补体系统C1q/C3介导的胶质细胞激活在小鼠抑郁样行为中的作用

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摘　要:【目的】探讨小鼠的杏仁核中C1q/C3补体系统在诱导抑郁样行为过程中的作用途径。【方法】本实验分3部分:① 采用慢性束缚压力方法建立小鼠抑郁模型,将12只8周龄雄性C57BL/6小鼠随机分为对照(WT)组和抑郁模型(CRS)组,各6只,抑郁模型组造模2周。行为学测试系统悬尾实验(TST)、强迫游泳实验(FST)测试小鼠抑郁状态,采用免疫荧光染色法检测小鼠杏仁核中突触素(Syn)和突触后致密物(PSD95)变化情况,小胶质细胞(Iba-1)和星形胶质细胞(GFAP)细胞荧光强度,C1q、C3含量。② 6只8周*CX3CR1-GFP*雄性鼠,随机分为*GFP*组以及*GFP*模型(*GFP*+CRS)组,各3只,采用免疫荧光染色法观察小胶质细胞及星形胶质细胞相互作用情况。③ 12只8周龄雄性C57BL/6和12只*C1q*$^{-/-}$小鼠随机分为对照(WT)组,抑郁模型(CRS)组,*C1q*敲除(*C1q*$^{-/-}$)组,*C1q*敲除模型(*C1q*$^{-/-}$+CRS)组,各6只,模型组组造模2周,行为学TST、FST检测4组小鼠抑郁状态,采用免疫荧光染色法检测四组小鼠杏仁核部位突触Syn和PSD95变化。【结果】行为学分析结果显示:与WT组比较CRS组在TST和FST中不动时间明显升高(P<0.000 1);免疫荧光法测定,与WT组比较CRS组杏仁核内突触Syn、PSD95含量明显降低(P<0.000 1; P=0.003 8);与WT组比较CRS组杏仁核内Iba-1和GFAP明显激活(P=0.000 4, P=0.003)且与WT组比较CRS组杏仁核内小胶质细胞与星形胶质细胞相互作用明显;与WT组比较CRS组杏仁核内C1q与C3产生明显增多(P=0.000 2, P=0.011 9);行为学分析,WT、CRS、*C1q*$^{-/-}$、*C1q*$^{-/-}$+CRS 4组综合比较,在TST中,与CRS组比较,WT组不动时间明显升高,*C1q*$^{-/-}$+CRS组不动时间也明显增高(均P<0.001);在FST中,与CRS组比较,WT组不动时间明显升高,*C1q*$^{-/-}$+CRS组不动时间也明显增高(均P<0.001); 与CRS组比较WT组与*C1q*$^{-/-}$+CRS组杏仁核内突触Syn明显增多(P<0.001;P<0.05),PSD95明显增多(P<0.05)。【结论】抑郁模型小鼠杏仁核内发生神经炎症,胶质细胞激活,表达补体C1q、C3,进一步激活小胶质细胞和星形胶质细胞修剪突触,导致突触含量降低,促使小鼠产生抑郁样行为;*C1q*基因缺陷阻断CRS诱导的小鼠突触丢失和抑郁样行为。

关键词:慢性束缚刺激;杏仁核;补体
中图分类号:Q189　　**文献标志码:**A　　**文章编号:**1672-3554(2021)03-0328-10
DOI:10.13471/j.cnki.j.sun.yat-sen.univ(med.sci).2021.0102

The Role of Glial Cell Activation Mediated by Complement System C1q/C3 in Depression-like Behavior in Mice

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Abstract:【Objective】To investigate the pathway of C1q/C3 complement system in the amygdala of mice in the induction

收稿日期:2021-02-26
基金项目:广东省科技发展项目(2016A020215036),广东省自然科学基金(2019A1515011184, 2020A1515010012),广州市科技计划项目(202002030441)
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of depressive-like behavior.【Methods】Three parts were included. Firstly, the depression model of mice was established by chronic restraint stress. Twelve 8-week-old male C57BL/6 mice were randomly divided into control (WT) group (n = 6) and depression model (CRS) group (n = 6). The depression model group was established for 2 weeks. The depressive state of mice was tested by tail suspension test (the tail suspension test, TST) and forced swimming test (the forced swimming test, FST). The fluorescence intensity of the changes of synaptic Synaptophysin (Syn) and post synaptic density protein 95 (PSD95), microglia (Iba-1) and astrocytes (GFAP) and the contents of complement component (C1q) and C3 in amygdala were detected by immunofluorescence staining. Secondly, six 8-week-old *CX3CR1-GFP* male mice were randomly divided into *GFP* group (n = 3) and *GFP* model group (*GFP*+CRS) with 3 mice in each group. The interaction between microglia and astrocytes was observed by immunofluorescence staining. Thirdly, twelve 8-week-old male C57BL/6 and $C1q^{-/-}$ mice were randomly divided into control (WT) group, depression model (CRS) group, *C1q* knockout ($C1q^{-/-}$) group and *C1q* knockout model ($C1q^{-/-}$+CRS) group, with 6 mice in each group. depression model was established for 2 weeks. Behavioral test TST and FST were used to detect the state of depression in four groups. Immunofluorescence staining was used to detect the changes of synaptic Syn and PSD95 in the amygdala of the four groups.【Result】The results of behavioral measurement showed that the immobility time in TST and FST in CRS group was significantly higher than that in WT group (P < 0.000 1); the result immunofluorescence assay shows that the content of Syn and PSD95 in amygdala in CRS group was significantly lower than that in WT group (P < 0.000 1; P=0.003 8); the activation of Iba-1 and GFAP in amygdala in CRS group was significantly higher than that in WT group(P=0.000 4, P=0.003); and the interaction between microglia and astrocytes in amygdala in CRS group was more obvious than that in WT group; compared with WT group, the production of C1q and C3 in amygdala was significantly increased in CRS group (P=0.000 2, P=0.011 9); Comprehensive comparison of WT, CRS, $C1q^{-/-}$, $C1q^{-/-}$+CRS, the immobility time in TST in WT group was significantly lower than that in CRS group and in $C1q^{-/-}$+CRS group was also significantly lower than that in CRS group (both P <0.001), the immobility time in FST in WT group was significantly lower than that in CRS group and in $C1q^{-/-}$+CRS group was significantly lower than that in CRS group (both P<0.001) . The Syn in amygdala in WT group and $C1q^{-/-}$+CRS group were significantly higher than those in CRS group (P<0.001).The PSD95 in amygdala in WT group and $C1q^{-/-}$+CRS group were significantly higher than those in CRS group (P<0.05).【Conclusion】In the depressive model mice, neuroinflammation occurred in the amygdala, where glial cells were activated to express C1q and C3, which further activated microglia and astrocytes to prune synapses, resulting in the decrease of synaptic content and the depression-like behavior in mice. $C1q^{-/-}$ deficiency prevented CRS-induced synaptic loss and depressive-like behavior.

