INTRODUCTION

Neurosyphilis (NS) is a collective term encompassing a series of severe neurological diseases caused by *Treponema pallidum* subspecies *pallidum* (*T. pallidum*), which is the pathogen of syphilis (Marra 2009). Symptomatic NS is classified into syphilitic meningitis, meningovascular syphilis, general paresis, tabes dorsalis and gumma of the CNS (Ghanem 2010). Recently, there have been increasing reports on NS cases, especially in human immunodeficiency virus (HIV)-positive patients (Farhi & Dupin 2010). Moreover, the clinical manifestations of NS have changed (Chahine et al. 2011), and the misdiagnosis rate of NS is high, and that of neurosyphilitic ischaemic stroke was up to 80.95% (Liu et al. 2012).

*T. pallidum* have been found in the cerebrospinal fluid of syphilis patients at all stages of infection, which indicates that they can invade the CNS within days of infection (Chung et al. 1994). Clinical studies show that NS patients have abnormal humoral and cellular immunity (Wang et al. 2015, Pastuszczak et al. 2013, Li et al. 2013), and the inflammatory system in the CNS may be activated (Lu et al. 2016). The rabbit model of NS confirmed the existence of neuroinvasive *T. pallidum* strains, and the clinical manifestation of infected rabbits varied with the infecting strains (Tantalo et al. 2005). A scanning electron microscopy study showed...
that *T. pallidum* could directly attach to cultured nerve cells (Repesh et al. 1982). *T. pallidum* typing studies showed that the preferential strain types of NS were from different areas (Molepo et al. 2006, Marra et al. 2010, Dai et al. 2012). These data suggest that *T. pallidum* has the potential to attach to and cross the blood–brain barrier (BBB), which further causes CNS disorders. However, as an infectious agent, how *T. pallidum* adheres to and across the BBB is largely unknown. In recent years, due to the development of in vitro BBB models based on human brain microvascular endothelial cells (HBMECs), which are the major component of the BBB, the current understanding of the molecular interaction between the BBB and some pathogens has significantly improved (Stins et al. 1994, Greiffenberg et al. 1998, Weksler et al. 2005). Scientists have made great achievements in the identification of ligands and response receptors that are associated with bacterial binding to and invasion of the BBB (Kim 2008, 2010). Unfortunately, so far, little is known about the interaction between *T. pallidum* and the BBB.

The major objective of this study was to explore the response of HBMECs to *T. pallidum* at mRNA level and search meaningful genes for further research. Primary HBMECs were used as an in vitro BBB model, and gene microarray analysis technology was used to analyze the gene expression of HBMECs in response to infection with fresh and virulent *T. pallidum* for a period of 4 h. We further performed Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyze to analyze the biological functions of these differentially expressed genes.

**MATERIALS AND METHODS**

**Ethics statement**

Mature male New Zealand White rabbits (n=6) were obtained and housed in a 20°C temperature-controlled room with food and water available ad libitum at the China Nanjing Command Institute of Military Medicine. When orchitis was considered to be optimal, the rabbit was sacrificed with an intravenous injection of pentobarbital (90 mg/kg). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. This study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College (Permit Number: 2015-KY024).

