

PROTEOMIC PEPTIDE SCAN OF PORPHYROMONAS GINGIVALIS FIMA TYPE II FOR SEARCHING POTENTIAL B-CELL EPITOPES

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SUMMARY

Purpose. To identify potential antigenic targets for Porphyromonas gingivalis vaccine development.

Materials and methods. In the present study, we analyzed the Porphyromonas gingivalis, fimA type II primary amino acid sequence and characterized the similarity to the human proteome at the pentapeptide level.

Results. We found that exact peptide-peptide profiling of the fimbrial antigen *versus* the human proteome shows that only 19 out of 344 fimA type II pentapeptides are uniquely owned by the bacterial protein.

Conclusions. The concept that protein immunogenicity is allocated in rare peptide sequences and the search the Porphyromonas gingivalis fimA type II sequence for peptides unique to the bacterial protein and absent in the human host, might be used in new therapeutical approaches as a significant adjunct to current periodontal therapies.

Key words: periodontitis, bacterial *versus* human peptide overlap, low-similarity peptides, peptide vaccine.

Introduction

Porphyromonas gingivalis (Pg) is a gram-negative anaerobic bacterium, and its central role in the development of periodontal disease is well known (1).

Periodontitis is a chronic disease of the tissues which surround and support teeth (periodontal). It leads to irreversible bone reduction and, in its advanced stages to teeth loss. As it is an inflam-

matory disease, many studies have been performed to better understand its pathogenesis, focusing both on the human immune system and on bacterial flora. Analyses on the latter have led to identify, among numerous microorganism frequently found in sub-gingival plaque, Pg as the bacterium with the main role in both onset and progression of periodontal disease. Its presence in patients' saliva has showed high sensitivity in diagnosing parodontitis, in particular with its aggressive forms (2).

In addition to its role in initiation/progression/diagnosis of periodontal diseases, it has been showed how Pg-mediated periodontitis is commonly linked to major oral conditions such as mucositis, burning mouth syndrome, dysplastic lesions, lesions of gastroesophageal reflux, mucosal atrophy and lichen planus (3-9).

Pg expresses proteinaceous appendages on its outer surfaces called fimbriae. Fimbriae of Pg, which are recognized as major virulence factors (10), are classified into six genotypes (types I, Ib, II, III, IV, V), based on the genotype of the fimA gene encoding the fimbrial subunits. Among the six genotypes, fimA type II strains are thought to be most strongly related to advanced periodontitis.

Nakagawa et al. (11) showed that fimA genotype II has a greater ability to adhere to and invade human epithelial cells than other fimA genotypes. Moreover, mutants in which the fimA type I gene was substituted with type II presented enhanced bacterial adhesion/invasion, supporting the fact that type II fimbriae are a critical determinant of virulence.

Recent studies have showed with sufficient evidence that antibodies can protect against Pg infections. Animal models have showed passive immunization using poly- and monoclonal antibodies, and humoral immune-responses against specific Pg antigens, showing that antibodies may have an anti-Pg function (12). Moreover, fimA has been suggested as a vaccine candidate to control *P. gingivalis*-induced periodontal disease.

The search for specific epitopic features of Pg fimA, able to induce immunogenicity, is still an enigmatic topic and molecular bases of small-peptide sequences immunogenicity is still unclear (13), so increasing the difficulty coefficient of developing effective vaccines against such microorganisms.

In this study, we map Pg fimA type II protein, searching for unique small peptides which may have immunogenic potential and thus be used in Pg immunotherapy.

The rationale is to search for aminoacidic sequences rarely found in human proteins, which

may induce immunologic response(s). As a matter of fact, unique protein sequences are more likely to trigger a host immune response than peptide motifs which are highly repeated/present in the host proteome, as the latter are expected to be silenced by the host's self-tolerance mechanism. Relationship between sequence frequency and immunoreactivity has been validated in cancer, autoimmunity and infectious disease models. Peptide regions with no/limited similarity to sequences of the host proteome were demonstrated to be successful specific targets using mono- and/or polyclonal antibodies against desmoglein-3 (14, 15), HPV16 E7 (16), EC Her-2/Neu oncoprotein (17), melan-a/MART-1 (18), high-molecular-weight melanoma associated-antigen (19, 20), tyrosinase (21, 22), HIV (23), HCV (24), and influenza A (25) proteins. Ulterior evidence was supported from epitope mapping literature, showing that most peptide epitopes follow the low-similarity rule (26).

Materials and methods

P. gingivalis FimA type II protein, corresponding to GenBank accession number: D17798; NCBI Taxonomic identifier: 837; UniProtKB/Swiss-Prot accession number: Q51822; length: 348aa (27), was analyzed for pentapeptide sharing with human proteome as follows.

