论著·基础医学

布鲁氏菌性骨关节炎中Tfh/Tfr细胞失衡特征及关节损伤相关分子的变化

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［摘要］ **目的** 布鲁氏菌性骨关节炎急慢性期免疫炎症机制尚未明确,滤泡辅助性T细胞(T follicular helper cells,Tfh)和滤泡调节性T细胞(follicular regulatory T cells,Tfr)细胞在该疾病中的动态变化及其与免疫炎症反应无相关报道。本研究旨在探讨布鲁氏菌性骨关节炎患者外周血及滑膜组织中Tfh细胞与Tfr细胞急性期与慢性期的变化特点,及其相关细胞因子、转录因子与免疫球蛋白的关联性,为该疾病免疫机制及临床诊疗提供科学依据。**方法** 本研究采用横断面研究设计,共纳入2024年1月至2025年8月石河子大学第一附属医院及医共体医院骨科确诊的急性期布鲁氏菌性骨关节炎患者13例、慢性期患者21例,另选取20例健康人群作为对照。收集所有研究对象外周血样本,同时收集慢性期患者3例及非感染性关节损伤手术患者的滑膜组织3例作为局部组织水平验证,未纳入总体临床统计分析。采用流式细胞术检测外周血中($CD4^+CXCR5^+CD25^-CD127^+$)Tfh细胞与($CD4^+CXCR5^+CD25^+CD127^-$)Tfr细胞百分比及两者比值;ELISA法测定血清中IL-4、IL-21、IL-10、TGF-β等细胞因子及IgA、IgG、IgE、IgM等免疫球蛋白水平;qRT-PCR检测外周血单个核细胞(peripheral blood mononuclear cells, PBMCs)中Tfh相关转录因子BCL-6、细胞因子*IL-21*及Tfr相关转录因子*FOXP3*、细胞因子*IL-10*的mRNA表达;Western blotting检测滑膜组织中IL-21、FOXP3蛋白及关节基质代谢相关蛋白MMP-13、COLⅡ、ADAMTS5的表达。**结果** ①滑膜组织中相关蛋白检测结果显示,相较于对照组,慢性期患者FOXP3(P=0.020 1)、MMP-13(P=0.004 8)、ADAMTS5(P=0.002 3)蛋白表达均显著升高,而IL-21(P<0.000 1)、COLⅡ(P=0.009 2)蛋白表达则显著降低。②外周血流式细胞术检测发现,急性期患者Tfh细胞比例较对照组(P=0.001 4)、慢性期组(P<0.000 1)均显著升高;Tfh/Tfr比值分别高于对照组(P=0.020 4)及慢性期组(P<0.000 1);而Tfr细胞比例则低于对照组(P<0.015 5)和慢性期组(P<0.000 1)。③qRT-PCR检测显示,急性期患者PBMCs中Tfh相关细胞因子*IL-21*、转录因子*BCL-6*相较于对照组升高(P=0.000 8,P<0.000 1);慢性期患者细胞因子*IL-10*、转录因子*FOXP3*相较于对照组表达均明显上调(P<0.000 1)。④酶联免疫吸附测定(enzyme-linked immunosorbent assay,ELISA)检测结果显示,急性期患者血清IL-21、IL-4、IgG水平均显著高于对照组(P<0.000 1);慢性期患者较急性期三者呈下降趋势(P<0.05)。而IL-10水平在慢性期显著升高,高于对照组(P<0.000 1)及急性期(P=0.034 3)。患者血清IgA、IgM水平在急、慢性期均显著高于对照组(P<0.05),但在2组间比较无统计学差异。⑤Spearman相关性分析显示,急性期Tfh细胞比例与IL-21、IgA、IgM呈正相关(r=0.26、0.34、0.37),与Tfr细胞呈负相关(r=−0.54),IL-21与IgG、IgM呈强正相关(r=0.75、0.58);慢性期上述相关性整体减弱,Tfr相关指标与其余指标联动增强。**结论** 本研究初步揭示了Tfh/Tfr细胞失衡在布鲁氏菌性骨关节炎中的变化,急性期以Tfh细胞及其相关因子高表达为主;慢性期以Tfr细胞及其相关因子高表达为特征,且存在骨关节病理损伤。

［关键词］ 布鲁氏菌;感染性关节炎;滤泡辅助性T细胞;调节性T淋巴细胞

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Characteristics of Tfh/Tfr cell imbalance and changes in joint injury-related molecules in Brucella osteoarthritis

