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专论与综述

链球菌突破血脑屏障的作用机制研究进展

曹芯蕊[#1]，贾凯翔[#1]，方仁东[*1,2,3]

1 西南大学动物医学院 动物健康与动物性食品安全国际合作联合实验室，重庆 400715
2 西南大学医学研究院免疫学研究中心，重庆 402460
3 草食动物科学重庆市重点实验室，重庆 400715

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摘　要：血脑屏障(blood-brain barrier，BBB)是中枢神经系统(central nervous system，CNS)的天然结构和功能屏障之一，可有效阻止病原菌的入侵。然而病原菌能通过其自身毒力因子与脑内皮细胞相互作用，诱导宿主免疫应答反应，分泌大量细胞因子、趋化因子等，破坏紧密连接蛋白，最终突破血脑屏障，引起细菌性脑膜炎，产生不可逆的神经系统损伤。链球菌(*Streptococcus*)作为引起细菌性脑膜炎的重要病原菌，关于其突破血脑屏障分子机制研究已有显著进展。本文针对主要的链球菌，包括肺炎链球菌(*Streptococcus pneumoniae*)、猪链球菌(*Streptococcus suis*)、B 型链球菌(group B *Streptococcus*，GBS)、马链球菌等突破血脑屏障的作用机制研究进展进行综述。

关键词：血脑屏障；链球菌；细菌性脑膜炎

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#These authors equally contributed to this work

***Corresponding author:** E-mail: rdfang@swu.edu.cn

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Progress in mechanism of *Streptococcus* penetrating blood-brain barrier

CAO Xinrui[#1], JIA Kaixiang[#1], FANG Rendong[*1,2,3]

1 Joint International Research Laboratory of Animal Health and Animal Food Safety, College of Veterinary Medicine, Southwest University, Chongqing 400715, China
2 Immunology Research Center, Medical Research Institute, Southwest University, Chongqing 402460, China
3 Chongqing Key Laboratory of Herbivore Science, Chongqing 400715, China

Abstract: Blood-brain barrier (BBB), one of the natural structural and functional barriers of the central nervous system (CNS), can prevent the invasion of pathogenic bacteria. However, pathogenic bacteria can interact with brain endothelial cells via their virulence factors to induce host immune response and massive secretion of cytokines and chemokines and destroy tight junction proteins to penetrate the blood brain barrier, causing bacterial meningitis and irreversible nervous system damage. *Streptococcus* is a major pathogen causing bacterial meningitis. In recent years, significant progress has been achieved in the research on the molecular mechanism of *Streptococcus* penetrating the blood-brain barrier. This article reviews the progress in the mechanism of main *Streptococcus* species including *Streptococcus pneumoniae*, *Streptococcus suis*, group B *Streptococcus*, and *Streptococcus equi* in passing through the blood-brain barrier.

Keywords: blood-brain barrier; *Streptococcus*; bacterial meningitis

细菌性脑膜炎也称化脓性脑膜炎，常见的引起脑膜炎的细菌包括脑膜炎奈瑟菌(*Neisseria meningitides*)、肺炎链球菌(*Streptococcus pneumoniae*)、B 型链球菌(group B *Streptococcus*，GBS)、大肠杆菌 K1 (*Escherichia coli* K1)、克雷伯氏菌(*Klebsiella*)、金黄色葡萄球菌(*Staphylococcus aureus*)、单核增生李斯特菌(*Listeria monocytogenes*)及常被人忽视的猪链球菌(*Streptococcus suis*)[1]。菌血症是细菌性脑膜炎的重要原因，病原菌随着血液不断流动，最终会侵入中枢神经系统(central nervous system，CNS)，引起血脑屏障(blood-brain barrier，BBB)通透性增加、脑膜炎症和神经组织浸润[2]。随后，细菌分泌的有毒产物和宿主的炎症反应会进一步加剧脑损伤，引起脑缺血、水肿、颅内压增高、脑积水等症状，半数以上的治愈者会留下永久性的神经系统后遗症[3]。近年来，随着临床抗菌药物的滥用，细菌性脑膜炎在全球范围内仍有较高的发病率和病死率。

