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Attachment of bacteria to tonsillar epithelium during acute tonsillitis

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Abstract
Epithelial cells were scraped from the tonsillar surfaces of 15 patients with current acute tonsillitis (AT) and of 15 individually matched healthy persons. The cellular mixture was stained with acridine orange and bacteria seen to be attached to the epithelial cells under the fluorescent microscope were calculated. Conventional bacterial culturing was also performed simultaneously. Significantly more bacteria were attached to epithelial cells from the AT group than from the controls (>10 attached bacteria per cell $p = 0.0103$, >50 attached bacteria per cell $p = 0.0212$). *In vivo* determination of bacteria attached to epithelial cells offers prospects of gaining a better understanding of the aetiopathogenesis of acute tonsillitis.

Introduction

The aetiology of acute tonsillitis (AT) is a highly controversial topic. Although it is well-established that beta-haemolytic streptococci, mainly those of group A (GABHS), cause roughly 30 per cent of all AT cases (Brook *et al.*, 1981; Stjernquist-Desatnik *et al.*, 1987), in the remaining 70 per cent the aetiological agent(s) is never established. Streptococci other than GABHS, *Staphylococcus aureus, Haemophilus influenzae* as well as anaerobic bacteria (Brook *et al.*, 1981; Toner *et al.*, 1986), and viruses such as Epstein-Barr virus, influenza virus, para-influenza virus, adenovirus, rhinovirus (Henle and Henle, 1970; Yamanaka and Kataura, 1984) have been reported as causing AT. The fact that some of these microorganisms are harboured on the tonsillar surface without causing any clinical signs of tonsillar infection only adds to existing confusion surrounding the aetiology of AT.

TABLE I
AGE AND SEX OF THE PATIENTS INVESTIGATED

Pat no.	Age (years)	Sex
1	1	Male
2	1 8/12	Male
3	2 8/12	Male
4	4	Male
5	4 6/12	Male
6	4 8/12	Female
7	9	Female
8	15 6/12	Female
9	20	Male
10	24 1/12	Female
11	28 3/12	Male
12	29 5/12	Male
13	32 9/12	Female
14	44	Female
15	49	Female

The tonsillar surface facing the oropharynx is covered by stratified squamous epithelium intermingled with ciliated cells (Maeda and Mogi, 1984). Like all mucosal membranes, the tonsillar surface is covered with a film of mucus, which is continuously propelled forward by the action of cilia. To cause invasive disease, microorganisms must attach firmly to the epithelial cells, and avoid being transported away with the mucous film. Once attached, the microorganisms can proliferate, form colonies and release extracellular toxins which can injure the underlying cells (Ginsburg, 1972).

We have recently presented a method for the study of bacterial attachment *in vivo* to epithelial cells of the nasopharynx (Stenfors and Räisänen, 1990). The purpose of the present study was to chart the attachment of bacteria to the epithelial cells of the palatine tonsils during AT in order to get a better understanding of the pathophysiology of this common disease. Simultaneously, we performed conventional bacterial culturing for aerobes. No attempt was made to identify anaerobes or viruses.

Material and methods

The study was carried out on 15 consecutive patients suffering from AT (sore throat, fever, local inflammatory changes of the palatine tonsils) who were attending the ENT out-patient department. The age and sex of the patients are shown in Table I. Individually matched, healthy persons served as controls. None of the subjects had taken antibiotics within two weeks prior to the sampling.

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TABLE II

PERCENTAGE OF EPITHELIAL CELLS (N=50) WITH NO ADHERENT BACTERIA, 1–10 ADHERENT BACTERIA, 11–50 ADHERENT BACTERIA AND MORE THAN 50 ADHERENT BACTERIA AND FINDINGS AT CULTURING FROM THE TONSILLAR SURFACE DURING CURRENT ACUTE TONSILLITIS

	Cells with bacteria adhering				
Patietn no.	0%	1–10%	11–50%	>50%	Bacterial findings
1	12	14	20	54	Ps. aeruginosa
2	6	4	20	70	Other bacteria*
3	0	0	34	66	Gr B Streptococci
4	24	20	14	42	Other bacteria
5	2	10	18	70	Other bacteria
6	20	32	24	24	Other bacteria
7	8	6	34	52	Gr A Streptococci
8	26	24	18	32	Other bacteria
9	48	38	14	0	Other bacteria
10	54	22	22	2	Gr A Streptococci
11	70	8	12	10	Other bacteria
12	90	0	4	6	Other bacteria
13	16	16	70	14	Gr A Streptococci
14	14	42	30	14	Other bacteria
15	94	4	0	2	Gr G Streptococci
χ	31.2	16.0	22.3	30.5	

*'Other bacteria' means non-pathogenic aerobes.