**Bacterial strains**

In the present study, we used *T. pallidum* (Nichols strain). *T. pallidum* was a kind gift from Prof. Tian-Ci Yang, Zhongshan Hospital of Xiamen University, China and was maintained by intratesticular inoculation of rabbits, which were 3 months old and had well-developed testes. *T. pallidum* was passaged and harvested as described previously (Robertson et al. 1982). To remove gross debris and whole cells from the extract, freshly harvested treponemes were centrifuged at 700×g twice for 5 min. The bacterial suspension was centrifuged at 12,000×g for 30 min at 4°C. Then, the supernatant was discarded, and the pelleted treponemes were resuspended in 1 ml fresh cell culture medium. The bacterial suspension was centrifuged at 300×g for 3 min at 4°C again. The supernatant containing treponemes was collected and diluted to an optimal concentration with fresh cell culture medium.
Cell culture and infection
Primary HBMECs (ACBRI 376) were purchased from Cell Systems (Kirkland, WA, U.S.A.), and cultured with the recommended medium (CSC-Complete Medium Kit R, Kirkland, WA, USA). Endothelial cells were used at passages 4-7 in all experiments. HBMECs were seeded onto T25 flasks and cultured in a 5% CO₂ atmosphere at 37°C. When the HBMECs reached confluence, the cells were infected with T. pallidum at a ratio of 40:1 (Fig. 1 and 2). Control cells received fresh CSC-Complete medium. After 4 h of co-incubation, the media was removed, and HBMECs were washed with phosphate-buffered saline three times and harvested in TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.A.).

Microarray analysis, GO enrichment analysis, KEGG analysis
We adopted the Agilent SurePrint G3 Human Gene Expression 8×60K microarray (Agilent, Santa Clara, CA, USA) to identify the expression profiling of HBMECs to T. pallidum. Total RNA was extracted from HBMECs using TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.A.). A NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA) and a 2100 Bioanalyzer (Agilent, Santa Clara, CA, U.S.A.) were used to measure the concentration and purity of total RNA and 1% formaldehyde denaturing gel electrophoresis was used to determine the integrity of the RNA. We used the PrimeScript RT reagent Kit (TaKaRa Biotechnology, Otsu, Shiga, Japan) to synthesize and hybridize cDNA according to the manufacturer’s recommendations.

For quality control, data summarization and normalization, the GeneSpring software V12 (Agilent, Santa Clara, CA, USA) was used to analyze the array data from 3 biological replicate experiments. Threshold values of ≥2 and ≤–2-fold change and a Benjamini-Hochberg corrected P-value of 0.05 were used to select differentially expressed genes. Data were Log2 transformed and median centered by genes using the Adjust Data function of CLUSTER 3.0 software and then further analyzed by a hierarchical clustering approach with average linkage (Eisen et al. 1998). Java Treeview software (Stanford University School of Medicine, Stanford, CA, U.S.A.) was then used to perform tree visualization.

GO analysis and functional annotation of differentially expressed genes were performed on Gene Ontology (www.geneontology.org) and Web Gene Ontology Annotation Plot (WEGO) based on statistical significance, respectively. If the corrected P-values were <0.05, the resulting GO terms were considered significant. Pathway analysis was performed based on the latest KEGG database version to determine the biological functions of differentially expressed genes. P-values of <0.05 were considered statistically significant.

qRT-PCR verification
Seven genes of interest were validated using a qRT-PCR method. qRT-PCR was performed on a Bio-Rad CFX96 Real-Time PCR system using SYBR Green master mix (SYBR Premix Ex Taq II, TaKaRa Biotechnology). The primer sequences for each gene are listed in Table I. The conditions for PCRs were 95°C for 5 min followed by 40 cycles of 95°C for 30 s and 60°C for 60 s. Each sample was measured three times. The 2^ΔΔCt method was used to calculate the relative fold change of mRNA expression level. Gene expression levels of the target genes were normalized to the β-actin levels. Each experiment was independently performed in triplicate.
RESULTS

Gene expression profile of HBMECs induced by *T. pallidum*

In the present study, a total of 35,377 different human genes were examined by using the Agilent SurePrint G3 Human Gene Expression 8×60K microarray. We detected 249 differentially expressed genes that included 218 upregulated genes and 31 downregulated genes, among which 93 genes were uncharacterized (Table II). Fig. 3 shows the cluster analysis results.