血脑屏障作为中枢神经系统的重要屏障，对维持脑内微环境的稳定至关重要。血脑屏障完整性的破坏是病原菌入侵中枢神经系统引起细菌性脑膜炎的重要环节[4]。许多国内外的研究已经确定了相关病原菌突破血脑屏障从而诱导中枢神经系统损伤的作用机制。本文针对链球菌破坏血脑屏障完整性导致细菌性脑膜炎的机制进行综述，以期使保护血脑屏障作为一个细菌性脑膜炎潜在的治疗靶点，为细菌性脑膜炎的治疗提供新的思路。

1 血脑屏障的结构与生理功能

血脑屏障是机体维持中枢神经系统稳态的

重要组成部分，由脑微血管内皮细胞、星形胶质细胞、周细胞、细胞外基质等组成。中枢神经系统的脑血管多为连续的无孔结构，能够严格地调节分子、离子和细胞在血液和中枢神经系统之间的运动，从而保护中枢神经系统免受毒素、病原体、炎症、损伤和疾病的影响[5]。

大脑内皮细胞、脉络膜丛上皮细胞和蛛网膜上皮细胞之间的紧密连接(tight junctions，TJs)是血脑屏障的一个显著特征，紧密连接主要由跨越细胞间隙的蛋白质复合物(occludin 和 claudin)、连接黏附分子(junctional adhesion molecules，JAMs)和闭合小环蛋白(zona occludins，Zos)组成，对血脑屏障的完整性至关重要[6]。除内皮细胞外，周细胞、星形胶质细胞也在神经活动中发挥重要作用，如调节血管生成、血液流动，促进伤口愈合、激活免疫细胞等[7]。小胶质细胞作为中枢神经系统中主要的免疫细胞，参与调节神经元发育、先天免疫反应并可在适应性免疫中充当抗原提呈细胞[8]。此外，不同的血源性免疫细胞群，包括中性粒细胞、T 细胞和巨噬细胞被激活时可以与中枢神经系统血管神经元相互作用，以应对感染、损伤和疾病[9]。

2 链球菌感染突破血脑屏障

大量研究表明，病原菌黏附聚集在脑微血管内皮细胞上，通过破坏内皮细胞之间的紧密连接蛋白，引起血脑屏障通透性变化，突破血脑屏障，进入中枢神经系统[10]。同时，病原菌进入中枢神经系统后，激活机体的免疫应答反应、炎性小体及相关信号通路活化，分泌大量的细胞因子和趋化因子及其他的代谢产物(如活性氧和氮)，进一步破坏血脑屏障，最终引发细菌性脑膜炎[11]。

在链球菌感染引起的脑膜炎中，*S. pneumoniae* 是主要病原菌，属于 α 溶血或 β 溶血的兼性厌氧链球菌。*S. pneumoniae* 首先在鼻腔黏膜内定殖，本课题组前期研究发现在 *S. pneumoniae* 感染过程中，nod 样受体热蛋白结构域相关蛋白 3 (nod-like receptor thermal protein domain associated protein 3，NLRP3)和凋亡相关斑点样蛋白(apoptosisassociated speck like protein containing CARD，ASC)通过 STAT6-SPDEF 通路促进了小鼠气道黏膜的固有免疫[12]。随后，*S. pneumoniae* 逐渐发展到内耳腔、肺部或侵入组织内进入血液[13]，最终通过血脑屏障或血液-脑脊液屏障侵入中枢神经系统[14]。*S. suis* 是新出现的一种引起细菌性脑膜炎的人畜共患病病原体，可引起脑膜炎、败血症、心内膜炎和关节炎等多种感染[15]，在 35 种已知血清型中，血清型 2 型是分布最广泛和致病性最高的[16]。据相关文献报道，马链球菌兽疫亚种(*Streptococcus equi* subsp. *zooepidemicus*，SEZ)和 GBS 也可以突破血脑屏障，引起细菌性脑膜炎[17]。因此，本文对各种链球菌突破血脑屏障的机制进行了阐述。

2.1 破坏紧密连接蛋白

在中枢神经系统中，内皮细胞间的紧密连接对分子和离子形成了高阻力的细胞旁屏障，有效阻止了循环中内、外源性物质进入脑组织[18]。然而，许多脑膜炎病原菌可以通过自身毒力因子干扰紧密连接蛋白，影响血脑屏障的功能[19]。

