

# Immune response to *Fusobacterium nucleatum* and *Prevotella intermedia* in patients with infectious mononucleosis

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**The role of four oral flora organisms (*Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis* and *Actinobacillus actinomycescomitans*) was investigated in 22 patients with infectious mononucleosis. Immunoglobulin-G class antibody titres to these organisms were measured by enzyme-linked immunosorbent assay. Serum levels in the patients were determined at day 1 and 42–56 days later. Significantly higher antibody levels to *F. nucleatum* and *Pr. intermedia* were found in the second serum sample of patients as compared to their first sample. The elevated antibody levels to *F. nucleatum* and *Pr. intermedia*, known oral pathogens, suggest a potential pathogenic role for these organisms in the pharyngo-tonsillitis associated with infectious mononucleosis.**

## Introduction

Infectious mononucleosis (IM) is generally manifested by pharyngo-tonsillitis, lymphadenopathy, atypical lymphocytosis and a positive heterophil antibody reaction [1, 2]. Most patients require only supportive treatment; however, serious complications such as upper airway obstruction can occur [3].

Although the Epstein–Barr virus is the primary cause of IM, the exact aetiology of the pharyngo-tonsillitis associated with IM is not well understood. An involvement of oropharyngeal organisms in the pharyngo-tonsillitis has been suspected in severe or protracted illness and the surfaces of tonsils of patients with IM harbour more species of anaerobic bacteria during the acute stage of the illness than after recovery [4]. While acyclovir therapy did not reduce the symptoms of IM [5], nitroimidazole therapy was found in non-placebo, open studies to be effective in reducing the duration of pharyngo-tonsillitis [6–10]. The nitroimidazoles (e.g., metronidazole) are effective against anaerobic bacteria [11], and have no antiviral activity or ability to modulate Epstein–Barr virus immunity [9].

The potential role of anaerobic bacteria in the inflammatory process of IM was investigated in this study. The presence of antibodies of the immunoglobulin-G class to four organisms commonly found in

the oral flora (*Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis* and *Actinobacillus actinomycescomitans*) was investigated by enzyme-linked immunosorbent assay (ELISA) in patients in the acute and recovery stages of IM.

## Materials and methods

### Patients

Twenty-two consecutive patients with IM were studied between 1979 and 1991. Patients were aged 13–21 years (mean 16 years and 7 months) and 15 were female. Patients who had received antimicrobial or steroid therapy during the 2 months before they were first seen or during the 56-day follow-up period were excluded from the study. All patients had pharyngo-tonsillitis, lymphadenopathy, lymphocytosis, a positive heterophil antibody reaction (Monospot<sup>®</sup> slide test, Ortho Diagnostic, or Monosticon Dri-Dot<sup>®</sup> slide tests, Oregon Diagnostic) and a Paul-Bunnell Davidsohn test with titres  $\geq 40$ .

Two serum samples were obtained from each patient; the first was collected when the patient was first seen, and the second was taken 42–56 days later in a single run. The serum samples were stored at  $-70^{\circ}\text{C}$ .

### Immunological methods

Antibody titres were measured by ELISA on two separate occasions by a modification of the method described by Ebersole *et al.* [12]. The assay was run twice. Isolates of the following oral bacteria served as

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sources of antigens: *A. actinomycetemcomitans* strain Y4 serotype b, ATCC (American Type Culture Collection) strain 9710 serotype c, *Pr. intermedia* ATCC strain 25611; *P. gingivalis* strain 381 (obtained from Dr S. S. Socransky, Forsyth Dental Center, Boston, MA, USA) and *F. nucleatum* ATCC strain 25586. The processing of antigens and the ELISA test were performed as described previously [13].

### Statistical analysis

The median test [14] was used to compare the data sets. For this purpose, the data were transformed to conform to the positive and negative control readings for each batch of 20 data values. The positive control reading was assigned an arbitrary value of 100 units and the negative control reading the value of zero. Actual data values were then transformed as a straight line adjustment between these two values.

