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Designing a peptide-based vaccine against *Porphyromonas gingivalis*

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1. ABSTRACT

Using proteome databases and exploiting the concept that a rare sequence is a potential epitope, epitopic sequences derived from *Porphyromonas gingivalis* fimA type I protein were examined for pentapeptide sequence similarity score to the human proteome. We obtained data showing that most of the linear bacterial determinants are (or are formed by) peptide fragment(s) absent (or rarely found) in the human proteins. These results seem to confirm the hypothesis that low-sequence similarity may contribute to shape the epitope repertoire and provides a potential tool for designing new immunotherapeutic approaches to apply in *Porphyromonas gingivalis* infected periodontitis.

2. INTRODUCTION

Porphyromonas gingivalis, a black pigmented gram-negative anaerobic bacterium, is an important etiologic agent of chronic inflammatory disease of periodontium (1, 2). In particular, it has been associated with aggressive forms of periodontitis and its presence in patients' saliva is nowadays considered of high sensitivity in diagnosing parodontitis (3). In recent years, *P. gingivalis*-mediated periodontal disease has been linked to several systemic conditions such as atherosclerotic cardiovascular disease (4, 5), pregnancy complications (6), diabetes (7, 8), pulmonary disease (9, 10), osteoporosis (11) and cancer (12, 13).

P. gingivalis expresses fimbriae on the cell surface, which are recognized as major virulence factors influencing disease initiation and progression (14-18) and, consequently, are considered as promising candidate antigens for vaccine development (18). This consideration is supported by the following observations. First, anti-*P. gingivalis* fimbriae antibodies occur in the serum of patients with adult periodontitis (19, 20), and antibody-secreting cells specific for *P. gingivalis* fimbriae have been found in the inflamed gingival tissue of periodontitis patients (21, 22); second, monoclonal antibodies against a particular subunit of *P. gingivalis* fimbriae, the so-called major fimbrial subunit protein type-1 (fimA, type I) blocked bacterial adhesion to human buccal epithelial cells (23-25). As a further note, immunization in a rat animal model with purified *P. gingivalis* fimbriae or a fimbriae-derived synthetic peptide (aa sequence: GKTLAEVKALTTELTAENQE and VAPAADAPQGFTVLENDYSA, respectively), has been reported to induce protective immunity against periodontal destruction (26).

Fimbriae of *P. gingivalis* are classified into six genotypes (types I to V and Ib), based on the genotype of the fimA genes encoding the fimbrial subunits (27). FimA type I *P. gingivalis* strains, as well as synthetic fimA type I peptide segments, exhibit chemotactic activity, induce production of pro-inflammatory cytokines such as tumour necrosis factor-a, and interleukins 1, 6, and 8 in human monocyte and macrophage cultures, and haemagglutinating activity (28).

Given these premises, *P. gingivalis* fimbriae appear to be highly immunogenic and, consequently, might be an ideal target for vaccine. Recent studies have provided convincing evidence that antibodies can protect against *P. gingivalis* infections (16-18, 29). Passive immunization using mono- and polyclonal antibodies, and humoral immune responses against specific *P. gingivalis* antigens in experimental animals have shown that antibodies may play an anti- *P. gingivalis* role. However, in face of such encouraging data, the epitopic characterization of *P. gingivalis* antigens is performed antigen-by-antigen, searching for specific epitope features for each *P. gingivalis* protein antigen (1, 2, 14-18, 29, 30). More in general, the molecular basis that determine the immunogenicity of specific peptide sequences remain unclear (31, 32). Little is known about the structure-function relationship(s) of protein sequences in immunology. Such a context makes difficult the development of preventive/therapeutic vaccines against infectious agents. Within this framework, we follow the rationale that rare peptide sequences (that is, peptide motifs rarely found in the host proteins), have the potential to elicit immune response(s). Unique protein sequences are more likely to evoke an immune response than highly repeated motifs, which are expected to be immunologically silenced by the host's tolerance mechanisms. The relationship between sequence rarity and peptide immunoreactivity has been already validated in cancer, autoimmunity, and infectious disease models; specific targeting of peptide regions with no or limited sequence similarity to the host proteome was demonstrated using mono- and/or polyclonal antibodies against EC Her-2/Neu oncoprotein (33), desmoglein-3 (34), melan-a/MART-1 (35), high-molecular-weight melanoma associated-antigen (36, 37), tyrosinase (38, 39), prostate specific-antigen (40), HPV16 E7 (41), HCV (42), and influenza A (43) proteins. In addition, the data received substantial support from epitope mapping literature revealing that most peptide epitopes obey the low-similarity rule (44-48).