研究表明，*S. pneumoniae* 进入中枢神经系统后繁殖或自溶，诱导过氧化氢的产生，形成过氧亚硝酸盐，或被骨髓过氧化物酶转化为次氯酸，激活基质金属蛋白酶(matrix metalloproteinases，MMPs)，分解紧密连接蛋白[20-21]。MMPs 是一类内肽酶，可降解基底膜和周围细胞的细胞外基质，在细菌性脑膜炎患者的脑脊液中，MMP-8、MMP-9 明显增加[22]，而且抑制 MMP-2 和 MMP-9 均可减少血脑屏障的破坏[23]。类似地，

S. suis 的丝氨酸/苏氨酸蛋白激酶(serine/threonine kinase，stk)通过影响 E3 泛素连接酶的表达，降解紧密连接蛋白 claudin-5，使 *S. suis* 穿越血脑屏障进入中枢神经系统[24]。在 *stk* 基因被敲除后，菌株黏附侵入及穿过血脑屏障的能力均显著减弱[25]。此外，*S. suis* 可借助溶血素等毒力因子重塑细胞骨架与紧密连接蛋白，提高脑微血管的通透性，使得细菌侵入脑内发挥致病作用[26]。除 *S. pneumoniae*、*S. suis* 外，Kim 等[27]发现 GBS 在感染脑内皮细胞后诱导宿主转录因子 snail1 的表达，引起紧密连接蛋白流失，造成脑损伤。

综上所述，尽管血脑屏障功能障碍不仅限于紧密连接蛋白的流失，但紧密连接蛋白作为控制中枢神经系统分子交换的最重要环节，链球菌通过其自身小分子蛋白及其代谢产物等引起 ZOs、claudin、occludin 等蛋白的流失，最终侵入中枢神经系统，提示紧密连接蛋白的重建可能是治疗以血脑屏障受损为特征的感染性疾病的一种有前途的方法。

2.2 激活免疫应答反应

当链球菌进入中枢神经系统后，机体的天然免疫应答开始发挥作用，吞噬细胞被激活，分泌大量的细胞因子、趋化因子，上调血脑屏障中细胞内黏附分子的表达，促进中性粒细胞的浸润[28-29]。然而，为应对链球菌的感染，脑组织中发生的一系列过度炎症反应将对血脑屏障的完整性造成影响，引起神经元损伤。

2.2.1 分泌细胞因子、趋化因子

目前的研究已经证明了 *S. pneumoniae* 的肽聚糖、磷壁酸、荚膜多糖和溶血素等可以激活中枢神经细胞的 Toll 样受体[30-31]、核苷酸结合寡聚结构域样受体(nod-like receptors，NLR)[32]及经典补体通路[33]，引起中枢神经系统的炎症反应。临床和试验均证明，*S. pneumoniae* 入侵时，内皮细胞、小胶质细胞和星形胶质细胞会产生大量细胞因子，如 IL-6、TNF 和 IL-1β 等，严重时引起细胞因子风暴，并与脑膜炎死亡率相关[34-35]。Koedel 等[36]发现 caspase-1 基因敲除小鼠 IL-1β 水平降低与改善血脑屏障完整性相关。同样地，有研究发现 IL-1 受体敲除小鼠及 IFN-γ 基因敲除小鼠在 *S. pneumoniae* 感染时血脑屏障受到更大的破坏，中枢神经系统中 *S. pneumoniae* 数量增加[37-38]。*S. pneumoniae* 的表面神经氨酸酶 A (neuraminidase A，NanA) N 端凝集素结构能够充分活化内皮细胞，激活细胞因子如白介素 8 (interleukin 8，IL-8)，募集中性粒细胞，促进 *S. pneumoniae* 对脑微血管内皮细胞的侵袭[39]。