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[**Abstract**] **Objective** The immuno-inflammatory mechanisms underlying acute and chronic Brucella osteoarthritis (BOA) remain unclear, and the dynamic changes in T follicular helper (Tfh) cells and follicular regulatory T (Tfr) cells in this disease, as well as their associations with immune-inflammatory responses, have not been systematically reported. This study aimed to characterize the stage-specific changes in Tfh and Tfr cells in peripheral blood and synovial tissue of BOA patients, and to analyze their relationships with related cytokines, transcription factors, and immunoglobulins, thereby providing the immunological evidence for pathogenesis and clinical management of the disease. **Methods** A cross-sectional study design was adopted in this research. A total of 13 patients with acute BOA and 21 patients with chronic BOA, who were diagnosed in the Department of Orthopedics at the First Affiliated Hospital of Shihezi University and its medical consortium hospitals from January 2024 to August 2025, were enrolled, and another 20 healthy individuals were subjected and served as the control group. Peripheral blood samples were collected from all participants, while synovial tissue specimens were obtained from 3 chronic BOA patients and 3 patients with non-infectious joint injury undergoing surgery for local tissue-level validation, which were not included in the overall clinical statistical analysis. Flow cytometry was performed to determine the proportions of Tfh cells ($CD4^+CXCR5^+CD25^-CD127^+$) and Tfr cells ($CD4^+CXCR5^+CD25^+CD127^-$) in peripheral blood, as well as the Tfh/Tfr ratio. Serum levels of IL-4, IL-21, IL-10, TGF-β, and immunoglobulins (IgA, IgG, IgE and IgM) were measured by ELISA. The mRNA expression of Tfh-related transcription factor *BCL-6* and cytokine *IL-21*, as well as Tfr-related transcription factor *FOXP3* and cytokine *IL-10*, was assessed in peripheral blood mononuclear cells (PBMCs) using qRT-PCR. Western blotting was used to detect the expression of IL-21, FOXP3, and joint matrix metabolism-related proteins MMP-13, type Ⅱ collagen (COL Ⅱ), and ADAMTS5) in synovial tissues. **Results** ① In the synovial tissues, the chronic BOA group showed significantly higher protein levels of FOXP3 (P=0.020 1), MMP-13 (P=0.004 8), and ADAMTS5 (P=0.002 3), while lower levels of IL-21 (P<0.000 1) and COL Ⅱ (P=0.009 2) compared with the control group. ② Flow cytometric analysis of peripheral blood revealed that the proportion of Tfh cells in acute-phase patients was significantly higher than that in the control group (P=0.001 4) and the chronic-phase group (P<0.000 1); so was the Tfh/Tfr ratio when compared with that in the control group (P=0.020 4) and the chronic-phase group (P<0.000 1); whereas the proportion of Tfr cells was lower than that in the control group (P=0.015 5) and the chronic-phase group (P<0.000 1). ③ qRT-PCR in PBMCs showed that the mRNA levels of the Tfh-related cytokine *IL-21* and transcription factor *BCL-6* were upregulated in acute-phase patients than the control group (P=0.000 8, P<0.000 1); those of the cytokine *IL-10* and transcription factor *FOXP3* were also enhanced in chronic-phase patients compared with the control group (both P<0.000 1). ④ ELISA results showed that serum levels of IL-21, IL-4, and IgG were significantly elevated in acute-phase patients, all higher than those in the control group (all P<0.000 1); these 3 indicators showed a decreasing trend in the chronic phase compared with the acute group (P<0.05). In contrast, serum IL-10 level was significantly increased in the chronic phase, higher than that in the control group (P<0.000 1) and the acute-phase patients (P=0.034 3). Serum IgA and IgM levels in patients in both acute and chronic phases were significantly higher than those in the control group (P<0.05), but no significant difference was observed between the 2 phase groups. ⑤ Spearman correlation analysis indicated that, in the acute phase, the proportion of Tfh cells was positively correlated with IL-21, IgA, and IgM (r=0.26, 0.34, and 0.37, respectively), and negatively with Tfr cells (r=−0.54). IL-21 showed a strong positive correlation with IgG and IgM (r=0.75 and 0.58). In the chronic phase, the above correlations overall weakened, while correlations with Tfr-related indexes exhibited stronger associations compared with the other indicators.

Conclusion This study preliminarily revealed the changes in Tfh/Tfr cell imbalance in BOA, with the acute phase characterized by Tfh cells and high expression of related factors, and the chronic phase by larger proportion of Tfr cells and high expression of related factors, accompanied by pathological joint damage.

[**Key words**] *Brucellosis*; arthritis, infectious; T follicular helper cells; T-lymphocytes, regulatory

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布鲁氏菌病(Brucellosis,简称布病)是一种常见的人畜共患细菌感染性疾病,在中国布病发病率呈逐年上升趋势[1]。布鲁氏菌性骨关节炎(brucellar osteoarthritis,BOA)是布鲁氏菌病常见的并发症之一,在布病感染患者中占10%~85%[2]。布鲁氏菌感染后会造成持续的慢性骨关节病变,受累关节中最常见的是骶髂关节和脊柱关节,早期症状不典型[3],其次是膝关节等外周关节[4],大部分患者明确诊断已是晚期,造成严重后果,如何有效的诊治布鲁氏菌性骨关节炎是当前的难题。

布鲁氏菌感染人体后触发机体复杂的免疫应答。其中,T细胞介导的免疫起到了关键作用[5-6]。研究显示,在患者中存在Th1/Th2细胞的免疫偏移,Th1细胞反应在急性期为主,同时急性期患者IFN-γ水平升高,这是清除布鲁氏菌的关键,在疾病的慢性期对布鲁氏菌抗原反应减弱,IFN-γ产生显著降低,Th2细胞比例增加并且伴有Treg细胞的增多导致了免疫抑制[7-8]。

近年来,滤泡辅助性T细胞(T follicular helper cells,Tfh)和滤泡调节性T细胞(T follicular regulatory cells,Tfr)逐渐受到了关注[9]。Tfh细胞是$CD4^+$T细胞的一个亚群,其主要转录因子是Bcl-6、主要细胞因子是IL-21。研究表明,Tfh可以通过支持B细胞分化和促进高亲和力抗体的成熟和产生,从而清除细菌并维持长期免疫记忆,支持体液免疫[10-11]。然而,如果Tfh细胞反应过度,可能会诱发自身免疫性疾病、慢性炎症及过敏反应[12-13]。

滤泡调节T细胞是调节性T细胞的亚群,其主要转录因子是FOXP3,Tfr可抑制Tfh活化及B细胞生发中心形成,从而调控体液免疫[14]。其过度反应也可导致多种临床免疫性疾病[12,15-16]。在感染性疾病中,Tfh提供B细胞帮助驱动抗体产生,而Tfr则相反,防止免疫过度反应。