Adherence investigation

Using a wooden spatula dipped in physiological saline, scrapings were taken from the surface of both tonsils and tonsillar plaques. Each spatula was placed in a sterile glass jar containing 2 ml physiological saline. The jars were immediately taken to the clinical laboratory for processing. Any epithelial cells adhering to a spatula were loosened by rinsing with 8 ml physiological saline. The samples were homogenized by extruding the cell mixture twice through a 20 G needle in order to disrupt chains and clumps. The samples, consisting of roughly 10 ml of liquid, were then filtered (5 μm pore size, Sartorius, Ministart® NML). Epithelial cells adhered to the filter, whereas leucocytes, red cells, unfixed bacteria and mucus passed through. The epithelial cells were removed from the filter with physiological saline, and the cell mixture was centrifugated for 10 min at 1500 rpm. The precipitate was spread over glass slides and allowed to dry. Finally, the dried samples containing tonsillar epithelial cells with adhering bacteria were stained with acridine orange and examined under a fluorescent microscope (Leitz Labrolux microscope with standard fluorescence equipment).

Fifty epithelial cells were examined and grouped according to number of attached bacteria as follows: no adherent bacteria, 1–10, 11–50, and more than 50 adherent bacteria.

Bacteriological investigation

Culture specimens were obtained from both sides by rotating a cotton swab along the tonsils, taking care not to touch other parts of the oropharynx. Each swab was placed in Stuart's medium and taken to the laboratory where it was wiped evenly over blood-agar and chocolate-agar plates. Identification of the bacterial species was performed according to standard laboratory routines. Particular interest was focused on β-haemolytic Streptococci, *Streptococcus pneumoniae, Haemophilus influenzae, Branhamella catarrhalis, Staphylococcus aureus* and *Pseudomonas aeruginaos* (pathogens). Other aerobic bacteria growing on the above-mentioned media were not further classified. No attempt was made to identify anaerobic organisms.

Statistical analysis

The findings of the attached bacteria were compared

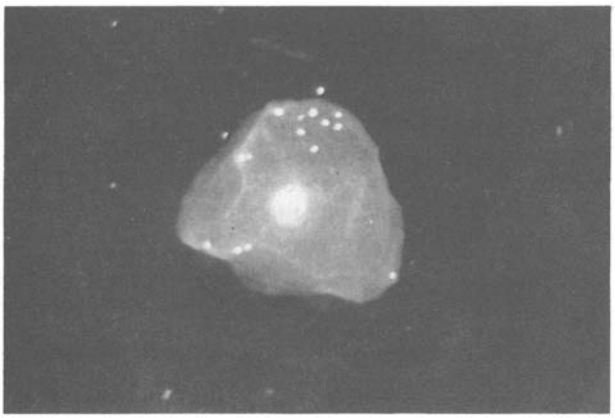

FIG. 1

Photograph of an epithelial cell obtained from the tonsillar surface with only few attached bacteria. Acridine orange stain. ×1250.

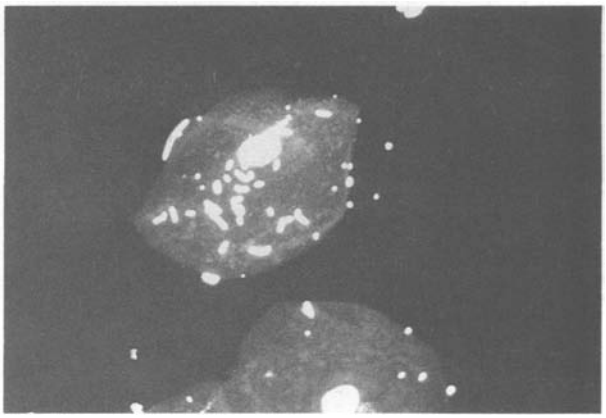

FIG. 2

Photograph of epithelial cells from the tonsiller surface with many attached bacteria. Acridine orange stain. ×1250.

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TABLE III

PERCENTAGE OF EPITHELIAL CELLS (N=50) WITH NO ADHERENT BACTERIA, 1–10 ADHERENT BACTERIA, 11–50 ADHERENT BACTERIA AND MORE THAN 50 ADHERENT BACTERIA AND BACTERIAL FINDINGS AT CULTURING FROM THE TONSILLAR SURFACE OF AGE AND SEX MATCHED HEALTHY INDIVIDUALS

