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Distinct cytokine pattern in response to different bacterial pathogens in human brain abscess

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ABSTRACT

Brain abscess is a focal suppurative process. Host inflammatory response in Gram type and specific bacteria has not been studied in brain abscess. A total of 57 brain abscess patients with monomicrobial infections were studied for Th1 (TNF-α, IFN-γ, IL1-β), Th2 (IL-4, IL-10) and Th17 (IL-17, IL-23) cytokine response by reverse-transcriptase PCR and ELISA. Th1 and Th17 cytokines were significantly elevated in Gram positive (*Staphylococcus aureus* and *Streptococcus intermedius*) and Th2 cytokine (IL-10) in Gram negative (*Bacteroides fragilis* and *Escherichia coli*) infections ($p < 0.05$). Cytokine levels were significantly higher in abscess than blood ($p < 0.001$). Elevated levels of several inflammatory cytokines (TNF-α, IFN-γ, IL1-β, IL-17 and IL-23) were associated with the duration of symptoms; predisposing factors also influenced the levels of several cytokines. The expression of inflammatory cytokines in abscess was influenced by the bacterial pathogen, duration of symptoms and predisposing factors. Local milieu of brain plays significant role in secretion of various cytokines.

1. Introduction

Brain abscess (BA) starts as a localized area of cerebritis and develops into a suppurative lesion enclosed by a well-vascularized fibrotic capsule. The abscess cavity contains inflammatory cells with intact cell membranes and nuclei, necrotic tissue debris, proteinaceous exudates, bacterial metabolites and cytosolic amino acids. MRI studies revealed that BA progressed through a series of well-defined stages in human patients (Poptani et al., 1995). The probable sequelae of BA include the replacement of the abscess area with a fibrotic scar, loss of brain tissue by surgical excision, or abscess rupture and death. The frequently isolated etiologic agents are *Staphylococcus aureus* and *Streptococcus* species; though a myriad of other organisms have also been implicated in brain abscess (Prasad et al., 2006). Bacterial pathogens produce an inflammatory reaction in the host, which plays an essential role in pathogen recognition and local production of soluble neuroinflammatory mediators *i.e.* cytokines. Cytokines are a large group of protein factors which play a central role in central nervous system (CNS) inflammation including modulation of blood–brain barrier (BBB) integrity, induction of adhesion molecules on cerebral microvascular endothelial cells, and subsequent activation of resident glia and infiltrating peripheral immune cells. Studies in experimental model of BA revealed that astrocytes, microglial and resident macrophages recognize invading pathogens through Toll-like receptors (TLRs). TLR signaling further stimulates NF-kappa B resulting in the production of various pro- and anti-inflammatory cytokines (Kielian, 2004). Besides the potential beneficial role these cytokines may exert on the antibacterial response in infection, if produced in excessive quantities may have detrimental effects on the integrity of surrounding normal brain tissue.

Earlier studies have demonstrated immunopathogenesis of BA in *in-vitro* and experimental model by using only *S. aureus* as causative agent (Kielian and Hickey, 2000; Kielian, 2004; Kielian et al., 2005; Gupta et al., 2008). However, a variety of organisms participate in the occurrence of BA. There is scanty data regarding cytokine (Th1, Th2 and Th17) response in human BA and relevant questions such as "are there any differences between cytokine response caused by Gram type specific pathogen and by each etiologic agent???" remains to be answered. Therefore local cytokine response in Gram type specific and pathogen specific infections were analyzed in human brain abscess. Furthermore cytokine response in abscess was compared with systemic response in blood to know whether only local milieu is involved in brain abscess. This comparative study is important because if there is a marked difference in cytokine response, this may have implication for immunomodulatory therapies which should be tailored differently for each pathogen.

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Table 1
Primers for RT-PCR analysis of cytokines.

Gene	Forward	Reverse	Product size (bp)	References
GAPDH	CAAGGTCATCCATGACAACTTTG	GTCCACCACCCTGTTGCTGTAG	496	NM_001256799.1
IFN-γ	TCGACCTCGAAACAGCATCT	TGTCCAACGCAAAGCAATAC	104	NM_000619.2
TNF-α	CAGAGGGAAGAGTTCCCCAG	CCTTGGTCTGGTAGGAGACG	325	NM_000594.3
IL-1β	AGATGAAGTGCTCCTTCCAG	CAACACGCAGGACAGGTACAG	468	NM_000576.2
IL-4	CTCTGGTTGGCTTCCTTCACA	CAGCAGTTCCACAGGCACAA	110	NM_172348.2
IL-10	AGTCGCCACCCTGATGTCTC	CCTGGGGGAGAACCTGAA	223	NM_000572.2
IL-17	ATGACTCCTGGGAAGACCTCATTG	TTAGGCCACATGGTGGACAATCGG	471	NM_002190.2
IL-23	CAGCAACCCTGAGTCCCTAA	TCAACATATGCAGGTCCCACT	150	NM_016584.2