Tfh细胞与Tfr细胞在自身免疫性疾病(如类风湿性关节炎等)中的作用被广泛研究。Tfh促进B细胞分化并产生大量自身抗体,加重关节的炎症反应和组织破坏[17]。此外,在沙门氏菌感染的患者中在疾病早期Tfh升高,晚期下降,Tfr则持续升高[18-19]。布鲁氏菌性骨关节炎作为布鲁氏菌病高发并发症存在早期诊断难、慢性化不可逆及治疗靶点匮乏的临床困境,其免疫机制尚未完全阐明,现有研究仅揭示在布鲁氏菌病存在Th1/Th2细胞免疫偏移现象。因此,本研究旨在探讨Tfh/Tfr及其相关因子在布鲁氏菌性骨关节炎中的变化,为布鲁氏菌性骨关节炎的免疫机制及临床诊断提供实验依据。

1 材料与方法

1.1 研究对象

本研究采用横断面研究设计,共纳入2024年1月至2025年8月收治的布鲁氏菌性骨关节炎患者34例(急性期n=13、慢性期n=21)及同期健康对照组20例。收集慢性期患者3例及非感染性关节损伤手术患者的滑膜组织3例作为局部组织水平验证,未纳入总体临床统计分析。所有研究对象均签署知情同意书。布鲁氏菌性骨关节炎患者的诊断需同时满足以下实验室检查及临床特征:①试管凝集试验滴度≥1∶100++,或病程持续1年以上仍有临床症状者滴度≥1∶50++;②补体结合试验滴度≥1∶10++;③抗人免疫球蛋白试验滴度≥1∶400++;④临床存在布鲁氏菌性骨关节炎典型表现,包括骨质破坏、关节肿胀、骨关节周围软组织肿胀等。纳入标准:①符合上述布鲁氏菌性骨关节炎诊断标准;②布鲁氏菌感染后3个月内出现骨关节症状且病程未超过3个月为急性期患者;③病程>6个月(排除3~6个月亚急性期病例),或布鲁氏菌感染后>6个月出现骨关节症状,伴持续性骨关节损害。排除标准:①伴有其他急性或慢性系统性疾病,如糖尿病、肿瘤等;②近期有使用免疫抑制剂、抗生素或其他干扰免疫系统的药物;③有其他感染性疾病史,或近期接受过其他抗感染治疗;④影像学检查显示明显的骨肿瘤、骨质疏松症、骨折或其他骨病变;⑤影像学检查发现明显的关节畸形、关节脱位或其他严重的关节结构异常;⑥确诊为结核、类风湿、强直性脊柱炎、退行性骨关节炎、感染性关节炎、痛风性关节炎或其他风湿性疾病。

1.2 患者样本收集与储存

受试者入院后,于清晨空腹状态下通过肘静脉采集全血5 mL,部分血液置于抗凝管中。采血后,将样本置于室温静置30 min以确保血液均匀处理,随后以600×*g*离心10 min,收集上清分装至离心管中,置于-80 ℃冰箱冷冻保存,其余样本置于肝素抗凝管用于流式细胞术检测。收集慢性期布鲁氏菌性骨关节炎患者的滑膜组织作为病例组,另选取同期行择期手术的非感染性关节损伤患者滑膜组织作为对照组。急性期患者多采取保守治疗,较少进行手术治疗,故未进行统计分析。本研究方案经石河子大学第一附属医院伦理委员会审查并批准(KJ2024-566-01),所有研究对象在入组前均签署书面知情同意书,研究过程严格遵循《赫尔辛基宣言》相关伦理原则。

1.3 Western blotting检测

取患者滑膜组织用剪刀剪碎后加入高效裂解液,在4 ℃下裂解1 h,随后研磨2 h。裂解液经4 ℃、11 300×*g*离心30 min后收集上清液,并使用BCA法测定蛋白浓度。蛋白提取成功后上样进行SDS-PAGE电泳、转膜、封闭、一抗孵育,一抗分别为:FOXP3(Abcam,ab154767)、IL-21(Abcam,ab215206)和MMP-13(Abcam,ab39012)、COL Ⅱ(Abmart,T510299)、ADAMTS5(Abcam,ab41037),4 ℃孵育过夜后经过洗涤、二抗(ZSG Bio,#ZB-2301)孵育及洗涤,使用ECL高效发光液显色,化学发光成像仪显影采图。条带定量以β-actin(Proteintech,#81115-1-RR)归一,并用ImageJ软件进行定量分析。

1.4 qRT-PCR检测

使用RNA提取试剂盒(RC113)从外周血提取单个核细胞(peripheral blood mononuclear cells,PBMCs)后提取总RNA,经NanoDrop测定浓度与纯度。采用RNA逆转录盒(R433)将RNA逆转录为cDNA。以cDNA为模板进行实时定量PCR,使用特异性引物(表1)。反应体系和循环程序按照试剂盒说明书设置,*β-actin*作为内参,采用$2^{-\Delta\Delta Ct}$方法计算相对表达量,同时设置空白对照以保证实验特异性。

表1 qRT-PCR引物序列

Table 1 Primer sequences in qRT-PCR

Gene names	Primer(5′→3′)
BCL-6	Forward:ACACATCTCGGCTCAATTTGC Reverse:AGTGTCCACAACATGCTCCAT
FOXP3	Forward:GTGGCCCGGATGTGAGAAG Reverse:GGAGCCCTTGTCGGATGATG
IL-21	Forward:GCACTTCCCACAGTGCCTAT Reverse:AGTTCTTCCCTTGATGGCCG
IL-10	Forward:TGCTCTTGCAAAACCAAACC Reverse:TTCACTCTGCTGAAGGCATC

1.5 ELISA检测细胞因子

血清中IL-21、IL-4、IL-10、TGF-β、IgA、IgE、IgG和IgM水平通过ELISA检测。使用酶标仪(Thermo Fisher 1510-02774)在450 nm波长下测定吸光度(*OD*值),并依据标准曲线计算样品浓度,所有实验严格按照相应试剂盒(上海江莱实业有限公司,中国上海)说明书操作,结果以试剂盒推荐单位表示(如ng/mL、pg/mL)。

