bonded dimers of synthetic peptides with Cys 913, peptides were synthesized spanning residues 907–926 and 912–931, in which Cys 913 was substituted by Ala. These peptides were not recognized in the assay (result not shown).

*Mapping of the haemagglutinating determinant*

The MoAb 61BG inhibits haemagglutination mediated by intact cells or culture supernatants of *P. gingivalis* [20]. We therefore investigated the relationship between the epitope recognized by the

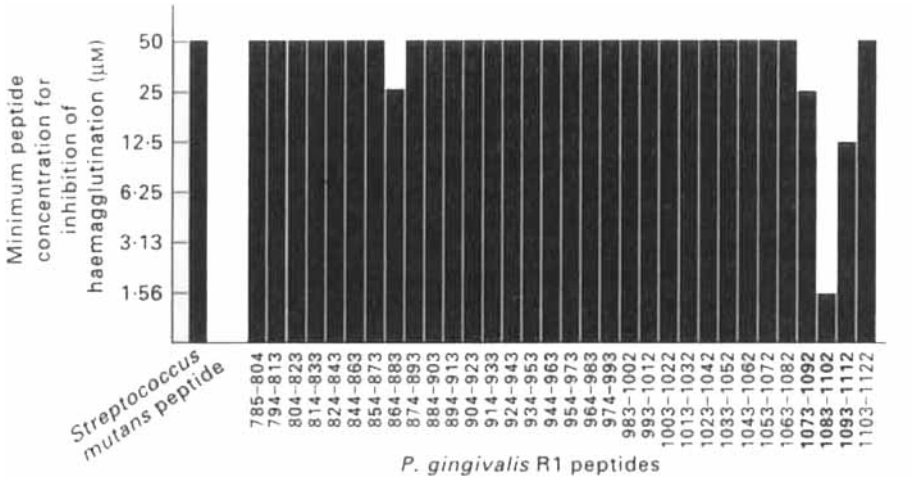



**Fig. 2.** Inhibition of haemagglutination by synthetic peptides spanning residues 785–1122 of PrpRl of *Porphyromonas gingivalis*. Synthetic peptides (20mers overlapping by 10 residues) were assayed for inhibition of haemagglutination of human erythrocytes mediated by intact cells of *P. gingivalis*.

MoAb and haemagglutinating determinants of the β adhesion component. The panel of overlapping synthetic peptides was assayed for inhibition of haemagglutination mediated by intact cells of *P. gingivalis*. As shown in Fig. 2, three overlapping peptides which spanned residues 1073–1112 of PrpRl in the C-terminal region of the β adhesion consistently inhibited haemagglutination. Peptide 1083–1102 (GVRSPEAIRGRIQSTWRQKT) inhibited most efficiently, with a minimum inhibitory concentration of $<1{\cdot}5\ \mu M$, while peptides 1073–1092 and 1093–1112 inhibited less efficiently, with minimum inhibitory concentrations of $25\ \mu M$ and $12{\cdot}5\ \mu M$, respectively. Partial lysis of erythrocytes by some of the peptide preparations was evident at the highest concentrations.

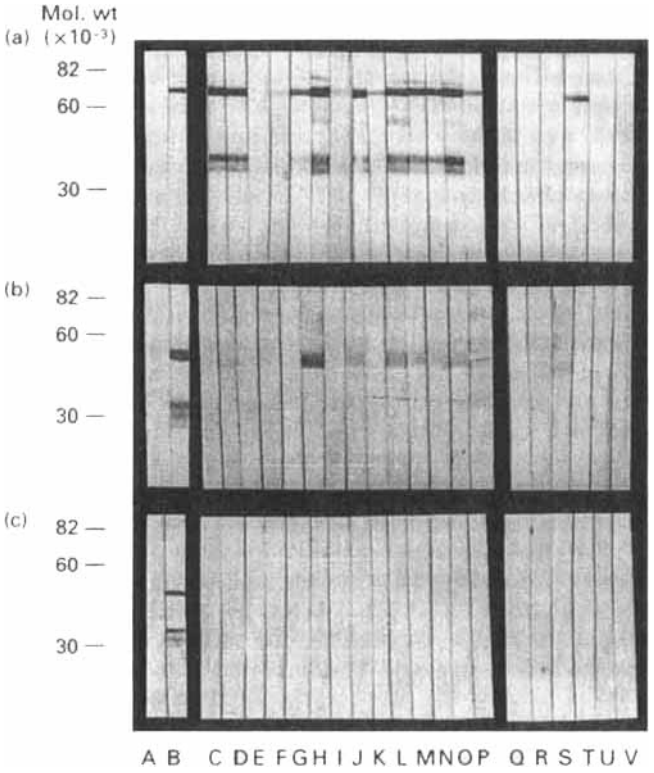



**Fig. 3.** Human serum recognition of β adhesion polypeptides. Purified glutathione-S-transferase (GST)-fusion proteins of recombinant polypeptides of PrpRl comprising residues 784–1130 (a), 784–931 (b) and 784–907 (c) were probed with sera from patients with periodontitis (lanes C–P), periodontally healthy subjects (lanes Q–V) or anti-GST MoAb (lane B) and anti-human papillomavirus (HPV) MoAb (negative control, lane A).

