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Immunoglobulin- and complement-coated bacteria in pus from peritonsillar abscesses

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Abstract

Fifty-five samples of pus were collected from 51 acute, non-perforated, two spontaneously ruptured and two recurrent peritonsillar abscesses (35 males and 18 females; median age 18 years) and analysed regarding (i) aerobic and anaerobic bacteria (standard culturing), (ii) morphology of bacteria and inflammatory cells (direct microscopy of acridine orange-stained material), and (iii) the percentage of bacteria coated with immunoglobulins IgG, secretory IgA (SIgA) and IgM and complement cleavage product C3b (immunofluorescence assay). Seventy-one per cent of the abscesses harboured a mixed bacterial flora of various aerobes and anaerobes. In none of the cases with a single bacterial species (27 per cent) could immunoglobulin- or complement-coated bacteria be found. In abscesses with a mixed flora, 18 per cent harboured IgG-coated, 15 per cent SIgA-coated, five per cent IgM-coated and five per cent C3b-coated bacteria, respectively. All pus samples contained inflammatory cells in abundance but they were mostly deformed and only occasionally could intracellular bacteria be recognized. Insufficient immunoglobulin-coating of bacteria might be an important aetiopathogenic factor in the development of a peritonsillar abscess. Bactericide in the abscesses is accomplished chiefly by protective mechanisms not dependent on antigen recognition by antibodies.

Key words: Peritonsillar abscess; Suppuration; Bacteriology; Fluoroimmunoassay

Introduction

A peritonsillar abscess (PTA) or quinsy is an acute and grave inflammatory condition of the human oropharynx. A PTA is a collection of pus between the fibrous capsule of the palatine tonsil and the superior constrictor muscle of the pharynx. It occurs as a complication of acute tonsillitis or as an abscess formation of the Weber's salivary glands in the upper pole of the tonsil (Passy, 1994). Whatever the aetiology, the first line of defence of the host's oropharynx has been breached and as a consequence microorganisms have penetrated into the oropharyngeal tissues. Bacteriology of the PTA pus usually reveals a mixed flora of various aerobes and anaerobes (Jokipii *et al.*, 1988; Brook *et al.*, 1991; Jousimies-Somer *et al.*, 1993; Lilja *et al.*, 1997).

When bacteria penetrate the tissues, the host naturally mobilizes several defence mechanisms. Ultimately most bacteria are killed by phagocytes attracted to the target site due to activation of complement via the alternative pathway and/or by cytokines released from macrophages (Rook, 1993). The complement cleavage products C3a and C5a attract and activate the neutrophils. Attachment to the bacteria of the complement derivative, C3b, is important in subsequent interactions with phagocytes (Walport, 1993). The main immunoglobulin isotype in serum, IgG, can also activate complement and act as an opsonin when attached to bacteria. The principal immunoglobulin on mucosal surfaces, secretory IgA (SIgA), serves an important purpose in hindering bacteria attaching to epithelial cells and thereby penetrating into the tissues. In contrast to IgG and IgM, SIgA cannot activate complement (Brandtzaeg, 1995). Antibodies definitely play a crucial role in dealing with bacterial toxins, e.g. diphtheria toxin is neutralized by blocking of the attachment of the binding portion of the toxin molecule to its target cells. Which role antibodies play in combating bacteria in acute peritonsillar inflammation is still not fully understood. The first step in their action on bacteria must, however, be to attach to the bacterial surface.

The purpose of the present study was to evaluate whether or not the bacteria occurring in PTAs are coated with immunoglobulins and/or complement. In addition, we focused on the occurrence of the inflammatory cells, in particular cells with intracellular bacteria. We used standard culturing techniques to identify aerobic and anaerobic bacteria, direct microscopy of acridine orange-stained

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pus for evaluation of the morphology of the microorganisms and inflammatory cells, and finally an immunofluorescence assay to study bacteria coated with immunoglobulins IgG, SIgA and IgM as well as C3b.

Material and methods

The study population consisted of 53 patients, 35 males and 18 females, with PTA. Their ages ranged from five to 46 years (median 18 years). A total of 55 pus samples were collected, of which 51 were from acute non-ruptured PTAs. Two samples were obtained from recurrent abscesses (in both cases two weeks after the primary abscess had been treated by needle aspiration and antibiotics). Two samples were obtained from abscesses that had ruptured spontaneously before hospital admission.

Pus samples were collected by means of direct needle aspiration (n = 25), after an incision of the abscess (n = 19) or in connection with a quinsy tonsillectomy (n = 11). Samples taken after the incision or tonsillectomy were aspirated from the mid-portion of pus into a sterile syringe.