1.6 流式细胞术

采集的肝素抗凝全血样本于4 h内处理,流式上样管中加入100 μLEDTA-全血,按照BD抗体说明书推荐浓度加入表面标志物抗体组合:FITC-CD4(货号:555346)、PE-CD25(货号:555432)、PE-Cy7 CD127(货号:560822)、APC-CXCR5(货号:558113)室温避光孵育15 min,加入红细胞裂解液室温避光裂解10 min,离心保留底层细胞,PBS洗涤重悬细胞沉淀,去上清再次离心,以PBS重悬细胞,并用流式细胞仪检测。Tfr定义为($CD4^+CXCR5^+CD25^+CD127^-$),Tfh定义为($CD4^+CXCR5^+CD25^-CD127^+$)[20-21]。

1.7 统计学分析

采用SPSS 26.0和GraphPad Prism 10.0进行统计分析及作图,计量资料以$\bar{x}\pm s$表示,淋巴细胞百分比及流式细胞结果比值等计数资料采用频数(%)表示,先对数据进行正态性检验,两组比较采用独立样本*t*检验,多组比较采用单因素方差分析、组间两两比较采用Tukey检验,采用Spearman相关分析Tfh、Tfr细胞比例与细胞因子、免疫球蛋白的相关性,以$P<0.05$为差异具有统计学意义。

2 结果

2.1 患者的基本资料

3组间性别构成差异无统计学意义($P=0.113\ 7$,表2),提示各组基线资料具有可比性。3组间红细胞沉降率(erythrocyte sedimentation rate,ESR)和C-反应蛋白(C-reactive protein,CRP)水平比较差异有统计学意义($P<0.005$),3组间淋巴细胞计数及淋巴细胞百分比差异均无统计学意义。影像学检查显示,病变主要累及L5/S1椎间隙及相邻椎体终板。MRI矢状位图像可见病变节段椎间盘信号异常,邻近终板完整性受损,局部骨质呈异常信号改变,提示该节段存在炎性水肿及感染性破坏(图1)。

2.2 Western blotting检测Tfh和Tfr细胞相关因子及软骨损伤标志物的表达

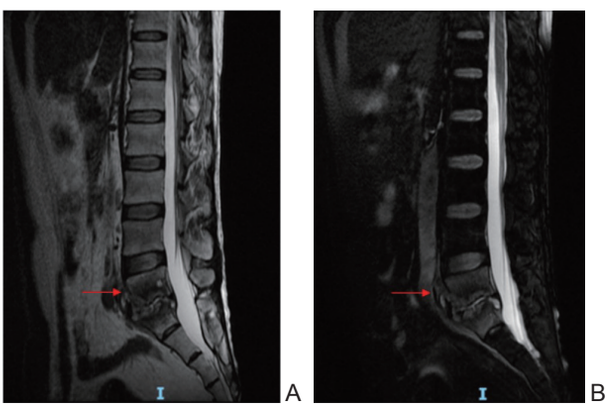
通过滑膜组织Western blotting检测发现,相较于对照组,慢性布鲁氏菌性骨关节炎患者IL-21表达降低($P<0.000\ 1$,图2B),COLⅡ表达下调($P=0.009\ 2$,图2D);而FOXP3($P=0.020\ 1$,图2C)、MMP-13($P=0.004\ 8$,

表2 基线及常规实验室检查结果

Table 2 Baseline data and results of routine laboratory tests

Items	Healthy Group (n=20)	Acute Phase Group (n=13)	Chronic Phase Group (n=21)	P
Gender (M/F)	8/12	10/3	13/8	0.113 7
ESR/(mm/h)	7.20±4.02	34.36±26.52	16.33±10.63	0.004 9
CRP/(mg/L)	1.13±0.79	41.54±28.46	12.53±9.79	<0.000 1
LC($\times10^9$/L)	1.99±0.46	2.00±0.99	1.80±0.66	0.284 7
Lym/%	32.06±8.09	37.19±11.77	37.47±10.08	0.399 5

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; LC: leukocyte count; Lym: lymphocyte percentage



A: T1-weighted image; B: T2-weighted image. The red arrows indicate abnormal bone changes in the L5 and S1 vertebrae accompanied by a paraspinal abscess

图1 布鲁氏菌性骨关节炎患者腰椎MRI

Figure 1 Lumbar spine MR images of a patient with Brucella osteoarthritis (BOA)

图2E)以及ADAMTS5(P=0.002 3,图2F)表达显著升高。上述结果显示在慢性布鲁氏菌性骨关节炎患者中存在着Tfh细胞与Tfr细胞的改变,并且伴有软骨损伤标志物的升高。

2.3 布鲁氏菌性骨关节炎患者和对照组外周血中Tfh和Tfr细胞变化特点

流式细胞术门控策略(图3A),急性期患者外周血中($CD4^+CXCR5^+CD25^-CD127^+$)Tfh比例显著高于对照组($P$=0.001 4,图3B),而($CD4^+CXCR5^+CD25^+CD127^-$)Tfr比例则低于对照组($P$=0.015 5,图3C)。在慢性期患者中,Tfh细胞比例下降,Tfr细胞比例显著升高(P<0.000 1,图B、C)。进一步计算Tfh/Tfr比值,发现急性期组显著高于对照组(P=0.020 4,图3D)。提示布氏杆菌骨性关节炎急性期以Tfh细胞比例升高为主,而慢性期Tfr细胞比例升高。