*Serum antibody recognition of β adhesion domain*

To determine whether human serum antibody responses were directed towards the epitope recognized by the protective MoAb 61BG1.3 or the haemagglutinating determinant, sera from patients with periodontitis were compared with those of a group of healthy subjects. Immunofluorescent analyses of subgingival plaque samples demonstrated that all patients harboured *P. gingivalis*. Western blot analyses of sera from 14 patients with periodontitis and six periodontally healthy controls using the recombinant polypeptides described above demonstrated that 13/14 patients recognized the polypeptide 784–1130 (Fig. 3a). Polypeptide 784–931 was recognized more weakly by 9/14 patients, but polypeptide 784–907 was recognized only by one patient. Polypeptide 784–1130 was recognized by only one healthy control, while the other polypeptides were not recognized by any control subject. Low levels of *P. gingivalis* (<1% of microbial flora) were present in plaque samples from the control subject whose serum recognized polypeptide 784–1130. These results indicate that the predominant antibody responses are directed towards epitopes C-terminal to residue 907.

Further epitope mapping was carried out by ELISA using the panel of overlapping synthetic peptides. The number of periodontally healthy controls was increased to nine for this assay. Intact cells of *P. gingivalis* were recognized by all 14 patients (Fig. 4), with titres of 1:20–1:640 and by 5/9 healthy subjects with titres of 1:40–1:80. The frequency of responses to each peptide is also shown in Fig. 4, where positive responses were determined by the criteria described above. In agreement with the Western blotting data, peptides spanning residues 934–973, 974–1042 and 1093–1112 in the central and C-terminal regions were recognized most frequently by sera from patients and to a lesser extent by controls. In addition, peptide 784–804 was recognized by 11/14 patients and by 5/9 healthy controls, while peptides spanning residues 794–843

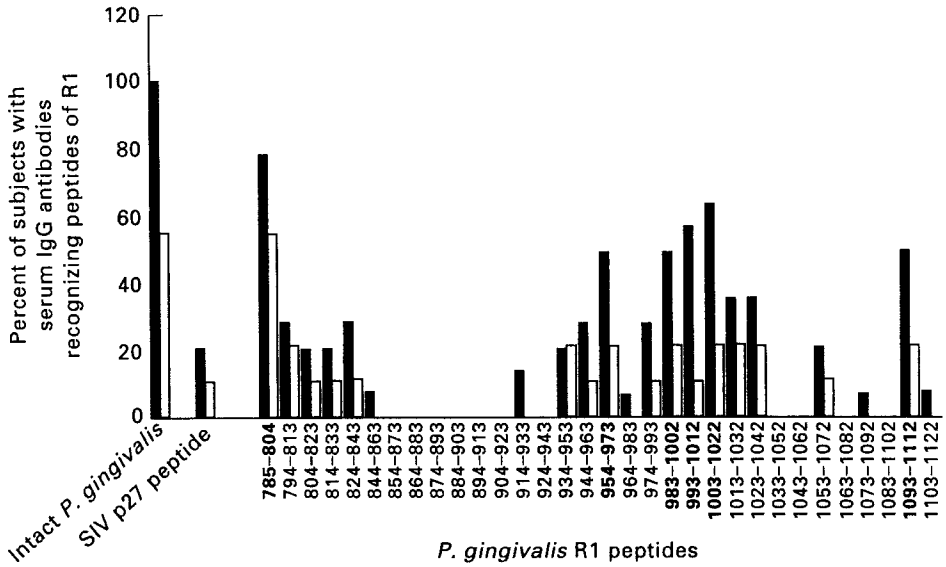



**Fig. 4.** Serum IgG antibody responses assessed by ELISA to *Porphyromonas gingivalis* Rl peptides in patients with periodontitis (■, $n = 14$) and periodontally healthy controls (□, $n = 9$). The frequency distribution of patients and controls responding to intact *P. gingivalis*, a control peptide from SIV p27 or the panel of 20mer peptides of *P. gingivalis* Rl is shown. Serum titres of ≥ 1:20 were regarded as indicating recognition (as described above). Significant differences in serum antibody responses to the peptides shown in bold were found between the groups.