In the present study, we analyze *P. gingivalis* fimA type I protein searching for potential immunogenic peptides that might be used in anti-*P. gingivalis* immunotherapies. We compare validated *P. gingivalis* fimA type I epitopes currently cataloged at the Immune Epitope Database and Analysis Resources (IEDB) (http://www.immuneepitope.org/) (49), and report that most of *P. gingivalis* epitopes involved in the immune response, and already experimentally validated by Ogawa (28), are characterized by a low level of similarity to the host proteome.

3. METHODS

The primary amino acid (aa) sequence of *P. gingivalis* fimA type I protein, corresponding to GenBank accession number: M19405.1; NCBI Taxonomic identifier: 837; UniProtKB/Swiss-Prot accession number: P0C940; length: 347aa (50); was used in the similarity analysis to the human proteome.

The bacterial sequence under analysis was dissected into 343 sequential pentapeptides, overlapping by 4 residues, that is MVLKT, VLKTS, LKTSN, KTSNS etc.

Each bacterial pentapeptide was used as a probe to scan the entire set of proteins forming the human proteome for identical matches (51). The pentapeptide matching analysis utilized PIR protein database and perfect peptide match program (http://pir.georgetown.edu/pirwww/) (52). 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 (33-44, 53). 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 (54, 55).

Immunological potential of bacterial pentapeptides was investigated by analyzing *P. gingivalis* fimA type I protein epitopes already experimentally validated and currently cataloged in the Immune Epitope Database and Analysis Resources (IEDB) (http://www.immuneepitope.org) (49). At the time of the analysis, IEDB contained numerous *P. gingivalis* epitopes

Table 1. Sequence similarity analysis to the human proteome of *P. gingivalis* fimA type I derived B-cell epitopes (28)

IEDB epitope ID[1,2]	Aa position[3]	Epitopic sequence[4]	Matches to the human proteome[5]
84789	101-110	k**TIVLK**agkn	3
84160	106-115	kagk**NYIGY**s	1
90768	111-120	**YIGYS**gtgeg	1
81211	126-135	dplk**IKRVH**a	2
84685	131-140	kr**vHARM**aft	1
88060	136-145	rm**AFTEI**kvq	1
86504	146-155	**MSAAY**dniyt	1
81191	151-160	dn**IYTFV**pek	0
82487	156-165	fv**PEKIY**gli	3
84138	161-170	iygl**IAKKQ**s	2
89436	186-195	tgsl**TTFNG**a	1
89403	191-200	t**FNGAY**tpan	0
91024	211-220	yvapa**ADAPQ**	2
80178	216-225	ada**PQGFY**vl	2
82633	221-230	gfyvl**ENDYS**	0
81709	226-235	**ENDYS**anggt	0
80414	231-240	angg**TIHPT**i	1
83498	236-245	ih**PTILC**vyg	1
87178	286-295	nyt**YDSNY**tp	0
88952	291-300	sn**YTPKN**kie	0
84584	296-305	knk**IERNH**ky	2
88083	301-310	r**NHKYD**iklt	0
80216	336-345	aewv**LVGQN**a	2

[1]*P. gingivalis* fimA type I IEDB epitope IDs were obtained from http://www.immuneepitope.org. (49),[2]Epitopes as described by Ogawa (28), [3]Aa position as reported by Ogawa (28), [4]Low similarity pentapeptides are given in bold capital in each epitope sequence,[5]Pentapeptide similarity is defined as number of occurrences (matches) in the human proteins.

