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Bacterial penetration into tonsillar surface epithelium during infectious mononucleosis

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Abstract

Bacterial penetration into epithelial cells, scraped from the palatine tonsils of 14 patients (10 males, four females; median age 16 years) with current infectious mononucleosis and concomitant membranous tonsillitis, was studied using the transmission electron microscopic (TEM) technique. Bacteria were seen to adhere to and penetrate the epithelial cells, some of which were completely filled with bacteria. This finding suggests intracellular proliferation of bacteria. Epstein-Barr virus, the causative agent of infectious mononucleosis, especially when associated with growth of β-haemolytic streptococci on the palatine tonsils, induces bacterial penetration into tonsillar tissue, that in turn might be a causative mechanism in the development of peritonsillar abscess.

Key words: Tonsil; Epstein-Barr Virus Infections; Streptococcal Infections; Peritonsillar Abscess; Tonsillitis

Introduction

Epstein-Barr virus (EBV), a ubiquitous human herpes virus originally described in cultured lymphoblasts from African Burkitt's lymphoma, is the causative agent of infectious mononucleosis (IM). EBV appears to be involved in the pathogenesis of a variety of diseases, e.g. IM, Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's disease, gastric carcinomas and other lymphomas and lymphoproliferative diseases. EBV has the ability to establish lifelong persistent infection, where epithelial cells and B cells play a major role in the viral strategy of EBV.[1]

One of the most striking symptoms during the course of acute IM is the involvement of the palatine tonsils, which become covered by a membranous exudate composed of various species of bacteria, bacterial remnants and detritus.[2] Recently Burstin and Marshall[3] reported that IM could cause severe airway obstruction due to bilateral quinsy. Penetration of bacteria into the peritonsillar tissue could naturally be a direct aetiological cause of this complication.

The purpose of the present study was to establish whether or not bacteria penetrated the epithelial cells of the tonsillar surfaces during membranous tonsillitis caused by EBV. Particular attention was paid to cases culture-positive for β-haemolytic streptococci. In this study we used electron microscopic techniques to examine cell samples obtained from the palatine tonsils of patients with active IM infection.

Patients and methods

The patient material comprised 14 individuals (10 males and four females; age range 11 to 21 years, median age 16 years) referred to our out-patient ENT department due to treatment-resistant acute tonsillitis (Table I). All patients fulfilled the criteria

TABLE I
AGE AND SEX OF SUBJECTS, AND BACTERIAL FINDINGS BY STANDARD AEROBIC CULTURING

Patient no.	Sex	Age (years)	Bacterial finding
1	Male	21	Normal flora
2	Male	21	Normal flora
3	Male	16	Normal flora
4	Male	17	Normal flora
5	Male	21	Normal flora
6	Female	15	Normal flora/*Streptococcus* C
7	Female	19	Normal flora
8	Male	19	Normal flora/*Streptococcus* C
9	Female	15	Normal flora/*Streptococcus* G
10	Female	14	Normal flora
11	Male	16	Normal flora
12	Male	11	Normal flora
13	Male	16	Normal flora
14	Male	20	Normal flora

Normal flora = growth of *Streptococcus viridans, Neisseria* spp., *Corynebacteria* spp.; *Streptococcus* C and G = β-haemolytic streptococci groups C and G.

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for IM according to Hoagland,[4] *viz* elevated body temperature, tonsillopharyngitis, lymphadenopathy, splenohepatomegaly, absolute lymphocytosis with more than 10 per cent atypical Downey-McKinley lymphocytes, detectable heterophil antibodies in serum according to Paul-Bunnell, and a positive rapid test for IM antibodies (Monosticon, Turnhout, Belgium). All patients also showed positive IgG and IgM antibody titres to EBV capsid antigen. Moreover, the IgG avidity was very low, hinting at a primary EBV infection of recent date.

To obtain epithelial cells both palatine tonsils were scraped twice using a wooden spatula that had been dipped in physiological saline. The cellular material was loosened from the spatula with physiological saline and then immediately processed as follows:

(1) Samples were smeared over blood-agar and chocolate-agar plates for standard aerobic culturing according to routine laboratory procedures (5 per cent CO_2 at 37°C for 48 hours).