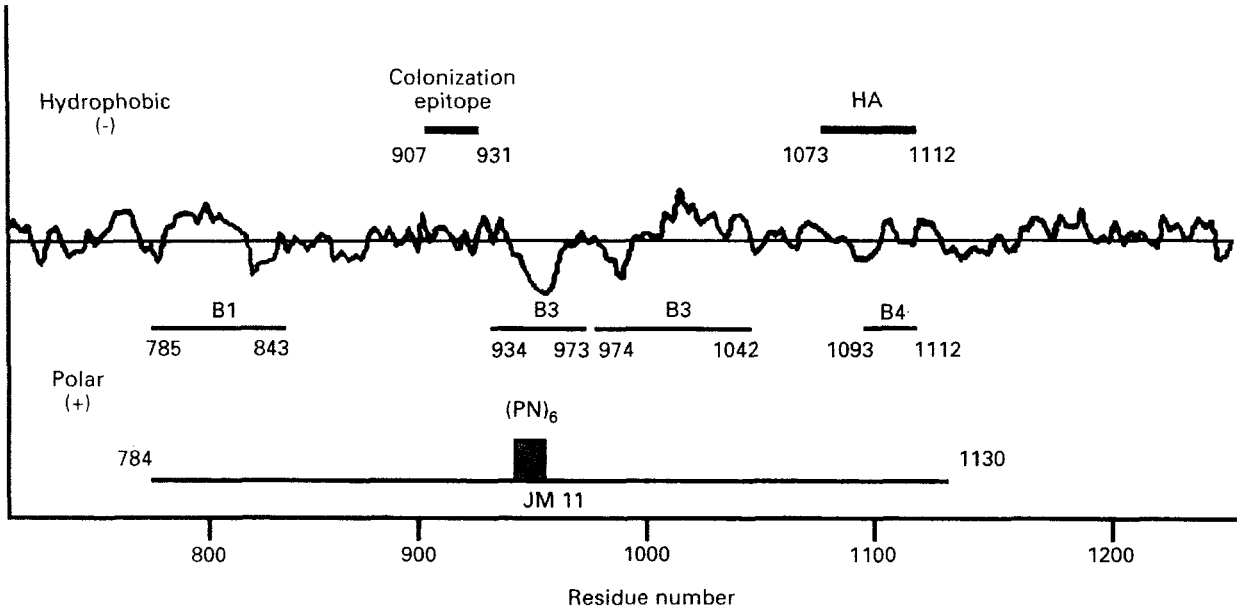
were recognized at lower frequency. Statistical analysis of individual serum titres against peptides indicated that peptides spanning residues 784–840, 993–1022 or 1093–1112 were recognized at higher titres than the control SIV peptide (Wilcoxon test, $P < 0.05$). Kruskal Wallis analysis of variance showed significantly elevated antibody responses in patients compared with healthy controls to the peptides highlighted in Fig. 4 ($P < 0.001$). Subsequent Mann–Whitney *U*-tests indicated that sera from patients had significantly higher antibody titres than those from healthy individuals against peptides spanning residues 993–1012 and 1093–1112 ($P < 0.05$) and against intact *P. gingivalis* ($P < 0.001$).

## DISCUSSION

In this study we have mapped an epitope (residues 907–931) which is recognized by MoAb 61BG1.3 which prevents colonization of *P. gingivalis in vivo*, a haemagglutinating epitope and B cell epitopes recognized by sera from patients with periodontitis naturally colonized with *P. gingivalis*. The findings are summarized in Fig. 5, together with a hydropathy plot [24] of the β adhesion domain. Polar sequences within the domain are indicated by positive values for the plot (below the axis) and hydrophobic sequences are indicated by negative values. Polar regions are more likely to be exposed on the surface of the molecule. The colonization epitope (recognized by MoAb 61BG1.3) is in a relatively hydrophobic portion of the domain, but immunofluorescence studies indicate it is exposed and accessible to antibody on the surface of intact cells of *P. gingivalis* [19]. In contrast, the haemagglutinating epitope includes the polar sequence spanning residues 1090–1105. B cell epitopes which were recognized by patients in this study show only partial correlation with polar portions of the sequence (except for the epitope(s) within residues 934–973) which may be exposed on the surface of the intact molecule. In addition, antibody recognition of more hydrophobic sequences, namely those within residues 785–843 and 974–1042, suggests these regions may also be partly exposed on the surface of the molecule.