Key words: chronic restraint stress; amygdala; complement

[J SUN Yat-sen Univ(Med Sci),2021,42(3):328-337]

抑郁症是较为常见的神经精神疾病,全球发病率逐年升高,目前患病率约为6%[1]。关于抑郁症的发病机制及干预办法已经开展了大量的研究,药物在一定程度上可以减缓相关症状,但是治疗效果仍然不理想[2]。因此寻找新的抗抑郁治疗靶点药物有重要意义。有文献报道杏仁核与社会情绪记忆密切相关[3]。杏仁核部位突触减少可能是小鼠产生抑郁样行为原因之一,中枢神经系统中小胶质细胞和星形胶质细胞作为免疫细胞,具有修剪突触的作用。文献报道在抑郁状态下中枢系统产生神经炎症[4],小胶质细胞在炎症状态下被激活,进一步诱导神经毒性星形胶质细胞的产生[5]。有文献报道小胶质细胞可以产生补体成分(complement component 1, C1q)[6],因此我们推测小胶质细胞激活、神经元突触修剪可能与补体系统有密切的关系。补体系统是人类体内免疫调节中重要的一环,研究表明补体系统在神经退行性疾病中发挥了重要作用[7],也有研究证实在精神疾病中脑内补体含量发生变化[8],补体C1q是补体系统中经典途径的起始蛋白,在补体系统中扮演重要的角色。研究证明,补体蛋白C1q和下游补体蛋白C3 (complement component 3, C3)定位于未成熟的突触,C3与小胶质细胞表达的补体蛋白C3a受体(C3aR)结合, 在无功能的网状突触的发育修剪方面起了重要作用[9]。在突触修剪过程中,这些受体表达在小胶质细胞中被上调,随后被下调。此外,有研究进一步

证明，星形胶质细胞表达的C3和小胶质细胞C3aR之间的交互作用调节C3/C3a-C3aR信号通路，从而动态调节小胶质细胞对神经元突触修剪过程[10]。这些发现提示抑郁症发病过程中的抑郁状态可能与补体C1q和C3激活小胶质细胞修剪突触有关。本实验研究杏仁核补体途径C1q/C3介导胶质细胞激活修剪突触诱发小鼠产生抑郁样行为的分子机制，为抗抑郁症药物的研发提供理论基础。

1 材料与方法

1.1 实验动物和饲养

本实验需要的C57BL/6小鼠购自广东省实验动物中心[许可证号：SYXK(粤)2018-0209]，*CX3CR1-GFP*转基因小鼠(B6.129P-CX3CR1tm1 Litt/J)购自Jackson Laboratory，*C1q*$^{-/-}$购自南方模式生物公司。实验动物饲养于SPF屏障环境：动物房环境在(22±2)℃，相对湿度在(55±5)%，光照时间12 h黑暗时间12 h，水和食物获取自由。选取12只8周龄雄性C57BL/6小鼠，12只8周龄雄性*C1q*$^{-/-}$小鼠群养5~7 d，使小鼠适应环境，随机分为对照(WT)组，抑郁模型(CRS)组，*C1q*敲除组(*C1q*$^{-/-}$)，*C1q*敲除模型组(*C1q*$^{-/-}$+CRS)每组6只，单笼单只饲养，中山大学实验动物伦理委员会机构审批并通过本实验所有动物实验操作。

1.2 抑郁模型建立

慢性束缚动物建立抑郁模型是目前公认的抑郁症动物模型之一，建立该动物模型的方法在参考文献[11]基础上，进行了适当修改。具体建模方法为：每天将模型组小鼠置于通风良好的50 mL圆锥形管内，采用一根3 cm长的空心管塞到锥形管的盖子内，将其尾部伸出锥形管外，防止小鼠尾部压伤。在此装置中小鼠不能向前或向后移动，每天束缚6~7 h，给予慢性束缚应激，对照组小鼠在笼内自由活动，连续14 d。*CX3CR1-GFP*转基因小鼠与*C1q*$^{-/-}$造模方法与上述方法相同。

1.3 行为学检测

1.3.1 悬尾实验 参照Depino等[12]报道的方法测定小鼠的抑郁状态，用胶带缠绕在小鼠尾部，将小鼠悬挂在空中，并将胶带固定在距离木质表面25 cm的线上。通过SuperTst高通量悬尾测试软件(上海欣软信息技术有限公司)记录小鼠5 min内的不动时间。

1.3.2 强迫游泳实验 参照Depino等[12]报道的方法测定小鼠的抑郁状态，将小鼠轻轻放入玻璃烧杯中(直径15 cm；高度25 cm)装入14 cm深的水(水温为25 ℃)用SuperFst高通量强迫游泳测试软件(上海欣软信息技术有限公司)记录小鼠5 min内不动的时间。试验结束时，用纸巾将小鼠擦干，放在有垫料的笼子里。

1.4 免疫荧光染色方法

腹腔注射戊巴比妥钠麻醉小鼠，注射剂量按照0.2 g/L注射。从剑突上方打开胸腔，暴露心脏，剪开右心耳，在左心尖处插管，生理盐水灌注，待流出血液转为澄清液体后改用4 ℃含多聚甲醛(paraformaldehyde，PFA)磷酸缓冲液灌注，固定组织，浓度为40 g/L，迅速取脑并在相同浓度的溶液中后固定24 h，蔗糖溶液梯度脱水后进行连续冰冻切片(厚度40 μm)。

