ors (41 men and 30 women) tested positive by ELISA screening test for HCV antibody but negative by RIBA-3. The majority (43; 30 men and 13 women) of these donors were previously negative by ELISA; the rest (28; 11 men and 17 women) were new donors.

Of the 43 seroconverting donors by ELISA, 29 had at least one negative pre-seroconversion HCV antibody screen by the third generation ELISA. The pre-index donations of the remaining 14 donors were screened by the second generation ELISA test for HCV antibody prior to August 1993. The archive serum samples relating to these donations were not available for testing by PCR or retesting by third generation ELISA for HCV antibody.

The paired archive serum samples ($n=58$) of the index (positive by third generation ELISA and negative by RIBA-3 HCV antibody tests) as well as the pre-index (negative by third generation ELISA HCV antibody test) donations relating to the unselected group of 29 seroconverting donors were all negative by nested PCR for HCV RNA.

Discussion

We have reported that in 1993, 52 established blood donors seroconverted by the second or third generation ELISA screening test for HCV antibody with negative RIBA-2 or RIBA-3 results.¹ In this communication we report similar seroconversion in a further 43 blood donors.

It can be argued that seroconversion by ELISA screening test for HCV antibody may not be genuine if pre- and postseroconversion samples of the same donors were analysed using different tests or different generations of the same test. Any "drift" in reactivity due to test

enhancement may confound changes in test results.⁴ However, seroconversion by ELISA in an unselected group of 29 donors described in this report is not subject to this technical consideration, as pre- and postseroconversion samples were tested by the same third generation ELISA screening test for HCV antibody.

All 29 seroconverting donors by ELISA with negative RIBA-3 tests were negative for HCV RNA by PCR. The absence of viraemia in the pre- and postseroconversion samples of all donors does not exclude HCV infection but argues strongly against this possibility. It follows that HCV antibody seroconverting donors by ELISA with negative RIBA-3 results—as a group with distinct laboratory results—are perhaps no different from previously untested donors with identical serological results. The explanation for false positive ELISA results is linked with test non-specificity⁵; however, the reason for seroconversion by ELISA in donors previously negative for HCV antibody remains unknown.

The authors are grateful to Dr F A Ala for critically reading the manuscript.

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J Clin Pathol 1996;49:255–257

Microbiological and serological investigations of oral lesions in Papillon-Lefèvre syndrome

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Accepted for publication
18 October 1995

Abstract

Microbiological and serological (enzyme linked immunosorbent assay) investigations were carried out, including karyotyping, on two Asian children with Papillon-Lefèvre syndrome. In case 1, a girl aged four years, the most prevalent putative periodontopathogens were *Eikenella corrodens*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia* (deciduous dentition) and *Bacteroides gracilis*, *E. corrodens* and *F. nucleatum* (permanent

dentition). In case 2, a boy aged nine years, they were *F. nucleatum*, *P. intermedia* and *P. loeschii* and *E. corrodens*. Serum from case 2 showed a raised specific IgG antibody response to *Actinomyces actinomycetemcomitans* serotype b. Thus, a wider range of species than hitherto reported may be associated with Papillon-Lefèvre syndrome, including *A. actinomycetemcomitans* and *F. nucleatum*.

(*J Clin Pathol* 1996;49:255–257)

Keywords: Papillon-Lefèvre syndrome, periodontopathogens, aetiology.

Papillon-Lefèvre syndrome is thought to be an autosomal recessive disease which can afflict offspring of parents showing consanguinity,¹ although some studies have found no consanguinity.² Thus, Papillon-Lefèvre syndrome is not a simple autosomal recessive trait. Papillon-Lefèvre syndrome is believed to occur with a frequency of only one per 2 million and so it might be regarded as a minor public health problem. However, its importance lies in the complete loss of teeth in children³ and in the continuing palmarplantar hyperkeratosis and other dermatological problems. As with other forms of periodontal disease, a microbial aetiology for Papillon-Lefèvre syndrome has been sought. Very few studies, however, have attempted detailed speciation of the microflora.⁴ *Actinomyces actinomycetemcomitans* has been associated with Papillon-Lefèvre syndrome and clinical improvement has followed its eradication.⁴ Other studies have found an association between Papillon-Lefèvre syndrome and *Bacteroides* spp., *Fusobacterium*, *A. actinomycetemcomitans*, *Eikenella corrodens*, *Capnocytophaga* spp., and spirochaetes.

The aim of this study was to gather more comprehensive microbiological information on Papillon-Lefèvre syndrome using both cultural and immunological methods.