**GO enrichment analysis**

To determine the classification and functional annotation of all differentially expressed genes, we identified significantly regulated GO biological process terms using WEGO software (Ye et al. 2006). The differentially expressed genes were annotated and classified into 38 functional groups, and the number of groups in three main categories (biological process, molecular function, and cellular component) was 27, 6, and 5, respectively (Fig. 4). Table III shows the top 10 significant GO terms in detail. The GO analysis results showed that most of the significantly enriched GO terms were involved in the biological process ontology. Other important functional groups included protein binding, extracellular region, extracellular space and extracellular region part.

The KEGG enrichment analysis results showed that only the TGF-beta signaling pathway (ko04350) was identified as likely to be relevant to *T. pallidum* infection. The gene encoding decorin was one of the genes upregulated in this pathway.

**Table I. List of primers used for the detection of ADAMTS5, CLDN4, DCN, F3, LDLR, RARRES2, MACF1 and β-actin by SYBR Green PCR.**

| Gene Symbol | Primer sequence |
|-------------|----------------|
| ADAMTS5 Forward primer | 5’-GTAAAGCATTTCCTATGTGTGAC-3’ |
| Reverse primer | 5’-TTATTATGCCCCACTGAACCCAC-3’ |
| CLDN4 Forward primer | 5’-CCCTCAGGACACTAATTGAG-3’ |
| Reverse primer | 5’-CAAAACAGAAACCACAAAGAAGG-3’ |
| DCN Forward primer | 5’-GCTTCTATCGGTTGATGT-3’ |
| Reverse primer | 5’-CTTATATCTGAGTAAATGGG-3’ |
| F3 Forward primer | 5’-TAATGTCAGGAGACATTGAT-3’ |
| Reverse primer | 5’-GTCAACCATAAGCTTAACTGACC-3’ |
| LDLR Forward primer | 5’-GTTTTAGTACGACTATCTCG-3’ |
| Reverse primer | 5’-GCCAGAAGCCACTACATAC-3’ |
| RARRES2 Forward primer | 5’TTCAGGAGACAGTGTGAG-3’ |
| Reverse primer | 5’-CATTTCCTCGTCCCTAGAT-3’ |
| MACF1 Forward primer | 5’TCCACACCTGTTGAACTAAC-3’ |
| Reverse primer | 5’TCAAATTTACACCCACACCTAT-3’ |
| β-actin Forward primer | 5’-CACGGACACGGGCGTATGG-3’ |
| Reverse primer | 5’TGATCCGTCGATGGGGG-3’ |
Table II. Top differentially expressed genes of HBMECs after infection with *Treponema pallidum* for 4 h.