单标染色：将全脑冰冻切片经磷酸盐缓冲液(phosphate buffered saline，PBS)洗片3次，5 min/次后，用含0.3% Triton X-100的牛血清白蛋白(bovine serum albumin，BSA) 37 ℃环境下孵育30 min，加入一抗在37 ℃孵育2 h后4 ℃孵育过夜，次日复温至室温后PBS洗3次，5 min/次，加入二抗，37 ℃孵育2 h，PBS洗3次5 min/次，晾干，荧光封片剂封片。

置于激光共聚焦显微镜(Zeiss 780)下拍照，使用Image J图像分析软件对Iba-1、GFAP、C1q、C3、PSD95和Syn荧光强度进行测量。

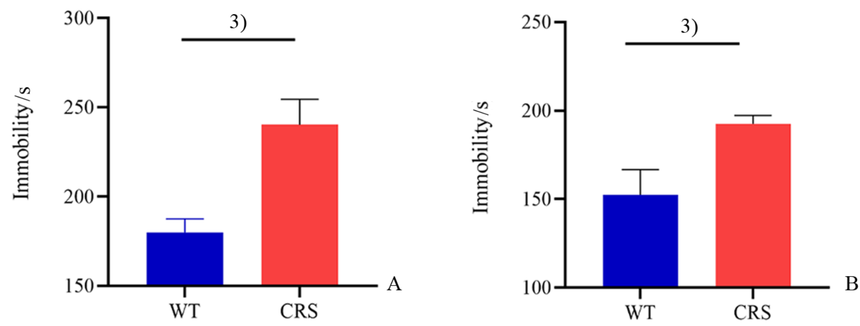
1.5 统计方法

采用SPSS25.0进行统计分析，数据均符合正态分布以均数±标准差表示，两组间采用两独立样本t检验，4组间数据通过组间方差齐性检验，采用双因素方差分析，采用LSD进行两两比较，$P<0.05$为差异有统计学意义。

2 结 果

2.1 抑郁模型小鼠行为学以及各项相关指标变化

2.1.1 两组小鼠抑郁状态比较 为检测WT组和CRS组小鼠的抑郁状态，在造模结束后，对两组小鼠进行悬尾和强迫游泳行为学检测。行为学结果显示两组结果有统计学差异(t=9.136，P<0.000 1；t=6.513，P<0.000 1)，5 min内CRS组摇摆不动时间明显高于WT组，具有抑郁样行为(图1)。

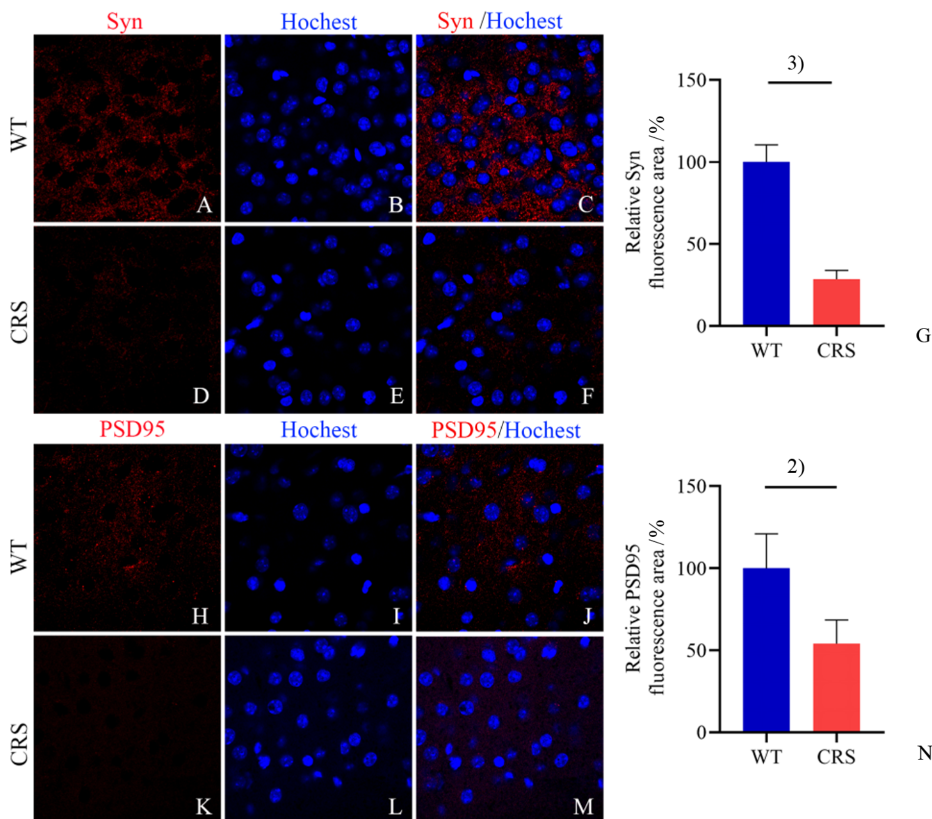


A: The time of immobility in tail suspension test. B: The time of immobility in forced swimming test. In tail suspension experiment and forced swimming experiment, the immobility time of CRS group was significantly longer than that of WT group (A: t=9.136, P<0.000 1; B: t=6.513, P<0.000 1). n=6 per group.

图1　悬尾实验和强迫游泳比较小鼠抑郁样行为

Fig. 1　Comparison of depression-like behaviors using the tail suspension test and the forced swimming test in mice

2.1.2　两组小鼠杏仁核内突触变化情况　免疫荧光染色检测小鼠杏仁核内Syn与PSD95荧光强度，两独立样本t检验结果显示差异有统计学意义。WT组杏仁核内Syn荧光强度明显高于CRS组(t=13.62, P<0.000 1;图2A~G),说明CRS组正常突触丢失更多。WT组杏仁核内PSD95荧光强度明显高于CRS组(t=4.032, P=0.003 8;图2H~N)。



A-C: Anti-Syn immunofluorescence of the amygdala in the WT group (630×). D-F: Anti-Syn immunofluorescence of the amygdala in the CRS group (630×). G: Syn-immunoreactivity intensity was increased in the WT group compared to the CRS group (t=13.62, P<0.000 1). H-J: Anti-PSD95 immunofluorescence of the amygdala in the WT group (630×). K-M: Anti-PSD95 immunofluorescence of the amygdala in the CRS group (630×). N: PSD95-immunoreacitivity intensity was higher in the WT group than that in the CRS group (t=4.032, P=0.003 8). n=5 per group