Methods

Two cases were studied: a four year old girl (case 1) and a nine year old boy (case 2). Subgingival plaque samples were taken for microbiological investigation between the ages of four years, six months and eight years, four months (case 1) using sterile paper points inserted into the pocket, following removal of supragingival plaque, and immediately placed into 0.01% w/v dithiothreitol in saline (simplified reduced transport fluid, RTF). Similar samples were taken from case 2.

For serological analysis by indirect enzyme linked immunosorbent assay (ELISA),⁵ subgingival plaque samples were placed in vials containing 1 ml phosphate buffered saline (PBS)/0.01% thiomersal (Sigma, St Louis, Missouri, USA) and 1 mm glass beads. Plaque samples were fixed to the wells of a 96 well plate using 0.25% glutaraldehyde. Standard suspensions of *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*, and *F. nucleatum* were also fixed to the wells. The plates were washed three times with PBS containing 0.05% Tween 20 (PBS-T) after each incubation step. Non-specific binding was blocked using 1% bovine serum albumin (BSA) by overnight incubation at 4°C. Specific bacteria were detected using horseradish peroxidase (HRP) conjugated specific monoclonal antibodies directed against *P. gingivalis* (clone CB5.C5), *P. intermedia* (clone 3B5/H2), and *A. actinomycetemcomitans* (clone 4B5/F2); *F. nucleatum* was detected using HRP conjugated sheep anti-mouse IgG antibody. The plates were incubated for 60 minutes, then developed with o-tolidine substrate and the reaction stopped with 3 M HCl. The absorbance was read at 450 nm in a TiterTek Multiscan. The optical density (OD) readings were compared with a standard preparation of bacteria.

A blood sample was also taken from case 2 for genetic karyotyping and for the measurement of specific serum IgG antibodies to putative periodontopathic bacteria, also using ELISA in which a 50 µl suspension of bacteria at 10 µg/ml was fixed to the wells of 96 well plates using 0.25% glutaraldehyde. The plates were washed three times with PBS-T and then after each incubation step. Non-specific binding was blocked with 1% BSA by an overnight incubation at 4°C. Serum (100 µl) diluted 1 in 1000 in PBS-T containing 0.1% BSA was added to the wells and the plates incubated for 60 minutes at room temperature. Wells with antibody buffer instead of serum were used to determine background. Then, 100 µl of a 1 in 1000 dilution of HRP conjugated anti-human IgG antibody was added to all wells and incubated for 60 minutes at room temperature. Finally, the assay was developed using o-tolidine in 100 mM citrate phosphate as the substrate, and 150 µl was added to all wells. The reaction was stopped by the addition of 50 µl 3 M HCl to all wells and the colour change was measured at 450 nm using a TitreTek Multiscan reader. The background levels were subtracted from mean OD readings of the triplicate wells and the OD readings were used to compare the serum reactivity with each bacterial species and strain.

All culture specimens were plated out within five minutes of initial sampling onto 5% horse blood fastidious anaerobe agar (FAA, Lab-M, Bury, UK) and onto Wilkins-Chalgren anaerobic agar (WCAA, Oxoid, Basingstoke, UK) which was supplemented with 0.001% w/v nalidixic acid and 0.1% w/v Tween 80 for the isolation of non-sporing, Gram positive anaerobes including *Actinomyces*. Samples were also plated onto FAA and onto WCAA supplemented with 0.08% gentamycin for the isolation of Gram negative anaerobes, except that neomycin replaced gentamycin as a selective agent in plaque samples from case 2. Anaerobic plates were incubated under an atmosphere of 80% N₂, 10% H₂ and 10% CO₂ at 37°C. A further plate of Columbia blood agar (Oxoid) was incubated aerobically. The bacterial species in each sample were determined using commercial identification kits: Minitek II (BBL, Cowley, UK), RapID-NH (Mercia Diagnostics, Guildford), API 20 Strep (Bio-Mérieux, Basingstoke, UK), RapID-ANA (Mercia Diagnostics).

Results

Microscopy revealed the presence of high numbers of bacteria typical of a mixed periodontal flora (table). Species isolated from case 1 are shown separately for wholly deciduous and wholly permanent dentitions. The most commonly isolated putative periodontopathogen was *E. corrodens* followed by *F. nucleatum* with the replacement of deciduous by permanent teeth being apparently associated with the appearance of *B. gracilis* but loss of *P. gingivalis*. Using an ELISA, *A. actinomycetemcomitans* was detected in most sites in case 1; neither *P. gingivalis* nor *P. intermedia* were found at any of eight sites sampled.