| Gene Symbol | FC<sup>abs</sup> | Gene or protein description/name | GenBank accession no. |
|-------------|-----------------|--------------------------------|----------------------|
| RARRES2     | 14.04           | retinoic acid receptor responder (tazarotene induced) 2 | NM_002889 |
| NR4A1       | 10.30           | nuclear receptor subfamily 4, group A, member 1 | NM_002135 |
| TMC1        | 8.14            | transmembrane channel-like 1 | NM_138691 |
| MYCN        | 8.12            | v-myc myelocytomatis viral related oncogene, neuroblastoma derived (avian) | NM_005378 |
| OR6B2       | 8.10            | olfactory receptor, family 6, subfamily B, member 2 | NM_001005853 |
| ADAMTS5     | 7.30            | ADAM metallopeptidase with thrombospondin type 1 motif, 5 | NM_007038 |
| LINCO0113   | 6.94            | long intergenic non-protein coding RNA 113 | AI796012 |
| DCN         | 6.49            | decorin | NM_001920 |
| GSTA5       | 6.46            | glutathione S-transferase alpha 5 | NM_153699 |
| NR4A3       | 6.32            | nuclear receptor subfamily 4, group A, member 3 | NM_173200 |
| HLA-DQB1    | 6.22            | major histocompatibility complex, class II, DQ beta 1 | NM_001243962 |
| PMCHL1      | 6.03            | pro-melanin-concentrating hormone-like 1, pseudogene | NR_003921 |
| 1-Mar       | 6.01            | mitochondrial amidoxime reducing component 1 | NM_022746 |
| DKK2        | 5.97            | dickkopf 2 homolog (Xenopus laevis) | NM_014421 |
| HLX         | 5.77            | H2.0-like homeobox | NM_021958 |
| F3          | 5.46            | coagulation factor III (thromboplastin, tissue factor) | NM_001993 |
| SOCS2       | 5.37            | suppressor of cytokine signaling 2 | NM_003877 |
| NDRG2       | 5.25            | NDRG family member 2 | NM_201535 |
| HLA-DQA1    | 4.99            | major histocompatibility complex, class II, DQ alpha 1 | NM_002122 |
| SPRY1       | 4.93            | sprouty homolog 1, antagonist of FGF signaling Drosophila | NM_199327 |
| PMCH        | 4.91            | pro-melanin-concentrating hormone | NM_002674 |
| MESTIT1     | 4.70            | MEST intronic transcript 1, antisense RNA (non-protein coding) | NR_004382 |
| NR4A2       | 4.59            | nuclear receptor subfamily 4, group A, member 2 | NM_006186 |
| COL11A2     | 4.27            | collagen, type XI, alpha 2 | NM_001163771 |
| IGF1        | 4.21            | insulin-like growth factor 1 (somatomedin C) | NM_000618 |
| MACF1       | 4.12            | microtubule-actin crosslinking factor 1 | NM_012090 |
| AREG        | 4.07            | amphiregulin | NM_001657 |
Verification of the microarray analysis results by qRT-PCR

Seven differentially expressed genes identified by microarray analysis were selected for validation by qRT-PCR, of which five were upregulated genes: RARRES2, ADAMTS5, F3, MACF1, and DCN and two were downregulated genes: CLDN4 and LDLR. These upregulated or downregulated genes have been reported to have antibacterial activity and are involved in the formation of the cytoskeleton, metabolism and coagulation. The results of the microarray analysis and qRT-PCR are shown in Fig 5. We observed that the results of both analysis methods were highly correlated. Therefore, the technique used in the present study was reliable and accurate. Besides, the differences in fold changes between the microarray analysis results and qRT-PCR results might be due to their different detection methods.
Figure 1. The graph of HBMECs co-incubated with *T. pallidum* with an ordinary optical microscope (400x). We could not see the *T. pallidum*.

Figure 2. The graph of HBMECs co-incubated with *T. pallidum* with a dark-field microscopy (1000x). We could see *T. pallidum* attached on the HBMECs.
### Table III. The top 10 significant GO terms.