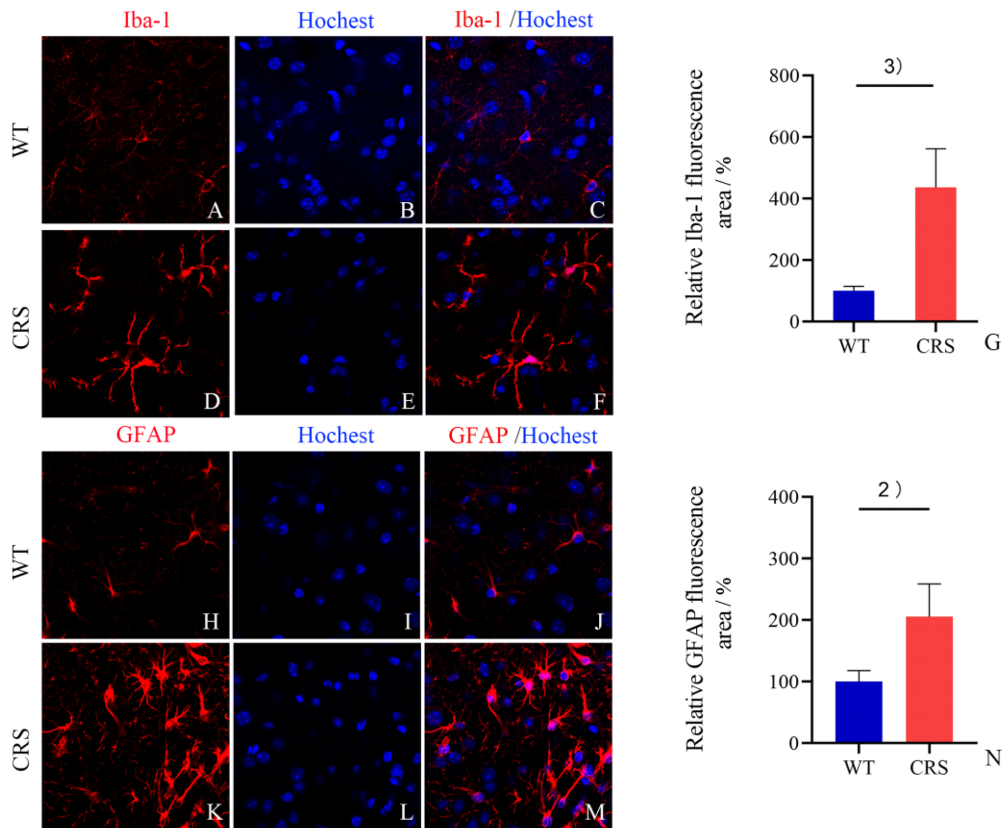
图2　免疫荧光法检测杏仁核内Syn和PSD95荧光强度

Fig. 2　Fluorescence intensity of Syn and PSD95 in the amygdala by immunofluorescence

2.1.3 两组小鼠杏仁核内小胶质细胞及星形胶质细胞变化 本实验已经证明在抑郁模型小鼠杏仁核部位突触含量减少,在中枢神经系统内炎症状态下通常伴有小胶质细胞及星形胶质细胞激活,修剪细胞周围突触。因此造模结束后,通过免疫荧光染色检测小鼠杏仁核的小胶质细胞(Iba-1)与星形胶质细胞(GFAP)荧光强度,两独立样本 t 检验结果显示差异有统计学意义。CRS组杏仁核内Iba-1荧光强度明显高于WT组(t=5.921, P=0.000 4;图3A~G)。CRS组杏仁核内GFAP荧光强度明显高于WT组(t=4.189, P=0.003;图3H~N)。

星形胶质细胞表达的C3和小胶质细胞C3aR之间的交互作用调节C3/C3a-C3aR信号通路,从而动态调节小胶质细胞修剪功能[10]。通过免疫荧光染色检测 *GFP* 小鼠杏仁核的小胶质细胞与星形胶质细胞相互作用情况。结果显示,与 *GFP* 组相比,*GFP*+CRS组星形胶质细胞与小胶质细胞相互作用明显,小胶质细胞分支变粗,突起增多(图4)。

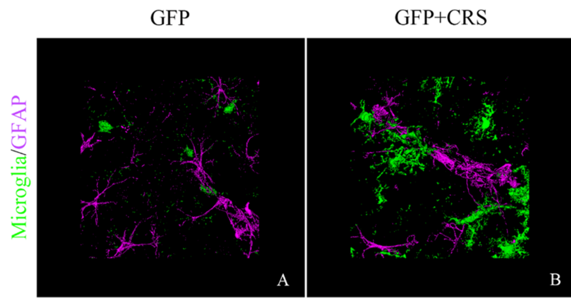
2.1.4 两组小鼠杏仁核内C1q、C3变化情况 上述结果已经证实CRS组星形胶质细胞与小胶质细胞均明显激活,且相互作用明显,接下来,观察两种胶质细胞产生补体情况。免疫荧光染色检测小鼠杏仁核内C1q与C3荧光强度,两独立样本 t 检验结果显示差异有统计学意义。CRS组杏仁核内C1q荧光强度明显高于WT组(t=6.667, P=0.000 2;图5A~G)。CRS组杏仁核内C3荧光强度明显高于WT组(t=3.24, P=0.0119;图5H~N)。



A-C: Anti-Iba-1 immunofluorescence of the amygdala in the WT group (630×). D-F: Anti-Iba-1 immunofluorescence of the amygdala in the CRS group (630×). G: Iba-1-positive fluorescence intensity is increased in the CRS group compared with the WT group (t=5.921, P=0.000 4). H-J: GFAP immunofluorescence of the amygdala in the WT group (630×). K-M: GFAP immunofluorescence of the amygdala in the CRS group in (630×). N: GFAP-positive fluorescence intensity in the CRS group is enhanced compared with that in the WT group (t=4.189, P=0.003). n=5 per group

图3 免疫荧光检测杏仁核内小胶质细胞及星形胶质细胞荧光强度

Fig. 3 Fluorescence intensity of microglia and astrocytes in amygdala detected by immunofluorescence