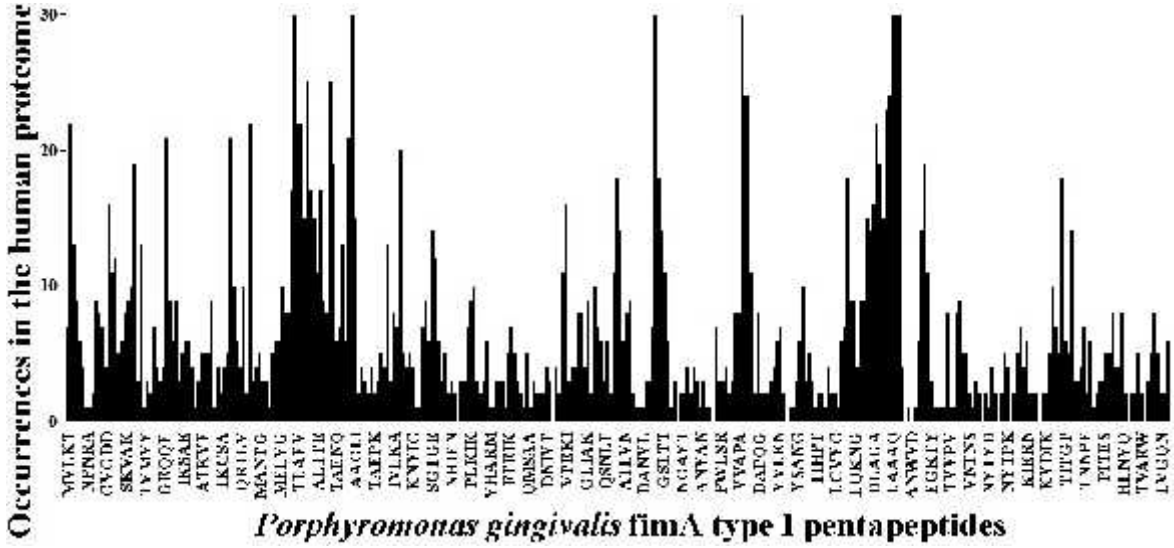

Figure 1. Pentapeptide identity profile of the *P. gingivalis* fimA type I protein *versus* the human proteome. The columns indicate the number of occurrences in the human proteome for each bacterial pentapeptide.

derived from various strains and with different characteristics. Specifically, the IEDB contains B- and T-cell epitopes that are (a) linear or conformational; (b) derived from *P. gingivalis* fimA; and (c) have sequences that were negative, positive, or produced mixed negative/positive results in immunoassays. The present report focused on B-cell linear epitopes derived from *P. gingivalis* fimaA type I protein that were reported to be positive in immunoassays.

4. RESULTS

4.1. Peptide-peptide profiling of *Porphyromonas gingivalis* fimA type I protein *versus* the human proteome

The histogram in Figure 1 shows how many times each bacterial pentapeptide occurs in the human proteome. Immunologically, Figure 1 indicates that utilizing the entire fimbrial antigen in anti-*P. gingivalis* immunotherapeutic

approaches carries a high risk of potential cross-reactions with the human proteins. In fact, it can be seen that a vast pentapeptide sharing exists between *P. gingivalis* fimA type I protein and the human proteome. Only 14 out of 343 bacterial fimbrial pentapeptides are uniquely owned by the bacterial protein. The 14 zero-similarity pentapeptides are, in the order: IENDP, IYTFV, FNGAY, NVPWL, ENDYS, QAANW, ANWVD, SNNYT, YDSNY, YTPKN, NHKYD, NVQCT, VAEWV, and QNATW.