| GO ID         | Term                                      | P       | Corrected P* | Gene                                                                 |
|---------------|-------------------------------------------|---------|--------------|----------------------------------------------------------------------|
| GO:0044707    | single-multicellular organism process      | 1.07E-05| 0.017713     | TLL1, RSP03, SIK1, RARRES2, SPRY1, DUSP5, DKK2, PBX2, DLX2, OR2H2, STC1, OR10A5, PMCH, DIEXF, NTS, HOXB8, NR4A3, TLL1, ZNF287, INHA, KLF5, TULP2, CCIN, DCN, TNN13, EPHA7, PER1, SEMA6D, LDLR, BCL2L11, SEMA3A, CRYAB, IGF1, MBP, ENC1, KIF5C, BDNF, OR6B2, F3, NEFM, DGKK, CALCRL, PRRX1, STAT1, NLRP3, FOX5, NR4A2, COL1A1, MRVI1, NR4A1, HOXD9, NDRG2, SHRoOM3, CXCL10, HTR1D, JPH1, CSGLNACT1, UNC13C |
| GO:0005576    | extracellular region                       | 1.09E-05| 0.02587      | TLL1, RSP03, RARRES2, PZP, CXXC6, DKK2, GZMK, AREG, STC1, PMCH, NT, STL1, INHA, TULP2, DCN, IL23A, SEL, LDLR, SEMA3A, IGF1, FREM3, IL1, COL1A1, F3, THST1, COL1A1, CXCL10, PRG4, ADAMTS5, AREG |
| GO:0005589    | collagen type VI                          | 0.0013  | 0.00129      | DCN                                                                 |
| GO:0005615    | extracellular space                       | 0.00228 | 0.01593      | PZP, DKK2, AREG, STC1, INHA, DCN, IL23A, SEL, LDLR, IGF1, F3, COL1A1, CXCL10, AREG |
| GO:0044421    | extracellular region part                  | 0.000265| 0.01593      | PZP, DKK2, AREG, STC1, INHA, DCN, IL23A, SEL, LDLR, IGF1, FREM3, COL1A1, F3, COL1A1, CXCL10, ADAMTS5, AREG |
| GO:0032501    | multicellular organismal process           | 2.05E-05| 0.017712     | TLL1, RSP03, SIK1, RARRES2, PZP, SPRY1, DUSP5, DKK2, PBX2, DLX2, OR2H2, STC1, OR10A5, PMCH, DIEXF, NTS, HOXB8, NR4A3, TLL1, ZNF287, INHA, KLF5, TULP2, CCIN, DCN, TNN13, EPHA7, PER1, SEMA6D, LDLR, BCL2L11, SEMA3A, CRAYAB, IGF1, MBP, ENC1, KIF5C, BDNF, OR6B2, F3, NEFM, DGKK, CALCRL, PRRX1, STAT1, NLRP3, FOX5, NR4A2, COL1A1, MRVI1, NR4A1, HOXD9, NDRG2, SHRoOM3, CXCL10, HTR1D, JPH1, CSGLNACT1, UNC13C |
| GO:0007275    | multicellular organismal development       | 2.89E-05| 0.017712     | TLL1, RSP03, SIK1, RARRES2, SPRY1, DUSP5, DKK2, PBX2, DLX2, STC1, PMCH, DIEXF, HOXB8, NR4A3, TLL1, ZNF287, INHA, KLF5, TULP2, CCIN, DCN, TNN13, EPHA7, SEMA6D, BCL2L11, SEMA3A, CRAYAB, IGF1, MBP, ENC1, KIF5C, BDNF, NEFM, CALCRL, PRRX1, FOX5, NR4A2, COL1A1, NR4A1, HOXD9, NDRG2, SHRoOM3, CXCL10, JPH1, CSGLNACT1 |
| GO:0009653    | anatomical structure morphogenesis         | 3.76E-05| 0.017712     | RSP03, DUSP5, PBX2, DLX2, HOXB8, NR4A3, KLF5, DCN, TNN13, EPHA7, SEMA6D, BCL2L11, SEMA3A, CRAYAB, IGF1, MYPN, KIF5C, BDNF, CALCRL, PRRX1, FOX5, NR4A2, COL1A1, NR4A1, SROOM3, CSGLNACT1 |
Table III. Continuation

| GO:0005515       | protein binding      | 7.62E-05 | 0.022482 |
|------------------|----------------------|----------|----------|
| TL1, RAB27B, RSP03, CD200, S0CS2, SIK1, RARRES2, SPRY1, BCL2A1, CXC L6, PBX2, STC1, MEF2C, PMCH, NTS, TLL1, DIO2, INHA, DCN, TNN1, CCL1, IL23A, EPHA7, SELE, SEMA6D, LDLR, BCL2L11, CRYAB, RRAD, IGF1, DIO2, MYPN, TRIB2, MBP, PFKFB2, ENC1, TRAF1, MYCN, KIF5C, BDNF, IL1RL1, F3, PTCRA, MIA3, NEFM, CALCRL, PRRX1, STATH, NLRP3, FOXS1, NR4A2, LDB2, FEM1A, COL1A1, MRV1, NFIA, NR4A1, NDRG2, SHROOM3, CCL10, GPR37L1, CD200, ADAMT55, DCN |
| GO:0009605       | response to external stimulus | 7.5E-05  | 0.025223 |
| STC1, NR4A3, INHA, DCN, CCL1, EPHA7, PER1, SEMA6D, LDLR, SEMA3A, KIF5C, BDNF, NR4A2, COL1A1, NR4A1, NDRG2, SHROOM3, CCL10, GPR37L1, CD200, ADAMT55, DCN |