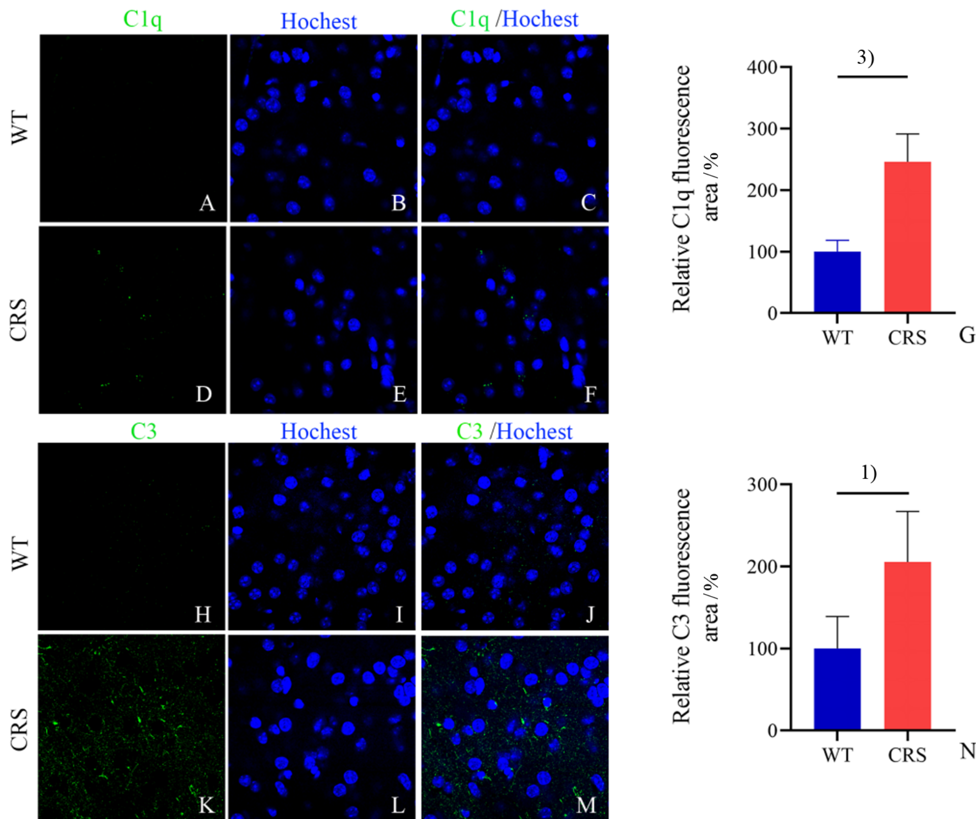
A, B: Immunofluorescence signal showing microglia-astrocyte interaction in the amygdala in the *GFP* group (630×, A) and the *GFP*+CRS group (630×, B).

图4　免疫荧光比较杏仁核内小胶质细胞与星形胶质细胞相互作用情况

Fig.4　Comparison of interaction between microglia and astrocytes in the amygdala by immunofluorescence

2.2　WT、CRS、$C1q^{-/-}$、$C1q^{-/-}$+CRS四组小鼠抑郁状态与突触的变化

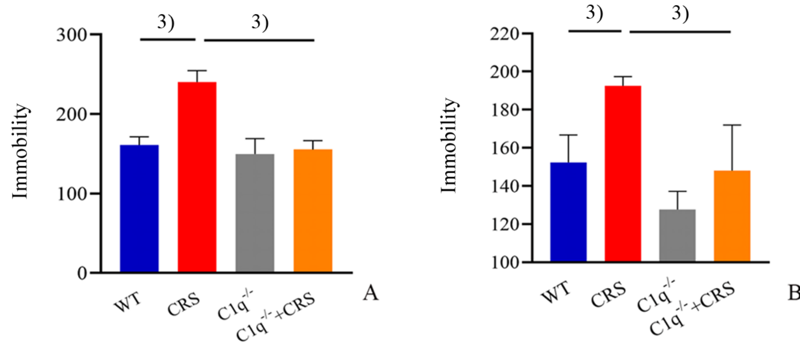
2.2.1　WT、CRS、$C1q^{-/-}$、$C1q^{-/-}$+CRS四组小鼠模型组抑郁状态比较　为评估4组小鼠的抑郁状态,悬尾实验双因素方差分析结果显示:基因型的主效应差异有统计学意义(F=24.032, P<0.001),抑郁模型处理的主效应差异有统计学意义(F=18.542, P<0.001),组间两两比较显示:与WT组比较,CRS组不动时间明显升高,差异有统计学意义(161.15±10.11 *vs.* 240.32±14.30, P<0.001)与CRS组比较,$C1q^{-/-}$+CRS组不动时间明显降低,差异有统计学意义(240.32±14.30 *vs.* 155.28±10.02, P<0.001);强迫游泳实验双因素方差分析结果显示:基因型的主效应差异有统计学意义(F=29.918, P<0.001),抑郁模型处理的主效应差异有统计学意义(F=22.862, P<0.001),组间两两比较显示,与WT组比较,CRS组不动时间明显升高,差异有统计学意义(152.39±14.32 *vs.* 192.55±4.79 , P<0.001)与CRS组比较,$C1q^{-/-}$+CRS组不动时间明显降低,差异有统计学意义(192.55±4.79 *vs.*148.03±23.95, P<0.001;图6)。



A-C: C1q immunofluorescence of the amygdala in the WT group (630×). D-F: C1q immunofluorescence of the amygdala in the CRS group (630×). G: C1q-positive fluorescence intensity was higher in the CRS group than that in the WT group (t=6.667, P=0.000 2). H-J: C3 immunofluorescence of the amygdala in the WT group (630×). K-M: C3 immunofluorescence of amygdala in CRS group (630×). N: C3-positive fluorescence intensity was higher in the CRS group than that in the WT group (t=3.24, P=0.0119). n=5 per group

图5　免疫荧光法检测杏仁核内C1q、C3荧光强度

Fig.5　Fluorescence intensity of C1q and C3 in the amygdala detected by immunofluorescence