Species identified in periodontal pockets of two cases of Papillon-Lefèvre syndrome by culture† and ELISA‡

Bacterial species	Case 1		Case 2
	Deciduous (n = 7)	Permanent (n = 5)	Permanent (n = 7)
<i>Actinomyces israelii</i>	0	0	0
<i>Actinomyces naeslundii</i>	1*	1	0
<i>Actinomyces odontolytica</i>	0	0	3
<i>Actinomyces viscosus</i>	0	3	1
<i>Arachnia propionica</i>	1	0	1
<i>Bacteroides gracilis</i>	0	2	0
<i>Bacteroides</i> sp.	0	0	1
<i>Capnocytophaga ochracea</i>	1	2	7
<i>Cardiobacterium hominis</i>	5	2	2
<i>Eikenella corrodens</i>	5	2	2
<i>Fusobacterium nucleatum</i>	4	2	7
<i>Fusobacterium necrophorum</i>	0	0	1
<i>Haemophilus aphrophilus</i>	0	2	6
<i>Haemophilus segnis</i>	0	1	0
<i>Haemophilus parainfluenzae</i>	0	3	5
<i>Leptotrichia buccalis</i>	0	1	0
<i>Leptotrichia</i> sp.	1	0	0
<i>Neisseria sicca/subflava</i>	0	3	7
<i>Pasteurella haemolytica</i>	0	1	0
<i>Prevotella intermedia</i>	1	1	6
<i>Prevotella loeschii</i>	0	0	6
<i>Prevotella oralis</i>	1	1	0
<i>Prevotella oris</i>	0	1	0
<i>Porphyromonas gingivalis</i>	2	0	0
<i>Peptostreptococcus micros</i>	1	1	0
<i>Selenomonas</i> sp.	2	0	0
<i>Streptococcus milleri</i> I	1	0	0
<i>Streptococcus milleri</i> II	0	0	5
<i>Streptococcus mitis</i>	0	3	2
<i>Streptococcus morbillorum</i>	2	0	0
<i>Streptococcus sanguis</i> I	1	1	1
<i>Streptococcus sanguis</i> II	5	2	6
<i>Veillonella parvula</i>	0	1	0

Species identified in periodontal pockets of two cases of Papillon-Lefèvre syndrome by ELISA (permanent teeth)

Bacterial species	Case 1 (n = 8)	Case 2 (n = 2)
<i>Actinobacillus actinomycetemcomitans</i>	5	2
<i>Porphyromonas gingivalis</i>	0	2
<i>Prevotella intermedia</i>	0	2
<i>Fusobacterium nucleatum</i>	0	2

* Number of sites with species.

† Culture was performed as described in the text.

‡ The ELISA determined the presence of specific bacterial species.

With case 2, *F nucleatum* and *P intermedia* were detected by culture and in addition ELISA demonstrated the presence of *P gingivalis* and *A actinomycetemcomitans*. The most frequently isolated presumptive periodontopathogen was *F nucleatum*, followed by *P intermedia* and *P loeschii*. The reactivity of a serum sample from case 2 against a bacterial panel revealed raised specific IgG antibody titres against all six strains of *A actinomycetemcomitans* tested (especially serotype b) but not against the single strains of *P gingivalis*, *F nucleatum* or *P intermedia* in the test panel.

Chromosome analysis of blood from case 2 showed a normal male karyotype. Case 1, who had presented one year earlier, had not been investigated for karyotype or serum antibody levels.

Discussion

The present data reveal a complex microbiological picture and also support the sug-

gestion that *A actinomycetemcomitans* and *P gingivalis* have a lower limit of detection using immunological reagents rather than culture.⁶ Previously, one study⁷ successfully identified 11 species, and unspiciated strains from other genera, from Papillon-Lefèvre syndrome periodontal pockets, including the presumptive periodontal pathogens *P melaninogenica* (was *Bacteroides melaninogenicus*), *Porphyromonas asaccharolytica* (was *Bacteroides asaccharolyticus*), *Capnocytophaga*, and *Fusobacterium*. Of these, *Capnocytophaga* is no longer universally regarded as a presumptive periodontal pathogen: its presence actually mitigates the activity of *P gingivalis*.⁸ Most other studies have assumed an aetiological role for *A actinomycetemcomitans*⁹ with scant regard for a causal role for other species. The present study found a wider range of putative pathogens than has been reported previously so that species may collectively contribute to the disease process. When Koch's postulates cannot be demonstrated, an increasing or high IgG antibody titre during the course of infection has been regarded as indicative of a role of a specific organism in disease. This approach is difficult to interpret in the case of Papillon-Lefèvre syndrome which is not an acute illness of rapid onset but a chronic infection. Nevertheless, studies have demonstrated raised levels of antibody to *A actinomycetemcomitans*,^{4,9,10} which is confirmed by the present study in which patients' serum reacted at high dilution with a panel of *A actinomycetemcomitans* strains though much less so with other periodontal bacteria. Interestingly, the reactivity is most pronounced with *A actinomycetemcomitans* serotype b, which has also been reported in a previous study.¹¹

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