GO, gene ontology. * Benjamini-Hochberg multiple testing was used for the corrected P-value.

Figure 3. The heat map of differentially expressed genes. Every column represents a sample, and each line represents a single gene. Different colours indicate different expression levels. The red, green and black indicate upregulation, downregulation and no change, respectively. C: control, S: stimulated.
DISCUSSION

This is the first study to characterize the gene expression pattern in HBMECs infected with the syphilis-causing pathogen *T. pallidum* based on gene expression microarray analysis technology. A total of 35,377 different human genes were examined in the present study, and significantly differentially expressed genes were observed 4 h post *T. pallidum* infection. This study demonstrated that *T. pallidum* infection induced 249 differentially expressed genes, most of which were upregulated. Further GO analyzed a majority of these significant GO terms classified into the biological process, other important functional groups included protein binding, extracellular region, extracellular space and extracellular region part. The number of differentially expressed genes was significantly less than other mRNA expression profile research of other tumor diseases or inflammatory diseases (Wang et al. 2015, Chen et al. 2015, Guo et al. 2015). The result suggests that the response of HBMECs induced by *T. pallidum* is weakly. The possible reason is that the outer membrane of *T. pallidum* contains a small amount of lipoid and glycoprotein, which may cause a weak immune response to avoid the surveillance of the host immune system. In clinic, patients of NS may be asymptomatic or with mild clinical symptoms.

Similar to other bacteria causing meningitis, *T. pallidum* adhesion to and crossing of HBMECs
is a prerequisite for successful disturbance of the CNS. However, until now, there has been no study revealing the interactions between *T. pallidum* and HBMECs. Our results indicated the likely ligands for *T. pallidum* binding to HBMECs for the first time. Among the differentially expressed genes induced by *T. pallidum* infection, we found several genes encoding proteins involved in extracellular matrix, such as decorin (DCN), collagen I and collagen XI. DCN is a small dermatan sulfate proteoglycan that decorates collagen fibers, regulates the production of fibronectin and thrombospondin-1, stimulates collagenase, and inhibits collagen I maturation (Scott & Orford 1981, Zhang et al. 2018). Moreover, DCN is an important ligand for *Borrelia burgdorferi* (*B. burgdorferi*), which is another pathogenic spirochete causing Lyme disease (Guo et al. 1995). Two proteins of *B. burgdorferi* (DbpA and DbpB) have been identified as corresponding adhesins that bind to DCN (Guo et al. 1998). Zambrano et al. reported that *B. burgdorferi* can bind to type 1 collagen lattices, but the corresponding adhesion molecule(s) remain unknown (Zambrano et al. 2004). The role of DCN and other collagens in the interaction between *T. pallidum* and HBMECs requires further research.

When endothelial cells are activated by pathogens or related proteins, they may express several kinds of cellular adhesion molecules and open intercellular junctions (Sumitomo et al. 2011). Our results showed that the expression of the gene coding for E-selectin was upregulated and that for claudin-4 was downregulated in infected HBMECs. E-selectin is a marker molecule expressed on the endothelial cell surface, and it plays an important role in the adhesion of leukocytes to the vascular endothelium (Wittchen et al. 2009). Moreover, E-selectin mediates the tethering and rolling of circulating leukocytes on the vascular endothelium during inflammation induced by infection (Kluger 2004). Claudin-4, encoded by *CLDN 4*, belongs to the claudin family, which coordinates with Zonula occluden-1 to maintain endothelial barrier function (Wang et al. 2019). However, how Claudin-4 plays a role in regulating intercellular junctions during bacterial infection is completely unknown. It has been reported that when human umbilical vein endothelial cells (HUVECs) are stimulated by recombinant *Treponema pallidum* protein 0965 (rTp0965), the expression of claudin-1 was decreased (Zhang et al. 2014). Our gene microarray analysis results suggest that HBMECs may be activated in the context of *T. pallidum* binding and invasion, but the details are unclear and require further study in the future.