2. Material and methods

2.1. Study subjects

A total of ninety patients with final diagnosis of brain abscess were included in the study. All the patients underwent magnetic resonance imaging (MRI) on a 3 T MR scanner (Signa HDxt, General Electric, Milwaukee, USA), using a 12 channel head coil (Tomar et al., 2011). The data (age, sex, duration of symptoms, antibiotic treatment and associated predisposing factors) related to the patients were recorded on predesigned proforma. The duration of symptoms was counted from the day when any of symptoms was reported by the patient or his relative. Further, information on prior antibiotic treatment was available in 76 patients and treatment range was 4 days to six months. However, all patients received antibiotics for prior to surgery that usually continued for one to two weeks. Institutional ethical approval as well as informed consent from all patients or their nearest kin was obtained prior to the investigation.

2.2. Sample collection

Pus was collected in a sterilized vial and snap frozen in liquid nitrogen immediately after surgery/aspiration. Blood was also collected from same patients and sera was separated. All samples were kept at −80 °C for further use.

2.3. Culture

All patients underwent surgery within 24 h of imaging. At the time of surgery 2 ml of aspirated pus was inoculated immediately into BACTEC plus aerobic and anaerobic media (Becton Dickinson Co., Sparks, MD, USA) to isolate the bacterial pathogens. The inoculated media were incubated at 37 °C and growth was monitored in BACTEC 9120 for 5 days. Media with positive growth were subcultured on appropriate solid media and incubated aerobically and anaerobically at 37 °C. Anaerobic incubation was carried out in jars filled with a gas mixture of N_2 (80–90%), CO_2 (5–10%), and H_2 (5–10%) through an Anoxomat system (Mart® BV Microbiology, Holland). All the isolates were identified by standard biochemical tests as described previously (Prasad et al., 2006).

2.4. RNA extraction

All the pus samples aspirated from brain abscesses were taken in RNA stabilization reagent (Qiagen, CA, USA) and snap frozen in liquid N_2 for cytokine study. Total RNA was extracted from snap frozen pus samples using Qiagen RNeasy kit (Qiagen, Inc., CA, USA) according to manufacturer's instructions.

2.5. RT-PCR (reverse transcriptase-polymerase chain reaction)

RNA was quantified by spectrometry so that equal amount of c-DNA can be synthesized. RNA (150 ng) were reverse transcribed by using c-DNA synthesis kit (first strand c-DNA synthesis kit, Fermentas, Germany) to make complementary DNA (c-DNA). RT-PCR reaction using c-DNA was conducted in 20 μl reaction mixture using Master Mix (DreamTaq Master Mix Fermentas, Germany) and primers specific for genes encoding human cytokines were used. Primer sequences used in the study are given in Table 1; c-DNA replaced by diethyl pyrocarbonate-water (DEPC, Sigma, MO, USA) was taken as negative control for PCR.

2.6. Densitometry analysis of PCR products

Ten μl of the PCR products were resolved on 2% agarose gel and were photographed under ultra violet illumination through Gel Doc XR+ (Bio-Rad, California, U.S.A.). Band intensities were quantified by densitometry analysis using IMAGE J (California, U.S.A.) software. To normalize mRNA levels, density of TNF-α, IFN-γ, IL1-β, IL-10, IL-17, IL-23 and GAPDH (housekeeping gene) bands from the same lane were scanned, and band density ratio of cytokines was calculated against GAPDH density.

2.7. ELISA

Brain abscesses were homogenized in 500 μl of PBS supplemented with a protease inhibitor cocktail tablets (Roche, Switzerland). Subsequently, homogenates were centrifuged at 14,000 rpm for 15 min at 4 °C and supernatants was collected and stored at −80 °C for ELISA (Garg et al., 2009). The levels of cytokines were determined by using commercially available ELISA kits (R&D System, USA) as per manufacturer's instructions. The results were expressed as picograms of cytokine/ml (pg/ml), based on the standards provided with the kits.

2.8. Statistical analysis

Values from densitometry and ELISA were the mean ± SD of experiments performed independently for each sample. Data were analyzed with SPSS statistical software, version 12.0 (SPSS Inc., Chicago, IL, USA). The mean values of the cytokine response (ELISA and RT-PCR) of different study groups were compared using one-way ANOVA, and $p \leq 0.05$ was considered significant. Paired sample T-test was performed to compare cytokine response between local and systemic (abscess and blood). Multivariate analysis was performed to find out any association of the cytokines with predisposing factors; $p \leq 0.05$ was considered significant.

3. Results

3.1. Study subjects

In 90 MRI confirmed patients (mean age 24 years) with brain abscesses, 67 were male. All the lesions appeared hyper intense on T2-weighted images with a peripheral hypo intense rim (Fig. 1A), and iso- to hypo-intense on T1-weighted images with iso-hyper intense rim (Fig. 1B). All these lesions showed a characteristic rim enhancement following administration of gadolinium contrast agent (Fig. 1C). Specimens collected from all these patients after surgical exploration were