2.4 布鲁氏菌性骨关节炎患者PBMC中Tfh/Tfr细胞相关转录因子、细胞因子mRNA表达

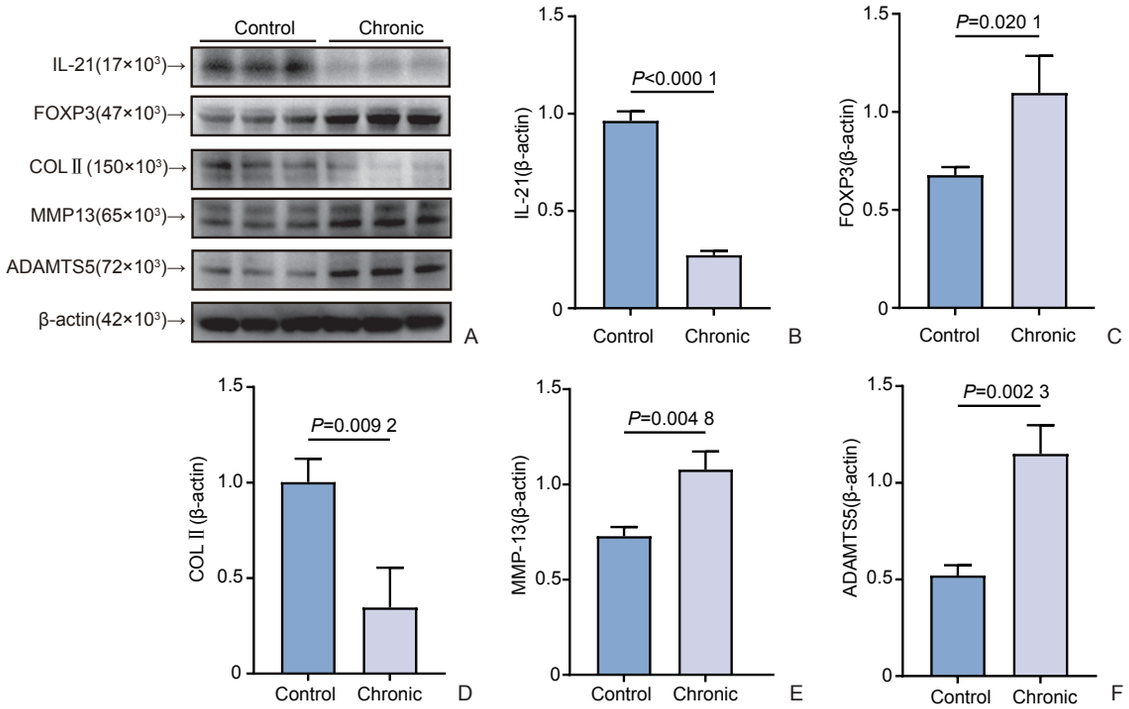
qRT-PCR结果显示,急性期患者PBMC中Tfh相关细胞因子*IL-21*和转录因子*BCL-6* mRNA表达上调,高于对照组(P=0.000 8,P<0.000 1,图4A、B);而在慢性期患者的PBMC中,Tfr的转录因子*FOXP3* mRNA和细胞因子*IL-10* mRNA较对照组均升高(P<0.000 1,图4C、D),以上结果提示急性期患者Tfh细胞转录因子BCL-6和细胞因子IL-21表达上调,在慢性期患者Tfr细胞转录因子FOXP3上调和细胞因子IL-10表达上调。

2.5 血清细胞因子与免疫球蛋白水平的变化

相较于对照组,急性期患者血清中IL-21、IL-4、IgG水平均显著升高(P<0.000 1,图5A、B、G),在慢性期三者呈下降趋势(P=0.029 0,P=0.001 4,P<0.000 1),慢性期患者TGF-β较急性期患者显著升高(P<0.000 1,图5D);IL-10、IgM在急性期及慢性期患者中均较对照组升高(P<0.000 1,图5C、F),IgA在急性期及慢性期患者中均较对照组升高(P=0.000 9,P=0.045 2,图5E),急、慢性期患者与对照组相比,IgE总体呈下降趋势(图5H)。提示布鲁氏菌性骨关节炎急慢性期存在不同的免疫应答特征,急性期以Tfh细胞相关的促炎因子高表达为主,慢性期则显示出以Tfr细胞相关的免疫调节因子升高的特点。