	Cells with bacteria adhering				
Individual no.	0%	1–10%	11–50%	>50%	Bacterial findings
1	26	22	28	24	H. influenzae/other
2	70	16	6	8	Other bacteria*
3	4	4	20	72	Gr G Streptoc.
4	86	14	0	0	S. aureus/Other
5	0	18	14	68	S. aureus/Other
6	6	10	20	64	S. epid/H. influenzae
7	28	16	36	20	Other bacteria
8	56	16	14	14	S. aureus/Other
9	46	40	14	0	Other bacteria
10	78	22	0	0	Other bacteria
11	36	58	6	0	Other bacteria
12	46	40	14	0	Other bacteria
13	62	30	8	0	Other bacteria
14	84	16	0	0	Other bacteria
15	76	20	4	0	Other bacteria
χ	46.9	22.8	12.3	18	

*'Other bacteria' means non-pathogenic aerobes.

using Wilcoxon signed rank test. To evaluate whether or not the number of attached bacteria was age dependent Spearman rank order correlation method was used. P-values below 0.05 were considered significant.

Results

The proportions of epithelial cells obtained by scraping from the tonsillar surfaces with no bacteria, 1–10 adherent, 11–50 adherent and >50 adherent bacteria in the AT group are shown in Table I and in the individually age and sex matched control group in Table II. Typical epithelial cells with few and with >50 attached bacteria are shown in Figures 1 and 2. It was noted that the attachment of more than ten bacteria to the tonsillar epithelial cells was significantly greater in the AT group than in the control group ($p = 0.0103$).

In the AT group, 30 per cent (5/15) of the patients had massive attachment of bacteria, i.e.>50 attached bacteria per cell in the majority of cells investigated (Fig. 2), *vis-à-vis* 20 per cent (3/15) in the control group. The difference was significant ($p = 0.0212$). Attachment of >50 bacteria to tonsillar cells appeared to be highly age dependent, as in both groups significantly more attached bacteria were observed in samples from young subjects, p-values being 0.0005 in the AT group and 0.0029 in the control group.

The bacterial findings in conventional aerobic cultures from the tonsils of the AT and control groups are listed respectively in Tables II and III. Beta-haemolytic streptococci were probably the causative agent in 33 per cent (5/15) of the AT cases. In the healthy control group, one individual harboured group G streptococci. In 60 per cent (9/15) of the AT cases, no respiratory pathogens at all (*S. pneumoniae, H. influenzae, B. catarrhalis, S. aureus* or *Ps. aeruginosa*) were found by conventional aerobic culturing. In the control group, some of these pathogens were found in 33 per cent (5/15) of the cases.

Especially in cases of massive bacterial attachment, two or more morphologically different bacteria were seen adhering to the cells simultaneously. Thus cocciformed bacteria and rods were attached to the same epithelial cell. Under these circumstances clusters of bacteria formed distinct microcolonies on the cell surface (Fig. 2).

Discussion

The importance of bacterial attachment to mucosal cells for the development of invasive infection has been emphasized by several workers (Ginsburg, 1972; Abraham and Beachey, 1985). To our knowledge, no article concerning bacterial attachment *in vivo* to epithelial cells of the palatine tonsils during current AT has previously been published. In this context it is worth mentioning that conventional bacteriological culturing methods do not distinguish between attached and non-attached bacteria. Thus the presence of an organism in a patient's throat and its subsequent culture from a swab does not necessarily establish that it is pathogenic.

In AT cases that prove positive for beta-haemolytic Streptococci by conventional culturing, it seems only natural to regard these microorganisms as the causative agents of the actual disease. However, in our material one individual harboured beta-haemolytic Streptococci on the tonsils without having visible tonsillar infection. It is a well-known fact that there are large numbers of healthy carriers of beta-haemolytic Streptococci who nevertheless show no signs of tonsillar or pharyngeal infection (Quinn *et al.*, 1957; Ross, 1971). It cannot be denied that in such cases the microorganisms do not attach to the epithelial cells but are only present in the mucous film covering the epithelium. Thus, in order to establish a conclusive bacteriological diagnosis it is necessary to identify separately which bacteria are attached to the epithelium and which are merely floating in the mucus. Such studies are already in progress in our laboratories.

In those AT cases where no pathogens can be identified by conventional culturing using aerobic methods but where there is massive attachment of bacteria to the epithelial cells, it seems natural to incriminate anaerobes as the causative agent(s) (Brook *et al.*, 1981; Toner *et al.*, 1986). Furthermore, in AT cases with rare bacterial

attachment, a virus may well be the culprit (Henle and Henle, 1970; Yamanaka and Kataura, 1984). It seems self-evident that further studies are necessary before we have conclusive answers regarding the aetiology of AT.

Conclusion

Conventional bacteriological culturing from the palatine tonsils during current AT infection does not give conclusive answers regarding the pathogen. By studying the bacteria attached to the epithelial cells according to the method outlined in this study however, it is possible to gain a better understanding of the aetiopathogenesis of acute tonsillitis.

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