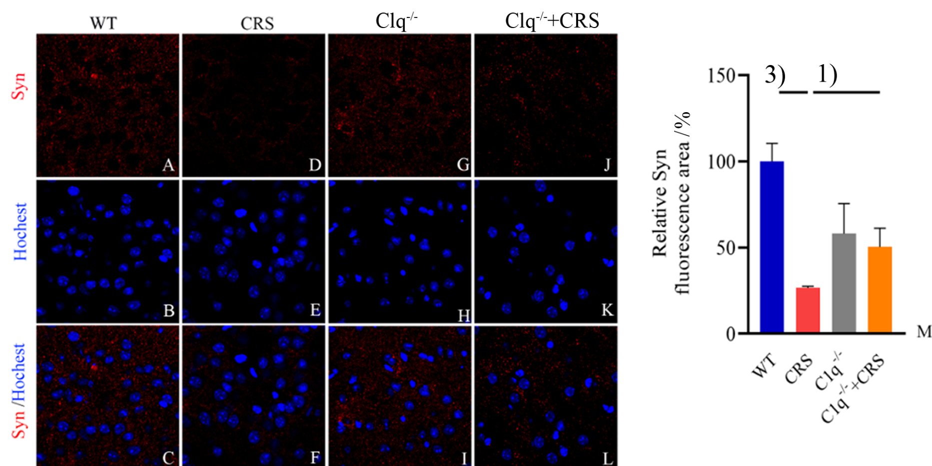


A: The time of immobility in tail suspension test. B: The time of immobility in forced swimming test. In tail suspension experiment and forced swimming experiment, the immobility time in the CRS group was significantly longer compared with the WT group and *C1q*$^{-/-}$+CRS group All the data represent the mean ± SD and were analyzed using two-way ANOVA of variance followed by LSD post hoc test(P_A<0.001 ; P_B<0.001). *n*=6 per group

图6　悬尾实验和强迫游泳比较各组小鼠抑郁样行为

Fig. 6　Comparison of depression-like behaviors between groups by using the tail suspension test and the forced swimming test

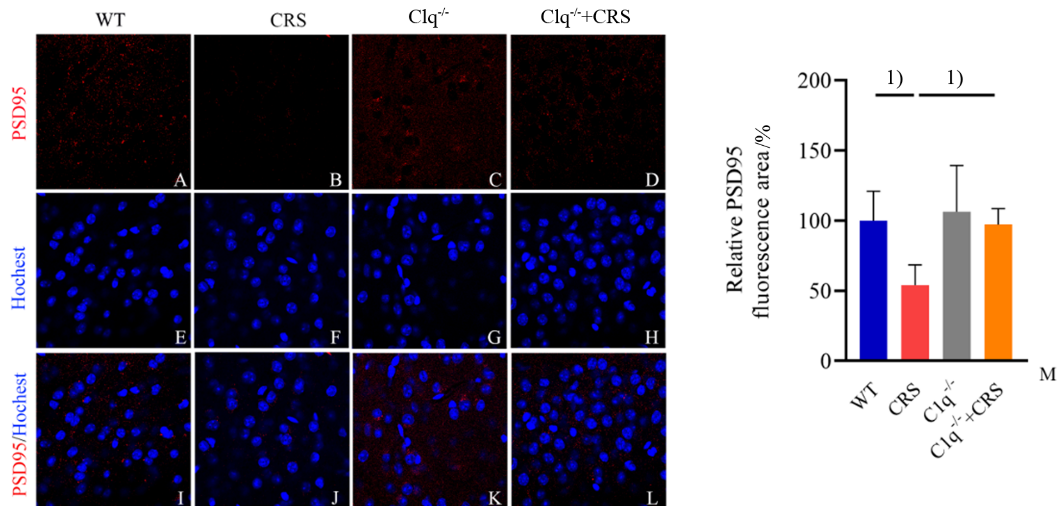
2.2.2　*C1q*敲除模型组与C57小鼠模型组杏仁核内突触变化情况　Syn荧光强度结果双因素方差分析显示:基因型的主效应差异无统计学意义(F=0.937, P=0.347),抑郁模型处理的主效应差异有统计学意义(F=18.647, P<0.001),组间两两比较显示:WT组杏仁核内Syn荧光强度明显高于CRS组,差异有统计学意义(100.00±10.41 *vs.* 26.67±0.87 , P<0.001);*C1q*$^{-/-}$+CRS组杏仁核内Syn荧光强度明显高于CRS组,差异有统计学意义(50.37±10.90 *vs.* 26.67±0.87 , P<0.05),说明*C1q*$^{-/-}$+CRS组正常突触消除减少(图7)。PSD95荧光强度结果双因素方差分析显示:基因型的主效应差异有统计学意义(F=5.672, P=0.029),抑郁模型处理的主效应差异有统计学意义(F=7.085, P=0.016),组间两两比较显示:WT组杏仁核内PSD95荧光强度明显高于CRS组,差异有统计学意义(100.00±20.99 *vs.* 53.97±14.53 , P=0.0185);*C1q*$^{-/-}$+CRS组杏仁核内Syn荧光强度明显高于CRS组,差异有统计学意义(97.09 ± 11.44 *vs.* 53.97±14.53 , P=0.0283;图8)。



A-C: Anti-Syn immunofluorescence of the amygdala in the WT group (630×). D-F: Anti-Syn immunofluorescence of the amygdala in the CRS group (630×). G-I: Anti-Syn immunofluorescence of the amygdala in the *C1q*$^{-/-}$ group (630×) J-L: Anti-Syn immunofluorescence of the amygdala in the *C1q*$^{-/-}$+CRS group (630×) M:Syn-immunoreacitivity intensity was increased in the WT group compared to the CRS group and also increased in the *C1q*$^{-/-}$+CRS group compared to the CRS group. All the data represent the mean±SD and were analyzed using two-way ANOVA of variance followed by LSD post hoc test (P<0.001, P<0.05). *n*=5 per group

图7　免疫荧光法检测各组杏仁核内Syn荧光强度

Fig. 7　Detection of Syn fluorescence intensity in the amygdala among groups by immunofluorescence