(2) Samples were homogenized by extruding the cell mixture twice through a 20-gauge needle. The sample was then pressed through a filter, 5 μm pore size (Sartorius, Göttingen, Germany). The tonsillar epithelial cells adhered to the filter, whereas leukocytes, unfixed bacteria and mucus passed through. The epithelial cells that were caught in the filter disc were released by counterdirectional injection by 5 ml physiological saline. The suspension was centrifuged for 10 min at 1,500 rpm, the supernatant was discharged carefully with a Pasteur pipette and the precipitate was adjusted to 0.5 ml with physiological saline. The epithelial cells were fixed by adding 0.5 ml eight per cent formaldehyde in 0.2 M HEPES buffer, pH 7.4. Cell suspensions were post-fixed in one per cent osmium tetroxide for 30 minutes and dehydrated in graded ethanol solutions. The material was then embedded in Epon/Araldite. Ultrathin sections were examined in a JEOL 1010 transmission electron microscope at 80 kV, after counterstaining with uranyl acetate and lead citrate.

Results

Aerobic culture

Generally speaking, aerobic culture of collected samples showed a prolific growth of non-pathogenic commensals such as *Diphtheroides* sp., *Neisseria* sp., *Corynebacterium* spp., and various α-haemolytic streptococi. In three patients, β-haemolytic streptococci were identified, two with Group C (patients 6 and 8) and one with Group G (patient 9).

Transmission electron microscopic (TEM) study

In all samples, bacteria and surface epithelial cells could be discerned. The tonsillar surfaces showed a stratified, squamous epithelium whose epithelial cells were held together by finger-like projections and further stabilized by desmosomes. Desquamated epithelial cells were also seen (Figure 1). Cellular debris was noted in abundance. The morphology of the bacterial flora was extremely varied, but were mainly coccoid bacteria of varying sizes intermingled with rods and cell remnants (Figure 2). Many bacteria were in intimate contact with the epithelial cell surfaces. Particularly in samples culture-positive for β-haemolytic streptococci, bacteria could be observed within the epithelial cells (Figure 3). Sometimes actin-like filaments gathered around the bacteria (Figure 4). Occasionally the bacteria were contained in vacuoles in the epithelial cells. In some areas, bacteria having the same morphology were found on both sides of the epithelial cells, suggesting penetration through the cells (Figure 5), or lay close to the epithelial cell surface, or within the cell surface, or within cells and then often surrounded by actin filaments (Figure 6). Also, long rods could be seen penetrating into cells, or located completely within epithelial cells (Figure 7).

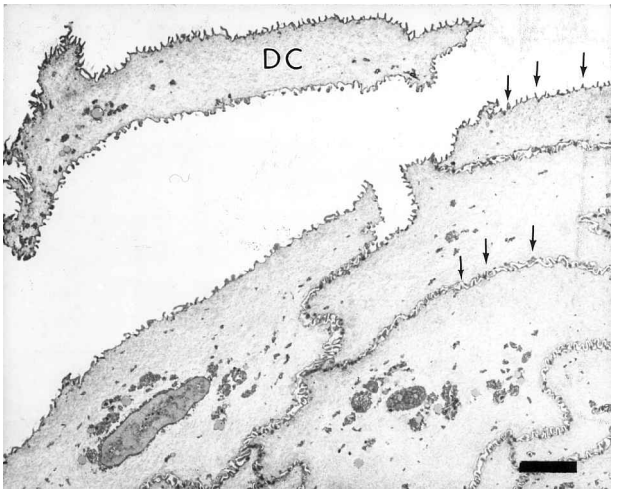

FIG. 1

Transmission electron micrograph of surface epithelium obtained by scraping the palatine tonsils (patient 6) showing the stratified, squamous epithelium. Note the desquamated cell (DC), and cellular projections on the apical surface and between the epithelial cells (arrows). Bar = 5 μm.