S. suis 感染后，小鼠大脑所有区域，特别是脑膜发生多灶性病变，中枢神经系统中的免疫细胞，如小胶质细胞会释放大量的促炎细胞因子，增加血脑屏障的通透性[40]。Lavagna 等[41]证明了 *S. suis* 能够被 MyD88 (myeloid differentiation factor88，MyD88)和 Toll 样受体 2 (Toll-like receptor 2，TLR2)受体识别，在树突状细胞内定殖激活黑色素瘤缺乏因子 2 (absent in melanoma 2，AIM2)，诱导大量 IL-1β 的产生，加剧了血脑屏障的损伤。*S. suis* 的重要毒力因子，如 TRIM32 基因、小 RNArss04 等均与 Toll 样受体的激活和细胞因子(如 IL-6 和 TNF-α 等)的产生有关，促进 *S. suis* 对脑微血管内皮细胞的粘附和侵袭，增加血脑屏障的通透性，诱导脑膜炎的发生[42-43]。在对 SEZ 入侵血脑屏障的研究中，Li 等[44]发现 SEZ 感染小鼠巨噬细胞可以激活 NLRP3/caspase-1 通路，引起 IL-1β 和 IL-18 的分泌，而 microRNA-223-3p 可以抑制 NLRP3 炎症小体的激活及其下游通路，以应对 SEZ 感染。

链球菌感染后，小胶质细胞等免疫细胞产生的趋化因子，如 C-C motif ligand 2 (CCL2)、

C-C motif ligand 3 (CCL3)、C-X-C motif ligand 8 (CXCL8)、C-X-C motif ligand 1 (CXCL1)参与了白细胞的募集和迁移，调节中性粒细胞、单核细胞和 T 细胞的趋化性，CXCL1 和 CXCL3 还与自然杀伤细胞募集相关[45]。

Xu 等发现，在 *S. pneumoniae* 感染小鼠巨噬细胞中，nod 样受体热蛋白结构域相关蛋白 6 (nod-like receptor thermal protein domain associated protein 6，NLRP6)炎症小体介导了 caspase-1、caspase-11 和 gasdermin D 的激活及 ASC 的寡聚化，NLRP6 敲除小鼠的存活率更高，细菌数量更少，炎症反应更温和，表明 NLRP6 在宿主防御 *S. pneumoniae* 中起负面作用[46]。此外，NLRP6 敲除小鼠的中性粒细胞在 *S. pneumoniae* 感染期间表现出较强的胞内杀菌能力，并形成中性粒细胞胞外诱捕网[47]。类似地，我们前期研究表明，AIM2 炎症小体、NLRP3 炎症相关通路和中性粒细胞丝氨酸蛋白酶在 *S. pneumoniae* 感染小鼠巨噬细胞、中性粒细胞中 IL-1β 的产生及保护小鼠免受肺炎链球菌的感染中至关重要[48-49]。有大量的文献表明，中枢神经系统血脑屏障的破坏与机体炎症小体的激活有关。小胶质细胞作为大脑中的常驻巨噬细胞，能够通过模式识别受体(如 TLR 和 NLR)感知病原体相关分子模式[50]。因此，我们推测链球菌感染中枢神经系统时会激活炎症小体，引起炎症反应，破坏血脑屏障的完整性。

2.2.2 免疫逃逸

研究表明，*S. pneumoniae* 的荚膜多糖有助于增强其免疫逃避能力，抵抗吞噬作用且在中性粒细胞胞外诱捕网黏附更少[51-52]。除荚膜外，*S. pneumoniae* 胆碱结合蛋白(choline-binding proteins，Cbp)可以通过破坏补体通路，增强对吞噬细胞的抵抗力[53-54]。最新的研究表明，*S. pneumoniae* 的溶血素具有异质性，高表达溶血素的 *S. pneumoniae* 通过破坏宿主自噬小体逃逸到细胞质中，与脑微血管内皮细胞泛素(ubiquitin，Ubq)发生共定位，通过泛素介导的自噬途径促进了 *S. pneumoniae* 的清除[55]。另外，低表达溶血素的 *S. pneumoniae* 会阻止自噬体成熟，避开所有细胞内防御机制，突破血脑屏障的成功率更高[56]。

相似地，*S. suis* 溶血素有助于提高大脑中的细菌密度，加剧大脑中的炎症反应，导致死亡率增加[57]。最新的研究发现，cryptotanshinone 可以在不影响细菌生长的情况下抑制溶血素的成孔活性，减少 *S. suis* 诱导的炎症反应，是治疗 *S. suis* 感染的潜在化合物前体[58]。此外，*S. suis* 感染可以触发小胶质细胞的自噬过程，增强自噬通量[59]。刘嘉楠等[60]研究发现 IFN-γ 可能通过诱导猪脑微血管内皮细胞自噬破坏血脑屏障的完整性，增强屏障的通透性，从而促进 *S. suis* 进入脑组织。