For aerobic and anaerobic culturing, roughly 0.5 ml of pus material was injected into a Portagerm® ampoule and transported to the bacteriological laboratory. Aerobic cultures were performed according to standard laboratory procedures using blood agar and chocolate agar plates and incubation in an atmosphere of four per cent CO_2 at 37 °C for 48 hours. For anaerobic culturing, non-selective enriched anaerobic blood agar plates were used. The anaerobic plates were incubated in an anaerobic atmosphere at 37 °C for three to seven days. The organisms were identified by standard procedures.

For direct microscopy of the pus samples, roughly 0.1 ml pus was flushed into a test tube and 1 ml physiological saline was added. Small amounts of this mixture were flushed onto a clean glass slide, spread uniformly using another glass slide and allowed to dry. The smear was fixed in 70 per cent alcohol for one minute and dried at 37 °C for two minutes. The dried slide was stained with acridine orange (Difco Laboratories, Detroit, Mich., USA) and examined under a fluorescence microscope (Leitz SM-Lux). The morphology of the inflammatory cells and bacteria was examined. Particular interest was paid to the occurrence of intracellular bacteria and/or bacteria in immediate contact with inflammatory cells. One hundred inflammatory cells were scrutinized on each slide. The staining method is described in detail elsewhere (Stenfors and Räisänen, 1988).

For immunofluorescence (IF) identification of immunoglobulins IgG, SIgA and IgM and the complement cleavage product C3b coating the pus bacteria, small amounts of pus mixture were flushed into four hydrophobic rings drawn on a clean glass slide. The slide was allowed to dry and the specimen was fixed in 70 per cent alcohol for one minute and dried at 37 °C for two minutes. These slides were kept at −70 °C until subsequent immunofluorescence processing. After thawing, two drops of fluorescein-conjugated goat anti-human IgG-serum (Cappel Labs; Westchester, Pa, USA; direct IF assay) were added into the first hydrophobic ring; two drops of fluorescein-conjugated $F(ab')_2$ fragment goat anti-human SIgA-serum (Cappel Labs; direct IF assay) into the second ring; two drops of fluorescein-conjugated goat anti-human IgM-serum (Sigma Chemical Co., St Louis, Mo, USA; direct IF assay) into the third ring; and two drops of rabbit anti-human C3 (reactive to both C3a and C3b; Serotec; Oxford, England) and fluorescein-conjugated anti-rabbit IgG-serum (Diagnostics Pasteur, Marnes-la-Coquette, France; indirect IF assay) into the fourth ring, respectively. Counterstaining was performed with 0.25 per cent Evans blue. All specimens were stained using the same dilutions of the antisera and then examined under the Leitz fluorescence microscope. One hundred consecutive bacteria within each hydrophobic ring were scrutinized. Bacteria expressing positive reactivity to the actual antiserum used stained green and those with negative reactivity stained red (Figure 1) (for details, see Stenfors and Räisänen, 1992).

Prior to the immunofluorescence studies, the antisera were titrated according to principles promulgated by the US Department of Health, Education, and Welfare, 1972. As positive controls for IgG- and SIgA-antiserum, we used bacterial samples obtained from the posterior wall of the nasopharynx of one of the authors; for IgM-antiserum, bacterial samples obtained from the palatine tonsils of a patient suffering from infectious mononucleosis; and for C3-antiserum bacterial samples obtained from a patient with a chronically draining ear, culture-positive for *Staphylococcus aureus*. For negative controls, five bacterial strains sampled from the normal flora of the oropharynx of one of the authors and incubated for two days on agar plates were used. All these samples were treated exactly as described above. The following dilutions of the various antisera were tested: 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512. Dilution of the antiserum to positive controls used was made according to the principle 'high specificity, low sensitivity'.

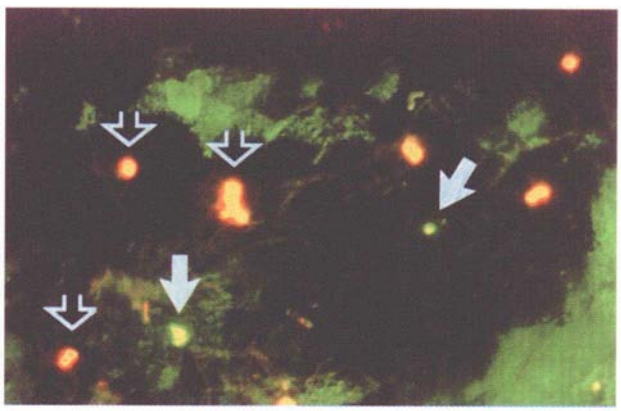

FIG. 1

Photograph of pus material showing bacteria expressing positive (green-stained bacteria; *filled arrows*) and negative (red-stained bacteria; *open arrows*) reactivity to human anti-IgG serum. (Fluorescein-labelled goat anti-human IgG-serum, counterstained with 0.25% Evans blue, × 3000).