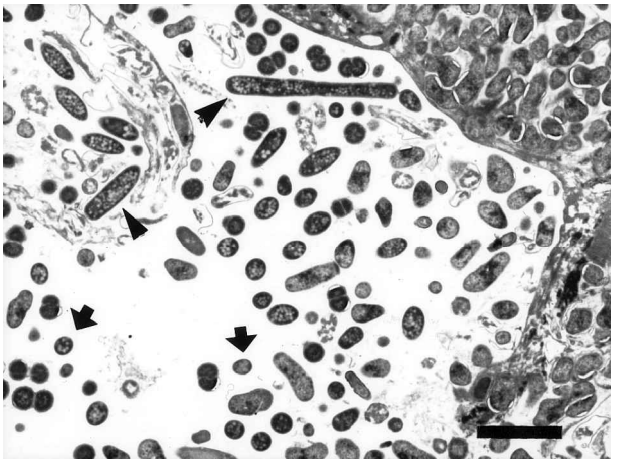

FIG. 2

Transmission electron micrograph showing cellular debris with numerous bacteria, coccoid bacteria (arrows) and rods (arrowheads). Patient 6; bar = 2 μm.

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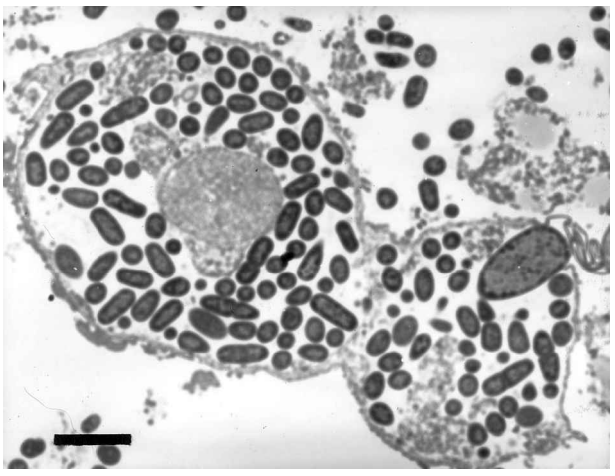

FIG. 3

Transmission electron micrograph of epithelial cells completely filled with bacteria. Note the shortness of the cellular actin filaments within the cells. Patient 9; bar = 1 μm.

Discussion

It is generally accepted that EBV is the causative agent of the IM infection.[1,5] However, it has become evident that in membranous tonsillitis, which occurs concomitantly, bacteria play an important role.[2,6] The 'fur', that completely covers the palatine tonsils during membranous tonsillitis namely consists of amassed bacteria, aerobes and anaerobes, plus bacterial and cellular detritus. Most of the bacterial species belong to the normal commensal flora, but well-known bacterial pathogens such as β-haemolytici streptococci, Groups A, C, G are also encountered.[2] In addition, bacterial anaerobes such as *Fusobacterium nucleatum* and *Prevotella intermedia*, known oral pathogens, can be associated with the pharyngotonsillitis during IM.[6]

By adhering to epithelial cells, the microorganisms can avoid being cleared from the mucosal surfaces. Furthermore, bacterial adherence is closely connected with induction of inflammatory changes in

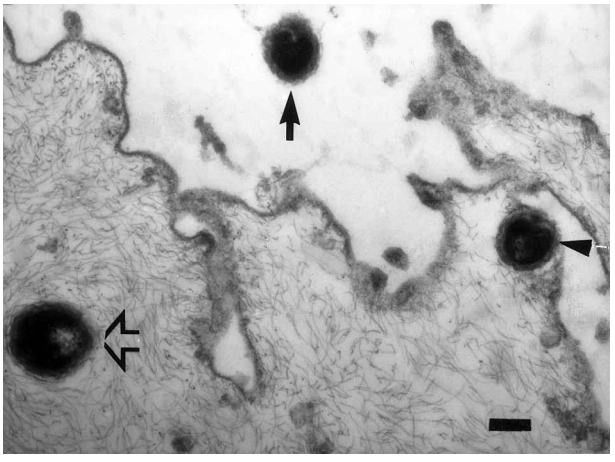

FIG. 4

Transmission electron micrograph showing coccoid bacteria outside the epithelial cell (arrow), penetrating into the cell (arrowhead) and within the cell (open arrow). Note the actin filaments within the epithelial cell. Patient 9; bar = 200 nm.