2.6 Tfh细胞与Tfr细胞数量变化与细胞因子、免疫球蛋白的相关性分析

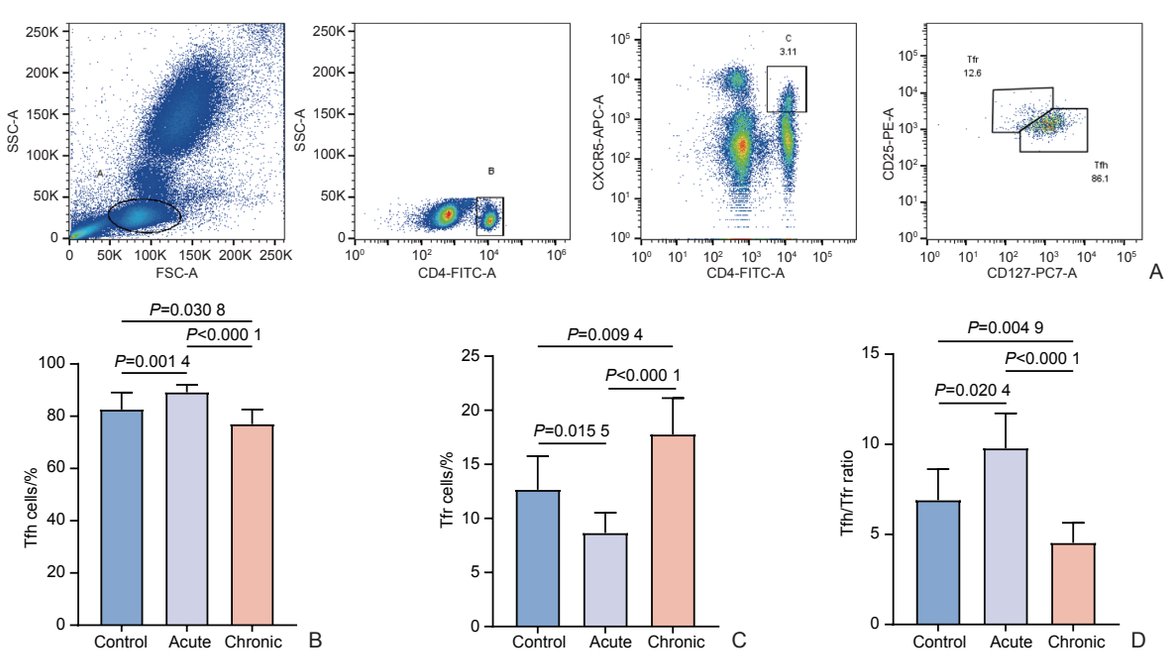
通过Spearman相关分析,急性期布鲁氏菌性骨关节炎患者中(图6A),Tfh细胞比例与IL-21(r=0.26)、IgA(r=0.34)、IgM(r=0.37)呈正相关,和IgG(r=0.18)也表现出一定程度的正相关趋势;而与Tfr细胞呈中等负相关(r=−0.54)。Tfr细胞比例与IL-4(r=−0.02)相关性较弱,但与IgA(r=−0.35)、IgE(r=−0.20)呈负相关趋势,IL-21与IgG(r=0.75)和IgM(r=0.58)呈强正相关,而TGF-β与IL-4(r=−0.73)呈显著负相关,提示在急性期,Tfh活化增强伴随Tfr功能下降。在慢性期患者中(图6B),各个指标相关性趋势较急性期减弱Tfh与IgM、IgA、IgG的相关性较急性期减弱,IL-21与IgG、IgM之间的相关性下降。



A: representative images of protein expression levels; B: decreased IL-21 expression in the chronic phase; C: increased FOXP3 expression in the chronic phase; D: reduced COL Ⅱ expression in the chronic phase; E: elevated MMP-13 expression in the chronic phase; F: elevated ADAMTS5 expression in the chronic phase

图2 Western blotting检测对照组和慢性布鲁氏菌性骨关节炎患者中Tfh细胞、Tfr细胞相关转录、细胞因子以及软骨损伤标志物

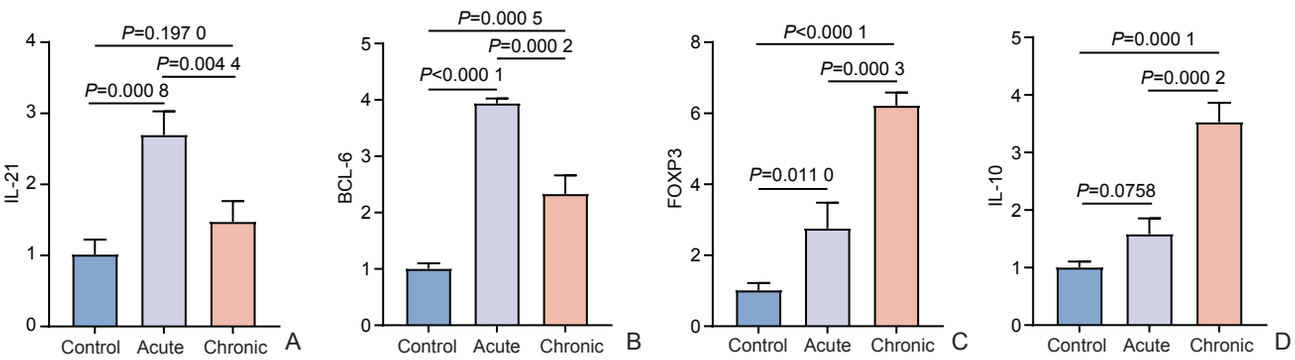
Figure 2 Expression of Tfh/Tfr-related transcription factors, cytokines, and cartilage injury markers in healthy controls and patients with chronic BOA by Western blotting



A: gating strategy for flow cytometric analysis; B: comparison of Tfh cell frequencies among the 3 groups; C: comparison of Tfr cell frequencies; D: comparison of Tfh/Tfr ratios