During microbial invasion, host cells produce a series of immune responses to prevent infection, such as chemokines, cytokines, and oxidative bursts. When HBMECs were infected with *T. pallidum* for 4 h, the expression of genes encoding CXCL-6, CXCL-10, and chemerin were increased. CXCL-6 is a CXC chemokine that exerts...
neutrophil-activating and angiogenic activities, and the overexpression of CXCL6 can promote the increased expression of pro-angiogenic genes, including IGF-1, VEGF-A, IL-8, and HGF (Kim et al. 2012). Helena et al. demonstrated that CXCL-6 itself was antibacterial, and its antibacterial activity was higher than that of CXCL-5 and CXCL-7 (Linge et al. 2008). Yong et al. also reported that CXCL-6 was significantly elevated in patients infected with some viruses (Yong et al. 2017). CXCL-10, also called interferon γ-induced protein 10 kDa, is another member of the CSC chemokine family and participates in a wide range of physiological and pathophysiology processes, such as chemotaxis, angiostasis, cell growth inhibition and apoptosis. It has been reported that levels of CXCL-10 in body fluids from individuals infected with bacteria, viruses, fungi and parasites are abnormal (Liu et al. 2011). For example, our previous study reported that CXCL-10 was elevated in HBMECs infected with HCV, which might be related to the enrichment of immunocytes (Wu et al. 2018). A study on neuroborreliosis revealed that the gene expression of CXCL-6 and CXCL-10 in HBMECs was significantly upregulated after 72 h of stimulation with B. burgdorferi and suggested that neutrophils attracted by chemokines expressed at the BBB may play an important role in the early inflammatory events involved in neuroborreliosis (Brissette et al. 2013). Chemerin is a secreted antimicrobial agent in human skin and is encoded by retinoic acid receptor responder gene 2 (RARRES2) (Banas et al. 2013, Nagpal et al. 1997). Paulina et al. demonstrated that chemerin displayed antibacterial activity against Escherichia coli and Klebsiella pneumonia and showed bactericidal properties at much lower concentrations (Kulig et al. 2011). Chemerin is also an attractant for leukocytes, including macrophages, dendritic cells, and NK cells, by which chemerin can acquire a pro-inflammatory role (Zabel et al. 2014, Vinci et al. 2012). It is emphasized that the inflammatory response is responsible for syphilis pathogenesis during the invasion and persistence of T. pallidum. By recruiting immune cells to sites of tissue damage, vasculitis invokes and ultimately leads to T. pallidum dissemination by destroying the endothelial barrier, dependent on the activation of the RhoA/ROCK and MAPK signaling pathways (Zhang et al. 2019). Our previous studies found that recombinant Treponema pallidum protein 17 (rTp 17) increased the expression of the gene encoding monocyte chemoattractant protein-1 (MCP-1) in HUVECs (Zhang et al. 2015). Although there is currently no experimental proof, we hypothesize that the chemokines induced by T. pallidum may be involved in the interaction between HBMECs and T. pallidum, and this hypothesis requires further verification.

In summary, the present study is the first report on global gene expression patterns in HBMECs in response to T. pallidum. Our results identified some differentially expressed genes associated with widespread biological processes. More importantly, our research will develop a new platform for further molecular and cellular experiments on the pathogenesis of NS.

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