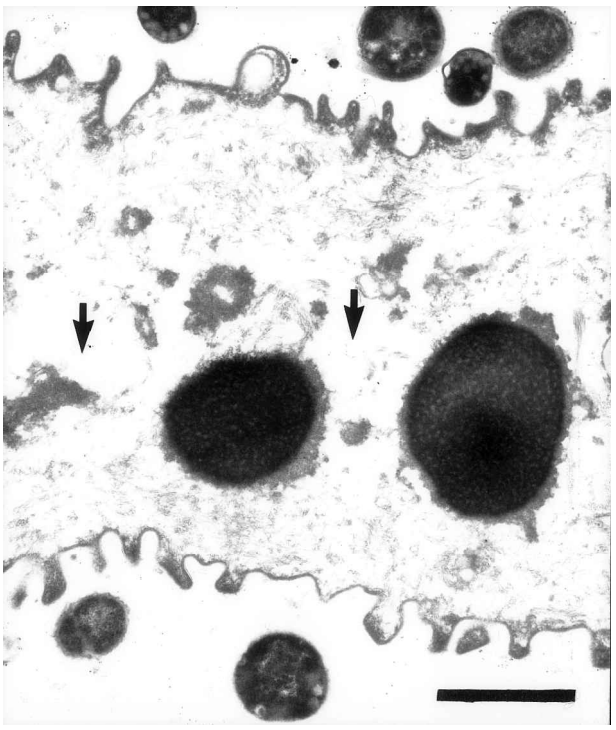

FIG. 5

Transmission electron micrograph of epithelial cell surrounded by coccoid bacteria. Note the vacuolization of the cellular stroma around the intracellular bacteria (arrows). Patient 6; bar = 500 nm.

underlying tonsillar tissues.[7] The interaction between bacteria and tissue cell surfaces in the adhesion process is mediated by non-specific (physiochemical forces) and specific bonds (adhesin-receptor binding).[8,9] The specific binding is a prerequisite for permanent adherence and colonization of bacteria.[10] Adherence of Group A β-haemolytic streptococci to epithelial cells is suggested to be mediated by various adhesins including lipoteichoic acid (LTA), M-protein, F-protein, and fibronectin-binding protein.[11–14] The chief receptor on epithelial cells is suggested to be fibronectin.[11]

The exact mechanism by which bacteria invade the mucosal lining to cause local and disseminated infections is still not fully understood. In a previous study,[15] the fine structure of the surface of palatine tonsils was characterized. It was found that the apical surfaces of the palatine tonsils consisted of an irregular pattern of microridges. *Streptococcus pyogenes* pathogens, when inducing disease, were adhering to the crests of these microridges, that were seen as finger-like projections when sections were examined by TEM (Figure 1). It seems self-evident that the site of invasion of the microorganisms into the epithelial cells ought to be at the same location.

The process of invasion is necessarily preceded by the step in which bacteria adhere to the cells. Valentin-Weigand *et al.*[16] showed that engulfment of the adhering bacteria was effected by cellular protrusions where actin microfilaments of the cells played a central role (macropinocytosis). McGee and

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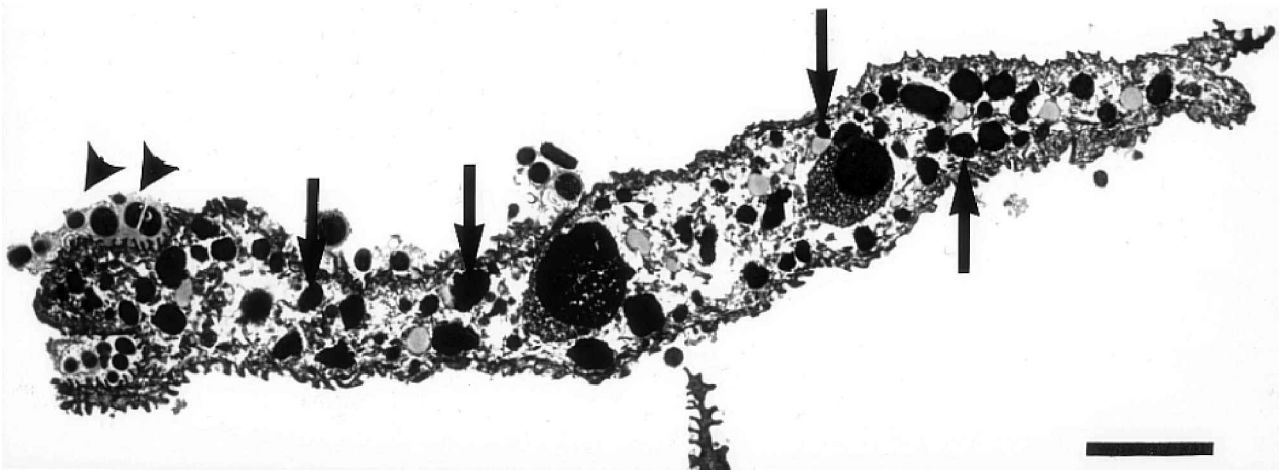