图3 对照组与急性期、慢性期布鲁氏菌性骨关节炎患者Tfh细胞百分比及Tfr细胞比例及比值比较

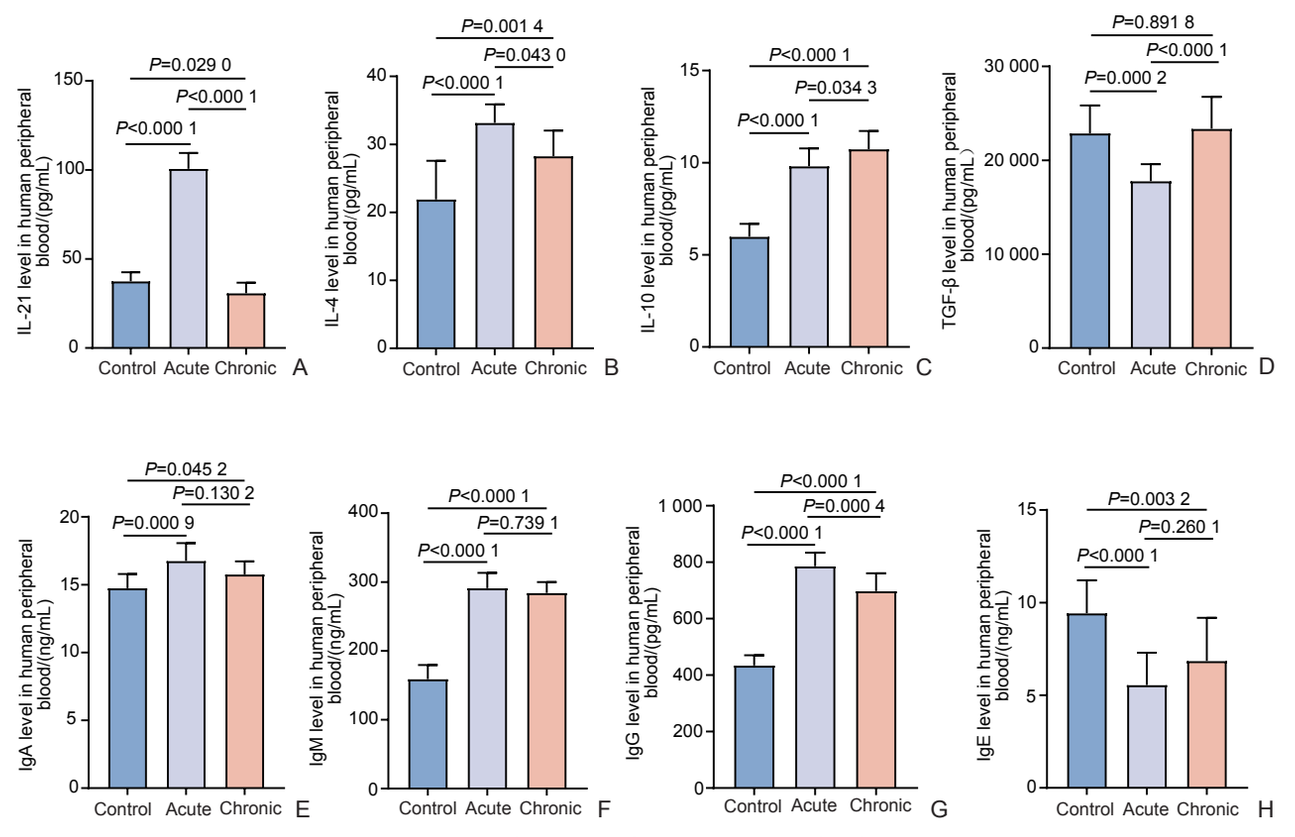
Figure 3 Comparison of Tfh and Tfr cell frequencies and their ratios among healthy controls and acute and chronic BOA patients



A: significantly increased BCL-6 expression in the acute phase; B: elevated IL-21 expression in the acute phase; C: elevated FOXP3 expression in the chronic phase; D: elevated IL-10 expression in the chronic phase

图4　PBMCs中Tfh细胞/Tfr细胞的转录因子、细胞因子mRNA表达

Figure 4　mRNA expression of Tfh/Tfr-related transcription factors and cytokines in peripheral blood mononuclear cells (PBMCs) from healthy controls and acute and chronic BOA patients



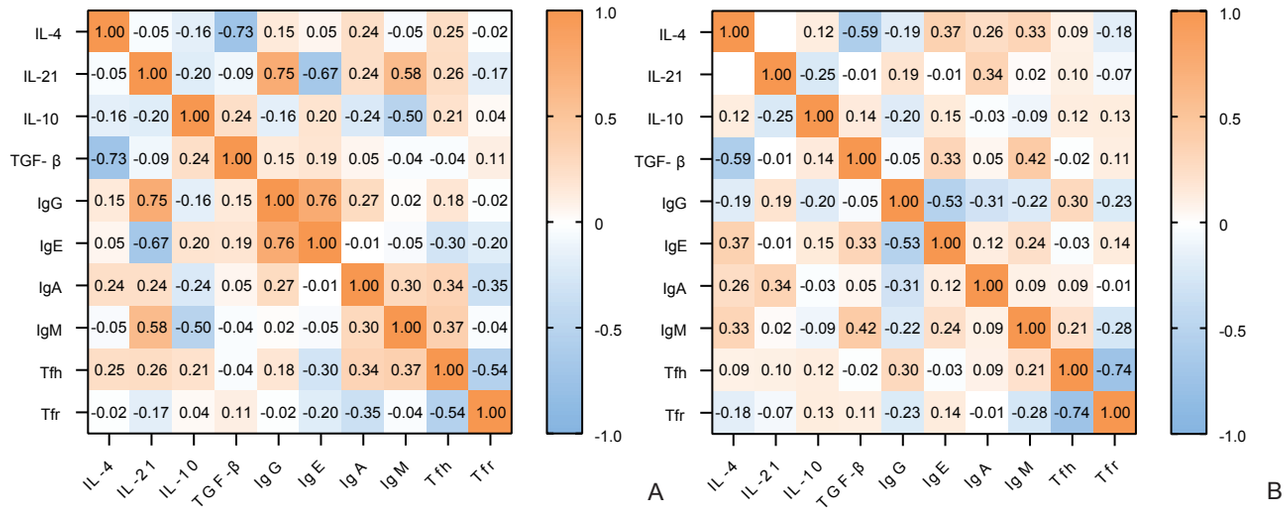
A: IL-21; B: IL-4; C: IL-10; D: TGF-β; E: IgA; F: IgM; G: IgG; H: IgE

图5　细胞因子和免疫球蛋白在各组中的变化

Figure 5　Serum levels of cytokines and immunoglobulins among healthy controls and acute and chronic BOA patients

3　讨论

布鲁氏菌性骨关节炎作为布鲁氏菌病的常见并发症,因早期症状不典型、疾病进展隐匿,临床确诊时多已处于晚期,且布鲁氏菌感染可导致持续性骨关节损伤,严重影响患者生活质量[1]。目前,布鲁氏菌性骨关节炎的免疫机制尚不清楚。Tfr细胞的组织定位依赖CXCR5介导的归巢,滑膜异位淋巴结构中的特异性分布是调控体液免疫的关键[22-23];且有研究表明直接检测类风湿关节炎(rheumatoid arthritis,RA)滑膜组织发现Tfh/Tfr相关分子在滑膜局部高表达,Tfh/Tfr细胞会选择性浸润这些炎症富集区域,且其分布密度与关