### Results

The serum antibody levels at day 1 and 42–56 days later for *P. gingivalis* and *A. actinomycetemcomitans* were similar and <30 units (Table 1). However, median antibody levels for *F. nucleatum* and *Pr. intermedia* showed a significant difference between the first and second samples ( $p < 0.05$ ) (Table 1), with the values doubling or more for *Pr. intermedia* in 12 (65%) patients, and for *F. nucleatum* in 15 (75%), (Fig. 1).

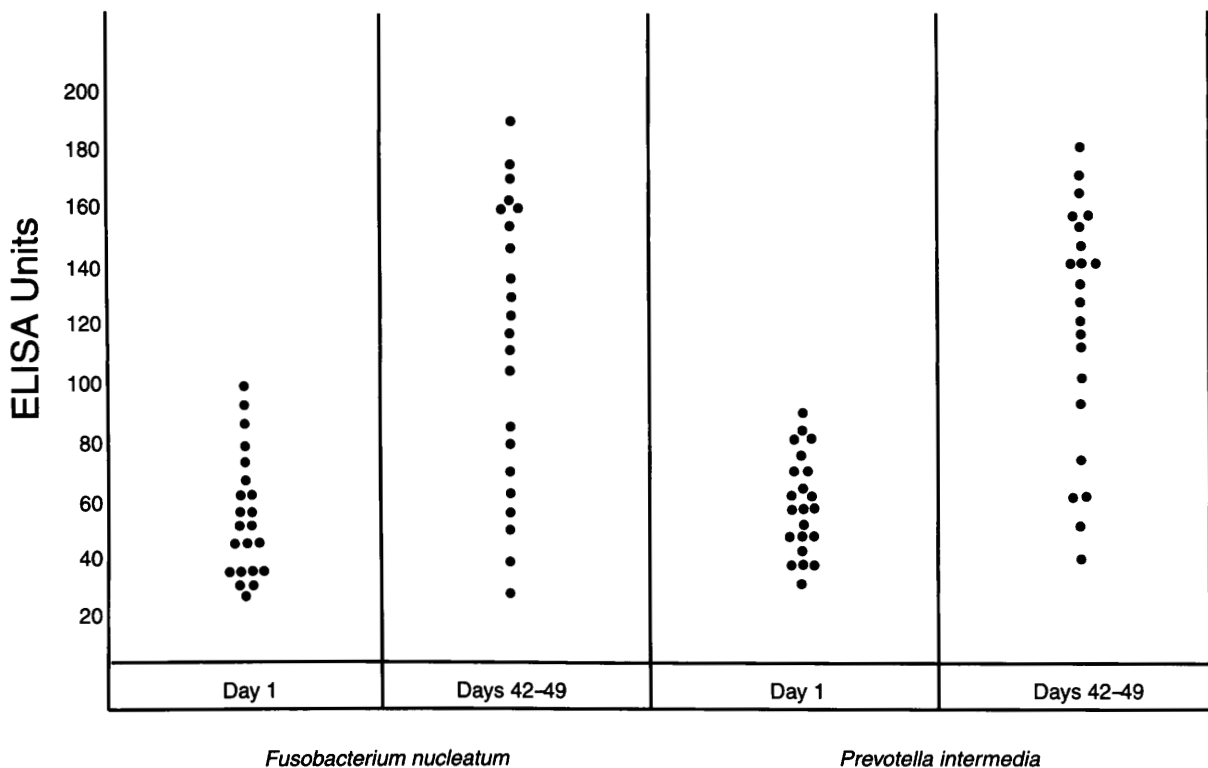
**Table 1.** Median serum levels as determined by ELISA in 22 patients with infectious mononucleosis

Organism	Days of illness when samples were collected—median (standard error)	
	Day 1	Days 42–56
<i>F. nucleatum</i>	53.3 (14)	118.3 (21)*
<i>Pr. intermedia</i>	58.4 (8)	122.2 (18)*
<i>P. gingivalis</i>	25.2 (4)	23.6 (4)
<i>A. actinomycetemcomitans</i>	19.2 (3)	21.0 (2)

\*Median test  $p$  value <0.05 compared to day 1.