FIG. 6

Transmission electron micrograph of a partly desquamated epithelial cell with many intracellular bacteria (arrows) and bacteria in close contact with the cell (arrowheads). Patient 6; bar = 6 μm.

co-workers[17] suggested that ingested bacteria were transported in membrane-bound vacuoles to the basolateral surfaces of the cells and released into the subepithelial tissue (transcytosis). Ward *et al.*[18] showed that gonococci, after internalization into epithelial cells, proliferated within and could be released from those cells. Our study suggests that a similar mechanism could also be responsible for the presence of the coccoid bacteria within the surface epithelial cells of the palatine tonsils during IM (Figures 3, 4 and 5). Furthermore, penetration into epithelial cells, subsequent proliferation of the bacteria, and their release into underlying palatine tissue could be the way in which a peritonsillar abscess develops.

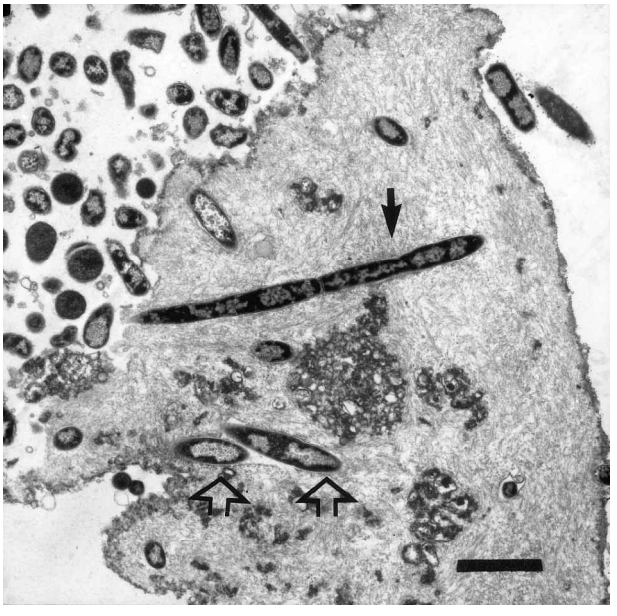

FIG. 7

Transmission electron micrograph showing an epithelial cell containing a rod (arrow) and small rods (open arrows) penetrating into the cell. Patient 9; bar = 2 μm.

Typically, in the early stage of the membranous tonsillitis phase is the suppression of the immunoglobulin-coating of the bacteria covering the tonsillar surfaces.[20] Secretory immunoglobulin A (S-IgA) is an important agent for preventing bacteria from adhering to mucosal epithelia,[21] whereas IgG appears to prevent bacteria from penetrating into epithelial cells.[14] A consequence of the reduced immunoglobulin-coating of the surface bacteria could be the immense bacterial colonization providing favourable opportunities for the bacteria to penetrate into the epithelial cells. Bacterial penetration of tonsillar tissue is, naturally, a prerequisite for the formation of a peritonsillar abscess. In a recent study,[22] β-haemolytic streptococci were found in the majority of pus samples obtained from peritonsillar abscesses. The findings of the present study suggest that the EBV causing IM infection and β-haemolytic streptococci together play a synergistic role in the development of serious upper airway disease.

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