A: acute phase; B: chronic phase

图 6 Spearman 相关分析显示急性期与慢性期患者 Tfh、Tfr 细胞比例与细胞因子及免疫球蛋白之间的相关性分析

Figure 6 Spearman correlation analysis between Tfh/Tfr cell frequencies and cytokine or immunoglobulin levels in patients with acute and chronic BOA

节损伤程度相关[24-25]。本研究排除亚急性期病例,避免亚急与慢性期混淆导致的结果偏倚,通过检测布鲁氏菌性骨关节炎患者急性期、慢性期外周血中 Tfh/Tfr 细胞比例、相关转录因子、细胞因子及免疫球蛋白水平,慢性期患者滑膜组织中相关蛋白的表达。

3.1 布鲁氏菌性骨关节炎急慢性期患者存在 Tfh/Tfr 细胞的失衡

在急性期患者中 Tfh 细胞比例增高,而在慢性期患者则存在 Tfr 细胞比例增多的情况。说明在布鲁氏菌性骨关节炎的疾病进展中存在着 Tfh/Tfr 的改变,BCL-6 为 Tfh 细胞的关键转录因子,IL-21 为其分泌的主要细胞因子,FOXP3 为 Tfr 细胞的关键转录因子,IL-10 为其分泌的主要细胞因子,本研究结果显示在急性期患者中 BCL-6、IL-21 mRNA 表达水平升高,而慢性期的患者中 FOXP3、IL-10 mRNA 表达升高。在 ELISA 检测中,Tfh 相关细胞因子 IL-21 和 IL-4 在急性期显著升高,而在慢性期下降;Tfr 相关细胞因子 IL-10 和 TGF-β 在慢性期明显升高。在抗体水平方面,急性期 IgM 显著升高,慢性期则以 IgG 升高为主,提示免疫应答逐渐转向慢性感染状态。

3.2 慢性期 Tfh/Tfr 失衡与关节损伤存在潜在关联

本研究通过蛋白水平功能发现了在慢性期患者滑膜组织中软骨损伤标志物的变化:软骨基质成分 COL Ⅱ 表达显著下调,而软骨降解相关酶 MMP-13、ADAMTS5 表达显著升高,以及在慢性期患者中存在 FOXP3 增多,IL-21 减少,这一结果直接证实了慢性期患者存在明确的关节软骨损伤。这种软骨损伤与慢性期 Tfr 细胞升高、Tfh 细胞降低的失衡状态同步存在。

3.3 Tfh/Tfr 细胞在布鲁氏菌性骨关节炎病程进展中的潜在机制

本研究还发现 Tfh/Tfr 细胞比例与相关细胞因子、免疫球蛋白存在显著相关性:急性期 Tfh 细胞比例与 IL-21、IL-4、IgM、IgA 正相关,慢性 Tfr 细胞比例与 TGF-β、IgG 正相关,提示 Tfh 细胞以及 IL-21、IL-4 的升高升高可能反映性期体液免疫的活化状态,Tfr 细胞可能通过分泌 IL-10、TGF-β 参与免疫抑制微环境的形成。这一结果与 Tfh/Tfr 细胞的已知功能一致,Tfh 细胞可通过 IL-21 等细胞因子促进 B 细胞分化为浆细胞并产生抗体,Tfr 细胞可通过 IL-10、TGF-β 等细胞因子抑制 Tfh 细胞的功能,同时调控 B 细胞抗体类型转换。已有研究表明,布鲁氏菌病在病程的进展中免疫应答呈现出由急性期的以 Th1 反应为主导逐渐转向以 Th2 为主的免疫应答,导致疾病的慢性化[8, 26-27]。并且,在慢性布鲁氏菌病患者外周血中髓系抑制细胞频率升高,伴随 IL-10、TGF-β 水平的增加,也导致了免疫抑制[28]。此外,在 RA,Tfh 细胞过度活化会促进自身抗体产生加重关节损伤,而 Tfr 细胞功能不足则无法有效抑制免疫紊乱[29]。与自身免疫性关节病类似,布鲁氏菌感染也涉及免疫调节失衡。结合这些研究结果和本研究,推测在布鲁氏菌性骨关节炎中,随着疾病的发展,Tfh/Tfr 细胞的改变可能加重免疫抑制,从而促进病原体持续存在并加重疾病进展。

3.4 本研究的局限性及未来研究方向

综上所述,本研究显示布鲁氏菌性骨关节炎患者Tfh/Tfr细胞比例在急慢性期发生改变,急性期Tfh细胞及其相关因子BCL-6、IL-21、IL-4升高,慢性期Tfr细胞及其相关因子FOXP3、IL-10、TGF-β升高。慢性期滑膜中FOXP3升高、IL-21下降以及软骨降解标志物的改变,从组织层面为外周血Tfh/Tfr变化在慢性期所反映的免疫抑制状态提供了间接支持。其具体机制仍需未来进一步研究。同时,本研究也存在一定局限性:①急性期患者多采取保守治疗,未获取其滑膜组织,未能对比急慢性期关节局部Tfh/Tfr细胞浸润及分子表达差异,限制了对关节局部免疫微环境的深入解析;②本研究缺乏体外验证,后续将结合布鲁氏菌感染小鼠模型及体外共培养体系,干预Tfh/Tfr细胞功能,系统阐明其在炎症通路调控与疾病进展中的因果机制。

利益冲突 本文所有作者声明不存在利益冲突

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