The bacterial amino acid (aa) sequence was dissected into 344 sequential pentapeptides, overlapping by four residues, that is MVLKT, VLKTS, LKTSN, KTSNP, TSNPN, etc.

Each bacterial pentapeptide was used as a probe to scan the entire set of proteins forming the human proteome for identical matches (19). The pentapeptide matching analysis utilized PIR protein database and perfect peptide match program (<http://pir.georgetown.edu/pirwww/>). The number of matches of each bacterial pentamer to the human proteome varied in a wide range, from no matches to hundreds of matches. A pentapeptide that has five (or less than five) perfect matches to the host proteome was considered to be a low-

similarity sequence, that is, a rare fragment (28). Pentapeptides were used because the literature indicates that five to six amino acids are a sufficient minimal determinant for an epitope-paratope interaction, and thus a pentapeptide can act as an immune unit and play a crucial role in cell immunoreactivity and antigen-antibody recognition.

Results

The peptide-peptide profiling of *Porphyromonas gingivalis* fimA type II protein versus the human proteome indicates how many times each bacterial pentapeptide occurs in the human proteome, as shown in Figure 1. Immunologically, Figure 1 seems to indicate that utilizing the entire fimbrial antigen in anti-*P. gingivalis* immunotherapeutic approaches carries a high risk of potential cross-reactions with the human proteins. Indeed, a vast pentapeptide sharing exists between *P. gingivalis* fimA type II protein and the human proteome.

Only 19 out of 344 bacterial pentapeptides are uniquely owned by the bacterial protein. The 19 zero-similarity pentapeptides are reported in Table 1.

It is also of note that a number of pentapeptides unique to the *P. gingivalis* are consecutively overlapping, thus forming hexapeptide sequences that may be particularly useful in *P. gingivalis* vaccine formulations. The following hexapeptide are formed by consecutive overlapping pentapeptides with no similarity to human proteins: **KYNFAP**, **LTNFNG**, **ANYTHV**, **SNNAPQ**, and **GYTPKN** (see also Table 1, sequences in bold).

Overall, the peptide-peptide profile of fimA type II compared to the human proteome shows that the *P. gingivalis* protein pentapeptide overlap to the human proteins in 325 bacterial pentapeptides for a total of 2,273 occurrences (Figure 1). Thus, it seems logical to postulate that a vaccine based on the bacterial pentapeptides not present in the human proteome might have the potential to exclusively hit the bacterial antigen without cross-reacting with the human host.

Discussion

Chemically synthesized small peptides, reproducing hi-antigenicity microorganisms' loci, are commonly used to produce effective vaccines, able to induce antibodies that react with full-

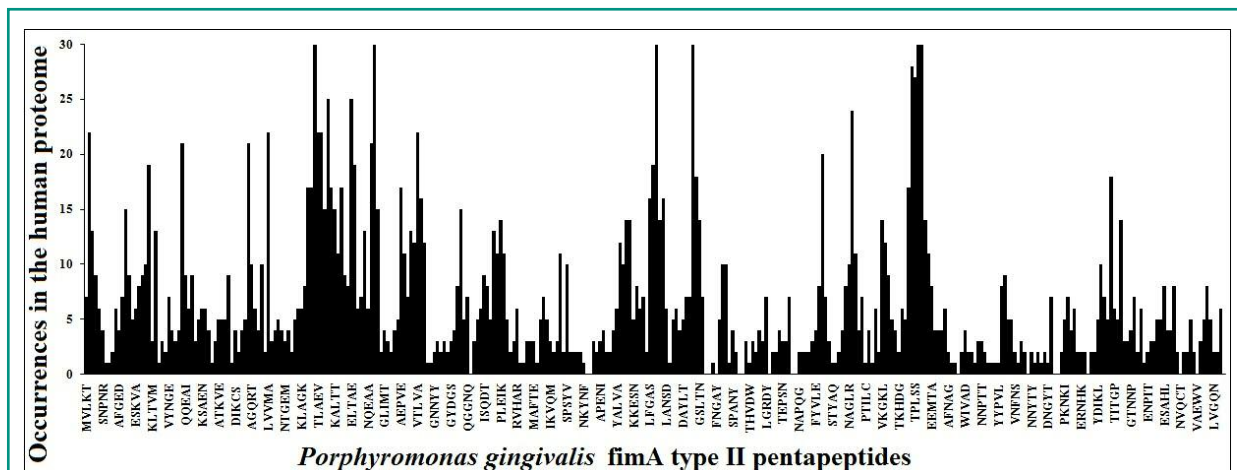


Figure 1 Pentapeptide identity profile of the *P. gingivalis* fimA type II protein versus the human proteome. The columns indicate the number of occurrences in the human proteome for each bacterial pentapeptide.