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TABLE I

BACTERIAL SPECIES IDENTIFIED IN 51 ACUTE NON-PERFORATED PTA EFFUSIONS; AND TWO RECURRENT AND TWO SPONTANEOUSLY PERFORATED PTAS

Acute (n = 51)		Recurrent or perforated (n = 4)	
Single flora (n = 14)	Mixed flora (n = 36)	Single flora (n = 1)	Mixed flora (n = 3)
GABHS 8	GCBHS 7	GABHS 1	*Corynebacterium* sp. 2
GFBHS 1	GFBHS 2		GCBHS 1
S. aureus 2	GGBHS 1		*Bacillus fragilis* 1
S. viridans 1	non-GBHS 2		*Neisseria* sp. 2
S. pneumoniae 1	*S. viridans* 14		*Fusobacterium* sp. 2
H. influenzae 1	*Staphylococcus* sp. 4		
	H. influenzae 3		
	S. aureus 2		
	Escherichia coli 1		
	Fusobacterium necrophorum 2		
	Fusobacterium sp. 3		
	diphtheroids 2		
	Bacteroides sp. 2		
	Proteus mirabilis 1		
	Veillonella sp. 1		
	Peptostreptococcus sp. 1		
	Corynebacterium sp. 1		

GABHS, GCBHS, GFBHS, GGBHS means Group A, C, F, G β-haemolytic streptococci, respectively. Non-GBHS means non-typable β-haemolytic streptococci
S. aureus = *Staphylococcus aureus*
S. viridans = *Streptococcus viridans*
S. pneumoniae = *Streptococcus pneumoniae*
H. influenzae = *Haemophilus influenzae*

Results

The bacterial findings obtained by standard aerobic and anaerobic culturing and direct microscopy of pus material from 51 consecutive non-ruptured, acute PTAs and two recurrent and two spontaneously ruptured PTAs are summarized in Table I. It appears that 50 (98 per cent) of the acute cases harboured bacteria; 14 (27 per cent) of the PTAs hosted a single bacterial species, of which ß-haemolytic streptococcus Group A (GABHS) was the most common, occurring in 16 per cent of the cases. Other bacterial species occurring as single microorganisms were *Staphylococcus aureus*, *Streptococcus viridans*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and ß-haemolytic streptococcus Group F. In most cases, however, the bacterial florae were mixed where other ß-haemolytic streptococci than Group A (in most cases Group C), *S. viridans*, *Staphylococcus* spp., *Fusobacterium* spp., *H. influenzae* and diphtheroids could be identified. In only one case were no bacteria found.

In two cases with a spontaneously ruptured abscess, a mixed bacterial flora of aerobes and anaerobes was seen. In two cases with recurrence (one caused by GABHS appearing as single species and the other by ß-haemolytic streptococcus Group C together with *Bacillus fragilis*), the same pathogenic organisms had been detected two weeks earlier in corresponding PTAs.

In the great majority of cases (87 per cent), no bacteria coated with immunoglobulins nor C3b could be found (Table II). This was the ultimate finding in PTA cases harbouring a single bacterial species. In the PTAs with a mixed bacterial flora, seven (18 per cent) displayed IgG-coated, six (15 per cent) SIgA-coated, two (five per cent) IgM-coated and two (five per cent) C3b-coated bacteria, respectively. The proportions of bacteria in corresponding effusion samples coated with immunoglobulins and C3b are shown in Table III. It can be seen that in none of the cases did all bacteria present in the effusions display immunoglobulin- or C3b-coating. It was also evident that in all samples with opsonized bacteria, anae-

TABLE II

NUMBER OF PTA EFFUSIONS HARBOURING IGG-, SIGA-, IGM- AND C3B-COATED BACTERIA IN PUS OBTAINED FROM ACUTE NON-RUPTURED PTAS (N = 51) AND FROM RECURRENT AND/OR SPONTANEOUSLY RUPTURED PTAS (N = 4)

Groups	IgG	SIgA	IgM	C3b
Acute non-ruptured PTAs				
Single flora (n = 14)	0	0	0	0
Mixed flora (n = 36)	5	5	1	2
Recurrent or spontaneously ruptured PTAs				
Single flora (n = 1)	0	0	0	0
Mixed flora (n = 3)	2	1	1	0