A-C: Anti-PSD95 immunofluorescence of the amygdala in the WT group (630×). D-F: Anti-PSD95 immunofluorescence of the amygdala in the CRS group (630×). G-I:Anti-PSD95 immunofluorescence of the amygdala in the *C1q*$^{-/-}$ group (630×) J-L:Anti-PSD95 immunofluorescence of the amygdala in the *C1q*$^{-/-}$+CRS group (630×) M:PSD95-immunoreacitivity intensity was increased in the WT group compared to the CRS group and also increased in the *C1q*$^{-/-}$+CRS group compared to the CRS group. All the data represent the mean ± SD and were analyzed using two-way ANOVA of variance followed by LSD post hoc test ($P<0.05$). $n=5$ per group

图8　免疫荧光法检测各组杏仁核内PSD95荧光强度

Fig. 8　Detection of PSD95 fluorescence intensity in the amygdala among groups by immunofluorescence

3　讨论

抑郁症影响全球近3.22亿人口的生活,严重影响人们日常生活[13],了解抑郁症作用机制有助于更好防治抑郁症,提高人们生活质量。目前有研究表明抑郁症与脑内的前额叶皮质[14]和前扣带皮质关系密切[15],杏仁核作为应对情绪潜在压力的重要中枢[16],严重抑郁症患者存在杏仁核基本结构和功能损害[17-18],但是关于抑郁症患者杏仁核部位的病理机制的相关报道较少,因此本研究将杏仁核作为研究部位。

抑郁症与炎症的发生密不可分,中枢神经系统炎症激活是抑郁症发病机制中的关键。已经有文献报道在焦虑症中可以在杏仁核引起神经炎症[19]。但是在抑郁症中杏仁核部位的神经炎症产生及机制尚不清楚,因此本研究探索在抑郁模型鼠杏仁核部位神经炎症相关指标的变化情况。

中枢神经系统炎症因子失衡会导致小胶质细胞功能损伤,小胶质细胞是中枢神经系统内的免疫细胞,参与神经系统内的突触修剪,修复神经损伤,脑内炎症反应,发挥先天免疫应答作用[20]。在本研究中也证实了这一观点,在抑郁模型组的小胶质细胞明显激活,细胞胞体变大,突起增多。星形胶质细胞被炎症介质和细胞因子激活,激活的星形胶质细胞产生许多可能影响中枢神经系统的调节因子,并向激活的小胶质细胞提供正反馈,而且在中枢神经系统损伤后会被诱导变成具有神经毒性的反应性星形胶质细胞[21]。本研究同样证明这一观点,在抑郁模型组的星形胶质细胞明显激活,胞体变大,突起同样增多。而且在本研究中还发现在抑郁模型组中小胶质细胞和星形胶质细胞具有明显交互作用。有研究发现在抑郁症动物模型中M1型激活的小胶质细胞增加[22]。结合本实验结果,小胶质细胞激活诱发神经毒性星形胶质细胞增多[5],进一步加重脑内神经炎症的产生。

小胶质细胞与突触的相互作用在维持神经元连接和影响神经元可塑性方面非常重要。在健康和发育中的脑中,小胶质细胞修剪突触前和突触后的突触,从而调节突触的神经支配。最近,已经有文献证明小胶质细胞自噬在突触修剪中的关键作用[23]。本研究对突触素蛋白和突触后蛋白进行荧光标记,也发现在抑郁模型组突触素蛋白和突触后蛋白明显减少。人们认为补体C1q和C3"标记"了这样的突触,从而将小胶质细胞导向它们进行吞噬[24],因此我们认为补体途径可能在抑郁症产生的神经炎症中扮演重要角色。

补体途径是先天免疫调节关键途径,补体途径中C1q是经典补体途径多亚单位复合体C1的识别成分。C1q不依赖于C1r和C1s,它可以增强凋亡细胞和细胞碎片的清除,下调吞噬细胞的促炎细胞因子的表达[25-27]。文献报道小胶质细胞是中枢神经系统C1q的主要来源[28],星形胶质细胞可以表达C3[5],星形胶质细胞表达的C3和小胶质细胞表达的C3aR之间的交互作用调节C3/C3a-C3aR信号通路,从而动态调节小胶质细胞吞噬功能,本研究中CRS组杏仁核部位C1q、C3均增多,因此我们推测补体级联反应进一步加剧神经系统炎症,促使小胶质细胞吞噬突触,加重小鼠抑郁样行为。研究结果表明C1q可能在抑郁症中杏仁核部位的神经炎症起到关键作用,因此本研究引入$C1q^{-/-}$小鼠,对*C1q*的作用进行进一步探究。

为了进一步确定C1q在抑郁症中的作用,我们对C57BL/6小鼠和$C1q^{-/-}$小鼠同时进行慢性束缚刺激,行为学结果显示$C1q^{-/-}$模型鼠抑郁样行为减轻,免疫荧光结果显示杏仁核区突触丢失减少。以上结果证明了我们的推测,C1q在抑郁症中起关键作用,$C1q^{-/-}$模型组行为更接近WT组,杏仁核内突触蛋白含量也明显增多。推测抑郁症产生的机制可能是杏仁核内产生炎症因子,激活小胶质细胞,产生C1q,激活的小胶质细胞诱导星形胶质细胞变为具有神经毒性星形胶质细胞,星形胶质细胞表达C3,与小胶质细胞表面C3aR受体相互作用,进一步激活小胶质细胞修剪周围突触,敲除*C1q*后小胶质细胞激活减轻,神经毒性星形胶质细胞减少,两胶质细胞相互作用减少,突触丢失减少,抑郁样行为减轻。

本研究结果表明抑郁样行为小鼠杏仁核内胶质细胞激活并表达补体蛋白,介导补体依赖的小胶质细胞突触修剪,导致神经元突触大量丢失,$C1q^{-/-}$模型小鼠杏仁核内突触丢失减少,抑郁样行为减轻,证实C1q在介导小胶质细胞突触修剪在抑郁症发生中具有关键作用。为抑郁症中补体作用途径提供新思路,补体途径在抑郁症中详细的作用机制有待进一步研究。

参考文献

[1] Sendi MSE, Zendehrouh E, Sui J, et al. Aberrant dynamic functional connectivity of default mode network predicts symptom severity in major depressive disorder [J]. Brain Connect, 2021, doi: 10.1089/brain.2020.0748. Epub ahead of print.