### Discussion

*F. nucleatum* and *Pr. intermedia* are known oral pathogens [11] and have been associated with adult periodontitis, but in contrast to *P. gingivalis* [15] and *A. actinomycetemcomitans* [16], they have also been isolated as a predominant species in several chronic respiratory tract infections [11]: aspiration pneumonia [17], lung abscesses [18], chronic otitis media [19], chronic sinusitis [20], retropharyngeal [21] and peritonsillar abscesses [22] and bacteraemia associated with these infections [23]. In addition, *F. nucleatum* and *Pr. intermedia* and other anaerobic bacteria have also been isolated from the cores of tonsils of children with recurrent group A  $\beta$ -haemolytic streptococcal (GABHS) [24] and non-GABHS [25] tonsillitis and peritonsillar abscesses [22]. Antibodies against *Pr. intermedia* were found to be elevated compared to controls in patients with non-streptococcal tonsillitis [13].



**Fig. 1.** Levels of serum antibodies to *F. nucleatum* and *Pr. intermedia* in 22 children with infectious mononucleosis.

The possible role of anaerobic bacteria in tonsillar inflammation is supported by several observations, including the isolation of anaerobes as predominant pathogens from tonsillar or retropharyngeal abscesses, in many cases without any aerobic bacteria [21, 22], and their isolation as pathogens in well-established anaerobic infections of the tonsils (Vincent's angina) [25]. In addition, studies have shown increased recovery of capsulate pigmented *Prevotella* and *Porphyromonas* spp. from acutely inflamed tonsils [26], their isolation from the cores of recurrently inflamed non-GABHS tonsils [27] and the response of antibiotics active against anaerobes in patients with non-GABHS tonsillitis [6–10, 28–31].

The results of this study suggest a potential secondary role for anaerobic bacteria in the pharyngo-tonsillitis associated with IM. In a previous investigation that included some of the patients in this study, the isolation of *F. nucleatum* and *Pr. intermedia* in 12 of 14 and 13 of 14 patients, respectively, during the acute inflammatory stage of IM and both species in four of 12 patients 2 months later were reported [4]. *F. nucleatum* and *Pr. intermedia* but not *P. gingivalis* or *A. actinomycetemcomitans* appear to be involved, but as the antibody levels for other aerobic and anaerobic bacteria previously reported within the tonsils were not investigated, it is possible that other species may be involved. The evidence that nitromidazoles alleviate the clinical symptoms of tonsillar hypertrophy and shorten the duration of fever in patients with IM also suggests such a role for anaerobic bacteria [6–10]. Metronidazole has no antiviral activity and is effective only against anaerobes. The effects of metronidazole in IM could be due to the suppression of the oral anaerobic flora. The increase in the number of anaerobic bacteria may be due either to the immunosuppressive effect of the Epstein–Barr virus or to direct synergy between the virus and the oral flora. Anaerobes may contribute to the inflammatory process induced by the Epstein–Barr virus.

Clearly, further studies are required to evaluate whether antibody titres to *F. nucleatum* and *Pr. intermedia* decrease following complete recovery and whether other aerobic and anaerobic bacteria play a role in the pharyngo-tonsillitis phase of IM. Prospective placebo-controlled, randomised and double-blind studies are warranted to evaluate the role of antimicrobial therapy in the alleviation of the signs and symptoms of IM. These studies should investigate whether a reduction in the number of anaerobic bacteria in the tonsils can modify the immune response to these bacteria. If such studies show efficacy, they may offer a mode of therapy that may alleviate some of the symptoms and complications of IM.

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