Table 1 - Peptide profile of *P. gingivalis* fimA type II primary sequence versus the human proteome: the unique *P. gingivalis* fimA type II identity spots at the pentapeptide level¹.

Position ²	Sequence ³
117-121	GGNQI
152-156	KYNFA
153-157	YNFAP
188-192	LTNFN
189-193	TNFNG
191-195	FNGAY
198-202	ANYTH
199-203	NYTHV
207-211	GRDYT
214-218	SNNAP
215-219	NNAPQ
264-268	AGWIV
285-289	SNNYT
293-297	GYTPK
294-298	YTPKN
303-307	NHKYD
331-335	NVQCT
336-340	VAEWV
344-348	QNATW

¹ A pentapeptide similarity analysis of the *P. gingivalis* fimA type II protein was conducted against the human proteome as described under Methods. Briefly, the number of matches scores the similarity level to the human proteome for each pentapeptide, and is obtained using PIR Perfect Peptide Match program (<http://pir.georgetown.edu>). The similarity level of each pentapeptide is calculated as the number of times the pentapeptide occurs in the human proteome, any such occurrence being termed a match. The similarity level is zero when the pentapeptide is absent in the human proteins. The *P. gingivalis* fimA type II pentapeptides with zero similarity to the human proteome are sequentially listed by aa position along the bacterial protein.

² Aa position along the bacterial protein.

³ Aa sequences given in one-letter code.

⁴ Consecutively overlapping pentapeptides given in bold.

length proteins. On this foreword, identification of peptide able to evoke the antibody recognition of Pg may be the first step toward specific vaccines, as it has already happened for several pa-

thologies and infectious diseases. Peptide-based immuno-stimulants are commonly used in oncology too. Tumor antigen-derived peptides triggered powerful anti-tumor immunity in the murine melanoma M-3; cytotoxic CD4+ and CD8+ T lymphocytes derived by mutant p21-ras (12Val) peptide vaccination were able to kill selectively autologous cancer cells carrying this mutation; Her-2/neu peptide (aa 657-665) is an immunogenic epitope of Her-2/neu oncoprotein with powerful anti-cancer activity; it has been showed that the 15-mer amino-acid sequence 101-115 (PPAYEKLSAEQSPPP) of the Melan-A/MART-1 melanoma antigen is an effective target for a vigorous and safe immunotherapy. Others peptide-based vaccine are used to prevent infective disease. Chemically synthesized peptides, which showed antigenicity on the S1 subunit of pertussis toxin, induced a peak of antibody title against native pertussis toxin in mice; linear peptide containing minimal T- and B-cell epitopes of plasmodium falciparum circumsporozoite protein showed immunogenicity against a transgenic sporozoite challenge.

Given these scientific bases, Pg fimA type II protein was analyzed in order to identify potential Pg immunogenic epitopes. Low-similarity and immunogenicity have a well-known relationship: on this premise, we observed that sequences of fimA type II made up of pentapeptide fragment(s) with low similarity to the human proteome could carry the immunogenic potential to evoke the humoral antibody recognition of Pg, and thus immune response is more likely to be triggered by antigenic motifs rarely found in human proteins.

In our previous study (9), the Pg fimA type I was used in the similarity analysis to the human proteome. This analysis showed the presence of 14 out of 343 bacterial fimA type I pentapeptides uniquely owned by the bacterial protein. Seven of these 14 zero-similarity pentapeptides are also present in fimA type II (FNGAY, SNNYT, YTPKN, NHKYD, NVQCT, VAEWV, and QNATW).

These peptide sequences might be particularly important in *P. gingivalis* vaccine formulations.

One of the current targets of periodontitis research is try to limit the infective potential of Pg. Results from this study could provide a method to understand the immune potential of this bacterium, facilitating the epitopic characterization of Pg (9). Proteomic peptide of porphyromonas gingivalis may also be influenced by prosthetic (29-33) and endodontic clinical outcome (33, 34).

The exact characterization of immunogenic Pg peptide sequences may be a successful approach to an effective active/passive immunotherapy anti-Pg, reducing risk of adverse side effects, which is a major issue regarding antibody-based therapies. Clearly, side effects may be induced by cross-reactivity resulting from using full-length antimicrobial agents, which may contain strains with high-similarity to human proteome (Figure 1). The use of low-similarity peptides help preventing undesirable adverse effects, as target sequence(s) are present in the microorganism(s) proteome only.

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