[2] Burrows K, Stewart JL, Kuplicki R, et al. Elevated peripheral inflammation is associated with attenuated striatal reward anticipation in major depressive disorder [J]. Brain Behav Immun, 2021, 93: 214-225.

[3] Martínez-Torres NI, Vázquez-Hernández N, Martín-Amaya-Barajas FL, et al. Ibotenic acid induced lesions impair the modulation of dendritic spine plasticity in the prefrontal cortex and amygdala, a phenomenon that underlies working memory and social behavior[J]. Eur J Pharmacol, 2021, 896: 173883. doi: 10.1016/j.ejphar.2021.173883.

[4] Menke A, Nitschke F, Hellmuth A, et al. Stress impairs response to antidepressants via HPA axis and immune system activation [J]. Brain Behav Immun, 2021, 93: 132-140.

[5] Liddelow SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia[J]. Nature, 2017, 541(7638):481-487.

[6] Fonseca MI, Chu SH, Hernandez MX, et al. Cell-specific deletion of C1qa identifies microglia as the dominant source of C1q in mouse brain[J]. J Neuroinflammation, 2017, 14(1):48

[7] Marinelli S, Basilico B, Marrone MC, et al. Microglia-neuron crosstalk: Signaling mechanism and control of synaptic transmission[J]. Semin Cell Dev Biol, 2019, 94:138-151.

[8] Dantzer R. Neuroimmune interactions: From the brain to the immune system and vice versa[J]. Physiol Rev, 2018, 98(1): 477-504.

[9] Yilmaz M, Yalcin E, Presumey J, et al. Overexpression of schizophrenia susceptibility factor human complement C4A promotes excessive synaptic loss and behavioral changes in mice[J]. Nat Neurosci, 2021, 24(2): 214-224.

[10] Lian H, Litvinchuk A, Chiang AC, et al. Astrocyte-microglia cross talk through complement activation modulates amyloid pathology in mouse models of Alzheimer's disease [J]. J Neurosci, 2016, 36(2): 577-589.

[11] Yang Y, Cui Y, Sang K, et al. Ketamine blocks bursting in the lateral habenula to rapidly relieve depression [J]. Nature, 2018, 554(7692): 317-322.

[12] Depino AM, Lucchina L, Pitossi F. Early and adult hippocampal TGF-β1 overexpression have opposite effects on behavior[J]. Brain Behav Immun, 2011, 25 (8):1582-1591.

[13] Chesney E, Goodwin GM, Fazel S. Risks of all-cause and suicide mortality in mental disorders: a meta-review[J]. World Psychiatry, 2014, 13(2):153-160.

[14] Liu X, Zhong S, Li Z, et al. Serum copper and zinc levels correlate with biochemical metabolite ratios in the prefrontal cortex and lentiform nucleus of patients with major depressive disorder[J]. Prog Neuropsychopharmacol Biol Psychiatry, 2020 ; 99: 109828. doi: 10.1016/j.pnpbp.2019.109828

[15] Woelfer M, Kasties V, Kahlfuss S, et al. The Role of Depressive Subtypes within the Neuroinflammation Hypothesis of Major Depressive Disorder[J]. Neuroscience, 2019, 403:93-110.

[16] Fonzo GA. Childhood Maltreatment and Amygdala Threat Reactivity in Young Adults-Timing Is Everything[J]. JAMA Psychiatry, 2019, 76(8):781-782.

[17] Malhi GS, Das P, Outhred T, et al. Default mode dysfunction underpins suicidal activity in mood disorders [J]. Psychol Med, 2020, 50(7):1214-1223.

[18] 张明，甘照宇，钟智勇，等.单双相抑郁智能损害的对照研究[J].中山大学学报(医学科学版)，2012，33(05):634-638.
Zhang M, Gan ZY, Zhong ZY, et al. A comparative study of mental impairment in unipolar and bipolar depression [J]. J Sun Yat-sen Univ (Med Sci), 2012, 33(05):634-638.

[19] Liu HY, Yue J, Hu LN, et al. Chronic minocycline treatment reduces the anxiety-like behaviors induced by repeated restraint stress through modulating neuroinflammation[J]. Brain Res Bull, 2018, 143:19-26.

[20] 袁丽芳，杨俊华，邢志伟，等.口服益生菌改善铝诱发的小鼠海马炎症[J].中山大学学报(医学科学版)，2020, 41(2):210-216.
Yuan LF, Yang JH, Xing ZW, et al. Oral probiotics improve aluminum-induced hippocampal inflammation in mice [J]. J Sun Yat-sen Univ (Med Sci), 2020, 41(2): 210-216.

[21] Liddelow SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia[J]. Nature, 2017, 541(7638): 481-487.

[22] 韩岳.小胶质细胞在早期生活应激所致行为异常中的作用机制研究[D].电子科技大学，2020，8-10.
Han Y. Mechanism of microglia in behavioral abnormality caused by early life stress [D]. University of electronic science and technology of China, 2020, 8-10.

[23] Kim HJ, Cho MH, Shim WH, et al. Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects [J]. Mol Psychiatry, 2017, 22(11): 1576-1584.

[24] Acharjee S, Verbeek M, Gomez CD, et al. Reduced Microglial Activity and Enhanced Glutamate Transmission in the Basolateral Amygdala in Early CNS Autoimmunity [J]. J Neurosci, 2018, 38 (42) : 9019-9033.

[25] Benoit ME, Clarke EV, Morgado P, et al. Complement protein C1q directs macrophage polarization and limits inflammasome activity during the uptake of apoptotic cells [J]. J Immunol, 2012, 188 (11) : 5682-5693.

[26] Roumenina LT, Sène D, Radanova M, et al. Functional complement C1q abnormality leads to impaired immune complexes and apoptotic cell clearance[J]. J Immunol, 2011, 187(8): 4369-4373.

[27] Fraser DA, Pisalyaput K, Tenner AJ. C1q enhances microglial clearance of apoptotic neurons and neuronal blebs, and modulates subsequent inflammatory cytokine production[J]. J Neurochem, 2010, 112 (3) : 733-743.

[28] Chen T, Lennon VA, Liu YU, et al. Astrocyte-microglia interaction drives evolving neuromyelitis optica lesion[J]. J Clin Invest, 2020, 130(8): 4025-4038.

（编辑　祁方昉）