TABLE III

BACTERIAL FINDINGS AND PROPORTIONS OF BACTERIA COATED WITH IMMUNOGLOBULINS IGG, SIGA, IGM, AND COMPLEMENT, C3B IN THE SEVEN CASES OF POSITIVE BACTERIAL OPSONIZATION

No.	Bacterial finding	IgG (%)	SIgA (%)	IgM (%)	C3b (%)
1.	GCBHS/Anaerobes	20	30	0	0
2.	GCBHS/Anaerobes	78	42	44	28
3.	*H. influenzae*/Anaerobes	6	38	0	0
4.	Aerobes/Anaerobes	48	56	0	24
5.	Aerobes/Anaerobes	72	32	0	0
6.*	Aerobes/Anaerobes	8	0	0	0
7.#	GCBHS/*B. fragilis*	70	38	40	0

GCBHS = Group C β-haemolytic streptococci
H. influenzae = *Haemophilus influenzae*
B. fragilis = *Bacillus fragilis*
6* was a spontaneously ruptured abscess
7# was a recurrent abscess

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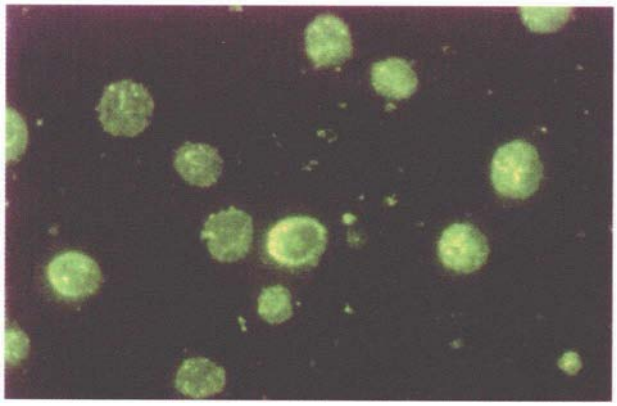

FIG. 2

Pus material showing inflammatory cells expressing positive reactivity to goat anti-human IgG serum. (Fluorescein-labelled goat anti-human IgG serum, counterstained with 0.25% Evans blue, × 3000).

robes were present. All bacteria displaying positive reactivity to human immunoglobulin- and C3b-antiserum were coccus-shaped. The most common single aerobic species was ß-haemolytic streptococcus Group C. In one of the recurrent PTAs, culture-positive for ß-haemolytic streptococcus Group C and *B. fragilis*, abundant IgG-, SIgA and IgM-coated bacteria could be observed. Furthermore, in this sample, inflammatory cells expressing positive reactivity to human IgG could also be identified (Figure 2).

In acridine orange-stained pus material, the inflammatory cells were mostly deformed and irregularly-shaped, frequently forming an amorphous mass. Intracellular bacteria or bacteria in immediate contact with the inflammatory cells were usually found only in recurrent PTA cases or those with spontaneous pus drainage (Figure 3). Generally speaking, only in these cases could intact polymorphonuclear granulocytes and/or macrophages be observed.

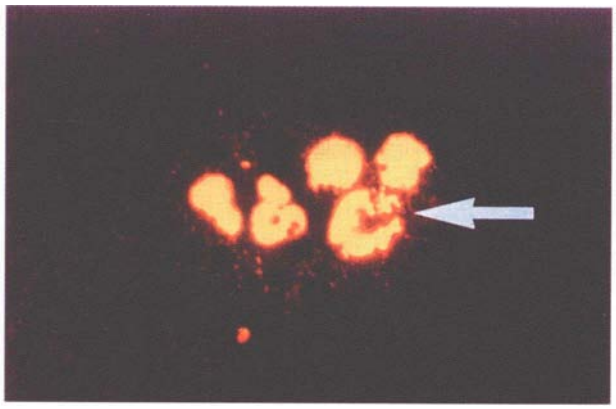

FIG. 3

Pus material showing leukocytes, one with intracellular bacteria (*arrow*). (Acridine orange, × 2800).

Discussion

Recent studies (Brook *et al.*, 1991; Jousimies-Somer *et al.*, 1993; Mitchelmore *et al.*, 1995; Lilja *et al.*, 1997) have reported that bacterial microorganisms were found in more than 85 per cent of PTA effusions. The present study confirmed these earlier findings. Here, however, we wanted to focus on certain essential circumstances of the host's defence in combating bacterial invasion. When attached to the bacterial wall, IgG antibodies can cause bacteriolysis directly, or opsonize the bacteria (as can also IgM) to effect phagocytosis (Rook, 1993). Opsonization for phagocytosis can be further potentiated if the complement cleavage product, C3b, is attached to the antigen-antibody complex (Walport, 1993).