On the whole, exact peptide-peptide profiling of the fimbrial antigen *versus* the human proteome shows that the *P. gingivalis* fimA type I protein pentapeptide overlap to the human proteins amounts to 329 bacterial pentapeptides repeatedly occurring throughout the human proteome for a total of 2,292 occurrences (Figure 1). Hence, it is logical to conclude that a vaccine based on the bacterial pentapeptides not present in the human proteome might have the potential of hitting exclusively the bacterial antigen without cross-reacting with the human host.

4.2. Peptide sequences unique to *Porphyromonas gingivalis* fimA type I protein: immunogenicity analysis

To test the feasibility of utilizing peptide fragments unique to the fimbrial antigen in anti-*P. gingivalis* vaccine design, we analyzed the similarity level of experimentally validated *P. gingivalis* fimA type I epitopes currently cataloged at the IEDB. At the time of the analysis, the IEDB contained 23 *P. gingivalis* fimA epitopes that had tested as positive in immunoassays (28).

Table 1 clearly shows that all *P. gingivalis* fimA type I immunoreactive epitopes are characterized by the presence of pentapeptides rarely (or never) found in human proteins.

5. DISCUSSION

Utilization of short peptide modules rather than full-length *P. gingivalis* antigens in vaccines may increase specificity and efficacy. It has been understood since the 1980s that chemically synthesized small peptides can induce antibodies that react with intact proteins (56). Furthermore, peptide-based vaccines have been successfully used against several pathologies and infectious diseases. For example, tumour antigen-derived peptides evoked potent antitumour immunity in the murine melanoma M-3 (57); cytotoxic CD4+ and CD8+ T lymphocytes generated by mutant p21-ras (12Val) peptide vaccination selectively killed autologous tumour cells carrying this mutation (58); Her-2/neu peptide (aa 657–665) is an immunogenic epitope of Her-2/neu oncoprotein with potent antitumour properties (59); synthetic peptides identified as antigenic sites on the S1 subunit of pertussis toxin induced especially high antibody titres against native pertussis toxin in mice (60); a linear peptide containing minimal T- and B-cell epitopes of *Plasmodium falciparum* circumsporozoite protein provided protection against a transgenic sporozoite challenge (61); the 15-mer amino-acid sequence 101-115 (PPAYEKLSAEQSPPP) of the Melan-A/MART-1 melanoma antigen has been shown to be a good target for a vigorous and safe immunotherapy (62).

Based on these scientific premises, this study explored the primary sequence of *P. gingivalis* fimA type I protein to define and characterize *P. gingivalis* epitopes that could be used for effective and safe anti-*P. gingivalis* immunotherapy. We observed that the immunological potential carried by the *P. gingivalis* antigens is localized in rare peptide fragments, thus confirming the relationship between low-similarity and immunogenicity (33-44). Sequences containing pentapeptide fragment(s) with low similarity to the host proteome appear to be those involved in the humoral antibody recognition of the *P. gingivalis* antigen, thus indicating that *P. gingivalis* antigenic motifs rarely found in host proteins are more likely to evoke an immune response. In short, these findings provide a method for investigating the immune potential of the *P. gingivalis* proteome, and, thus, may help fight *P. gingivalis* infection, one of the primary challenges in current periodontitis research. This study might also contribute to accelerate the epitopic characterization of *P. gingivalis* antigens which advances slowly, antigen-by-antigen (1, 2, 14-18, 28, 29).

Moreover, using exact immunogenic *P. gingivalis* peptide sequences may provide not only effective active or passive immunotherapeutic anti-*P. gingivalis* approaches but, in addition, might abolish the risk of adverse effects, which remains a major obstacle to antibody-based therapies (63-67). Adverse events are possibly caused by cross-reactivity that may result from using entire microbial antigens, because of the presence of peptide sequences common to microbes and humans (68, 69) (see also Figure 1). Using low-similarity peptides may help develop effective vaccines exempt of adverse side-effects (70), since the target sequence sites are present in the microbial organism only.

In conclusion, the findings of the present study may provide guidance in the analysis, identification, and utilization of the B-cell response in vaccination against *P. gingivalis*-associated periodontitis.

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