The colonization, haemagglutinating and human B cell epitopes are separated in the primary structure of the β adhesion component of the protease complex Rl, although there may be some overlap between the haemagglutinating epitope (within residues 1073–1112) and a B cell epitope (within residues 1093–1112). Although the colonization epitope was mapped to residues 907–931 by Western blotting, no recognition of 20mer synthetic peptides spanning this portion of the sequence was evident when binding was assessed by ELISA. Recognition by this MoAb may require correct formation of a disulphide bond linking Cys 913 to another Cys residue, which would not occur in the synthetic peptide. Alternatively, the epitope may be linear but formed by residues which are separated in the sequence. All residues required for recognition might therefore not be included in a synthetic peptide of 20 residues. We have previously reported that a further MoAb which inhibits *P. gingivalis*-mediated haemagglutination also recognizes the sequence 907–931 [23].

The topological relationship between the haemagglutinating epitope(s) and that recognized by MoAb 61BG1.3 in the native structure is not known. Inhibition of haemagglutination mediated by the MoAb may be the result of steric hindrance. The epitopes may also be in close proximity in the native molecule. Folding of distal sequence elements may be stabilized by disulphide bonds, since there are six Cys residues in this domain. The present study also suggests that the haemagglutinating epitope is linear. The structure of the cell surface adhesin, termed pertactin, from *Bordetella pertussis* provides a precedent for the limited conformational dependence of an adhesion epitope [25]. Pertactin forms an extended β-helix, from which loops protrude which do not possess defined secondary structure and which are associated with biological activity. One such loop includes the Arg-Gly-Asp tripeptide motif which may mediate attachment to integrins.




| Epitope | Residues | Sequence |
|---|---|---|
| Colonization | 907–931 | GVSPKVCKDVTVEGSNEFAPVQNLT |
| HA | 1073–1112 | ALLEETITAKGVRSPEAIRGRIQSTWRQKTVDLPAGTKYV |
| B1 | 785–843 | PSCSPTNMIMDGTASVNIPAGTYDFAIAAPQANAKIWIAGQGPTKEDDYVFEAGKKYHF |
| B2 | 934–973 | AVGQKVTLKWDAPNGTPNPNPNPNPNPNPGTTTLSESFEN |
| B3 | 974–1042 | GIPASWKTIDADGDGHGWKPGNAPGIAGYNSNGCVYSESFGLGGIGVLTPDNYLITPALDL PNGGKLTF |
| B4 | 1093–1112 | RIQSTWRQKTVDLPAGTKYV |

**Fig. 5.** Colonizing, B cell and haemagglutinating epitopes of the $\beta$ adhesion of *Porphyromonas gingivalis*. A hydropathy plot [24] for the $\beta$ adhesion domain (residues 720–1262 of PrpRl) is shown together with the positions of the recombinant polypeptide JM11, and epitopes determined in this study. Residue numbers (based on PrpRl) and assignment of the domain are from references [6] and [23]. $(PN)_6$: repeating Pro-Asn sequence; colonization epitope : epitope recognized by MoAb 61BG1.3; HA : haemagglutinating epitope; B1–B4 : human B cell epitopes. Amino acid sequences of the epitopes are listed.

In common with several bacterial adhesins [26], the $\beta$ component of Rl protease includes a proline-rich sequence. This sequence $(PN)_6$, which comprises residues 950–962 (Fig. 5), is within the region of the $\beta$ domain to which B cell responses were predominantly directed, namely residues 934–1042. This sequence includes one of the most polar regions of the domain (Fig. 5), which is consistent with it being an exposed portion of the molecule and therefore accessible to antibody. Similarly, the immunodominant region of pertactin overlaps with and is C-terminal to a proline-rich sequence which contains the motif $(PQP)_5$ in the C-terminal region of the molecule [27]. This part of the sequence is also in one of the loops which protrude from the predominantly $\beta$-helix structure of pertactin [25].

The presence of serum antibodies which recognize the $\beta$ component of Rl and other members of this sequence-related family of gene products may not be sufficient *per se* to confer protection against infection with *P. gingivalis*, since the sera which were analysed in this study were collected from patients with periodontitis and their plaque contained *P. gingivalis*. Properties of serum antibody, such as isotype or affinity, but especially the epitope which is recognized, may influence protection. Indeed, antibodies to the colonization epitope peptide 907–931 were not detected by ELISA in the sera of patients with periodontitis, although Western blotting suggested weak recognition of this epitope by some patients. This suggests that residues 907–931 might be a target to which protective antibodies should be directed. The limited overlap between haemagglutinating epitopes and those recognized by patients' sera may also be significant. Identification of colonizing and haemagglutination inhibiting epitopes of the $\beta$ component of the protease Rl may provide important targets to which protective antibodies should be directed. Induction of antibody responses to these epitopes may prevent infection with *P. gingivalis* and allow evaluation of the role of this microorganism in disease.

## ACKNOWLEDGMENTS

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