The present study showed that immunoglobulin- or C3b-coated bacteria could only be found in PTA effusions in a minority of cases. We found that in PTA cases where one single bacterial species invaded the peritonsillar space, none of the invaders were opsonized. This finding implies that bacterial coating with antibodies was incomplete, which could be one explanation for the development of a PTA. Only in cases with a mixed bacterial flora could immunoglobulin-coated bacteria be found. In these cases, however, we cannot say exactly which bacterial species were actually coated – only that these bacteria were coccoid in shape. In order to form a clear impression of this essential circumstance, other immunocytochemical techniques are needed. Such studies are already in progress in our laboratories.

In six cases, all with a mixed flora, SIgA-coated bacteria could be found. The anti-human SIgA-serum used in this study expressed positive reactivity to SIgA, harboured mainly on mucous membranes, and monomeric serum IgA. According to recent studies, monomeric serum IgA is extremely vulnerable to proteolytic destruction on mucous membranes (Brandtzaeg, 1995) and does not bind to some bacteria at all (Hammerschmidt *et al.*, 1997). However, PTA is not an inflammatory condition on mucosal membranes where SIgA definitely exerts its main mode of action. When SIgA-coated bacteria could be found in PTA pus, this finding might imply that these bacteria had been coated on the tonsillar surfaces prior to tissue invasion. We have recently shown that under healthy conditions SIgA-coated bacteria on tonsillar surfaces are generally the rule (Stenfors and Räisänen, 1996). Another possible source of origin of SIgA antibodies in such cases could naturally be secretion from the salivary glands of Weber, located in the upper pole of the palatine tonsils where most PTAs are to be found. Such a location could concur with Passy's (1994) findings, suggesting that these glands were the site of the PTAs.

Ultimately, most of the bacteria invading tissues are combated initially by the innate immune system without the need for a specific adaptive immune reaction (Rook, 1993). In the present study, we found numerous inflammatory cells by direct microscopy of acridine orange-stained pus material.

However, in most cases the inflammatory cells were deformed and disintregrated due to either programmed cell death (apoptosis) and/or rapid cell death following phagocytosis. This finding could have any of three possible causes: (i) the phagocytes may be destroyed immediately after phagocytosis and antimicrobial enzymes such as myeloperoxidase, cathepsin G, lysozyme, lactoferrin, elastase, and/or collagenase may be secreted from the intracellular granules of the phagocytes (Brook *et al.*, 1991); (ii) leukocidins secreted by bacteria may destroy the phagocytic cells (Brook *et al.*, 1991); (iii) it is well known that streptolysins O and S produced by GABHS inhibit chemotaxis, leukocyte mobility and phagocytosis and can even kill phagocytic cells (Volk *et al.*, 1986). Disintegrated and deformed leukocytes were particularly evident in cases culture-positive for GABHS, whereas those few cases with spontaneous rupture of the abscess exhibited phagocytes with intracellular bacteria (Figure 3). This finding hinted at the importance of draining of the PTAs. Aspiration of old pus from the abscesses allows new and fresh inflammatory cells to accumulate in the inflammatory target area and stimulates further phagocytosis.

Furthermore, the present study showed that microorganisms occurring in a single species of pus effusion are coated with neither immunoglobulins nor the complement cleavage product, C3b. The reason why we did not find immunoglobulin-coated bacteria could simply be that there were no such bacteria or that the phagocytic process immediately following the immunoglobulin coating of the bacteria was so rapid that this step in the bacterial killing procedure could not be visualized. The pus material revealed numerous disintegrated and deformed granulocytes. Only in PTA effusions harbouring a mixed flora could opsonized bacteria be found. In none of the cases, however, were all bacteria coated simultaneously (Figure 1). When taking into consideration that *S. viridans*, *Corynebacterium* spp. and *Neisseria* spp. are non-pathogens, these microorganisms can only have followed the real pathogens on their way to the peritonsillar space. Consequently, suppression of the immunoglobulin coating of the tonsillar bacteria might well be an important cause in the aetiopathogenesis of PTA. It must also be noted that PTA is an acute inflammatory condition that develops within a couple of days, too short a time for protective antibodies to develop. Whether or not patients with acute tonsillitis harbour immunoglobulins in their serum which are able to coat the actual pathogens during an acute PTA infection is an obvious topic for further study.

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