GBS 的荚膜多糖通过唾液化阻止补体沉积，抵抗吞噬细胞的吞噬作用，以保证在血液中存活，是 GBS 侵入血脑屏障的第一步[61]，转录调控因子 CovR 和 CiaR 参与了这一过程[62-63]。同样地，GBS 菌毛蛋白 PilA 可以与脑内皮上的 α2-β1 整合素结合，促进细菌附着和促炎趋化因子释放，增加血脑屏障通透性[64]。

链球菌通过破坏补体通路、抑制自噬等多种方式抵抗吞噬作用，破坏血脑屏障，进入中枢神经系统，诱发宿主炎症反应，但血脑屏障的连续无孔结构使得消炎抑菌药物无法穿过血脑屏障治疗链球菌感染引起的脑膜炎，这仍是细菌性脑膜炎治疗的一个难题。

2.3 其他方式

除破坏紧密连接蛋白、激活免疫应答反应外，链球菌溶血素(suilysin，Sly)在突破血脑屏障中发挥重要作用。Sly 是一种成孔胆固醇依赖

性溶细胞素，*S. suis* 的致病性与 *sly* 基因的完整性密切相关，含有完整 *sly* 基因的 *S. suis* 通过黏附内皮细胞，诱导 III 组分泌性磷脂酶 A2 (group III secretory phospholipase A2，PLA2G3)的分泌，损伤脑微血管内皮，增强血脑屏障的通透性，进入中枢神经系统[65-66]。Fang 等发现 *S. pneumoniae* 溶血素可以诱导小鼠巨噬细胞内钙的流入，继而激活钙蛋白酶，分泌 IL-1α，揭示了溶血素与宿主细胞对 *S. pneumoniae* 感染之间的相互作用[67]。同样地，也有研究表明 *S. pneumoniae* 溶血素可以结合宿主细胞膜形成前孔，随后穿过细胞膜引发宿主细胞构象变化，对上皮细胞和内皮细胞具有直接的细胞毒性作用[68]，并可诱导胶质细胞和神经元细胞死亡，增加血脑屏障的整体通透性[69-71]。

除溶血素外，*S. suis* 的烯醇化酶(enolase，Eno)可以与猪脑微血管内皮细胞表面的 40S 核糖体蛋白(40S ribosomal protein SA，RPSA)结合，激活细胞内 p38/ERK-eIF4E 信号通路，促进热休克蛋白家族 D 成员(heat shock protein family D member 1，HSPD1)在细胞内表达，引起细胞形态的不利变化，加速细胞凋亡，增加血脑屏障通透性，促进 *S. suis* 入侵[72-73]。此外，Eno 还可以通过细胞因子 IL-8 的释放，在体外破坏猪脑微血管内皮细胞和星形胶质细胞组成的血脑屏障的完整性[74]。

3 小结

综上所述，链球菌可以通过破坏紧密连接蛋白、诱导宿主免疫应答反应及其他多种方式损害血脑屏障的完整性，引发细菌性脑膜炎，诱导神经系统损伤。因此，保护血脑屏障的完整性是防治链球菌感染的关键。目前，针对链球菌感染引起脑膜炎的治疗方案主要是抗生素治疗，辅助皮质类固醇激素[75]。此外，杨梅素[76]、银杏素[77]、黄芩素[78]等均可以通过直接结合 *S. suis* 的重要毒力因子 Sly，破坏其二级结构，降低 Sly 的溶血活性，对治疗链球菌引起的脑膜炎可提供很大帮助。疫苗免疫是降低细菌性脑膜炎发病率的有效预防策略，但由于非疫苗血清型的出现和增多，细菌性脑膜炎仍然是威胁全球人类健康的重大疾病，特别是在婴幼儿、老年人和免疫功能低下的人群中发病率和病死率仍较高。目前，有关重要链球菌突破血脑屏障引起细菌性脑膜炎的分子机制研究已经取得了较大的进展，链球菌和宿主血脑屏障相应细胞靶点的确定将为相关疫苗以及拮抗剂的应用提供思路，阻断参与链球菌附着和转运的屏障位点将成为细菌性脑膜炎的重要研究方向之一。

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