研究进展

基于无菌动物研究肠道微生物与类风湿关节炎发病相关性

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【摘要】 肠道微生物与宿主的生长发育、免疫、代谢等方面均密切相关,但肠道菌群与宿主之间复杂的相互作用在很大程度上仍然是未知的。目前无菌动物已成为探索肠道微生物与宿主相互作用的重要工具,多项研究使用无菌动物模型探讨肠道菌群在宿主代谢、机体免疫系统的发育和成熟等方面的作用,其中包括肠道菌群在自身免疫性疾病发病及预后中的作用。研究发现,肠道菌群作为环境因素之一可能参与类风湿关节炎(rheumatoid arthritis, RA)发病,然而其因果关系未明。本文将对使用无菌动物探讨肠道微生物参与类风湿关节炎发病的相关性研究作一综述,为进一步深入研究肠道菌群在 RA 发病中的作用及机制研究提供理论依据。

【关键词】 无菌动物;肠道菌群;类风湿关节炎;免疫;动物模型
【中图分类号】Q95-33 【文献标识码】A 【文章编号】1005-4847(2017) 06-0667-05
Doi:10. 3969/j. issn. 1005 – 4847. 2017. 06. 015

Germ-free animal as a model to study the role of gut microbiota in the pathogenesis of rheumatoid arthritis

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【Abstract】 The relationship between intestinal microbiome and host growth and development, immunity, metabolism and other aspects is very close. But the complex interaction between intestinal microbiota and host is still largely unknown. At present, germ-free animal models have become an important tool for exploring the interaction between intestinal microbiome and host. Many studies used germ-free animal models to explore the role of gut microbiome in host metabolism, the development of the immune system, including the role of gut microbiome in the pathogenesis and prognosis of autoimmune diseases. It has been found that intestinal microbiome as one of the environmental factors may be involved in the pathogenesis of rheumatoid arthritis, but its causal relationship is unknown. This paper will review the correlation between the intestinal microbiota and pathogenesis of rheumatoid arthritis by using germ-free animal models, and provide a theoretical basis for further study of the role of intestinal microbiome in the pathogenesis of rheumatoid arthritis.

【Key words】 Germ-free animal; Gut microbiome; Rheumatoid arthritis; Immunity; Animal models
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类风湿关节炎是一种以慢性、对称性关节炎为主要表现的自身免疫性疾病,其基本病理改变为滑膜炎、血管翳的形成,并逐渐出现关节软骨和骨的破坏,最终可导致关节畸形和功能障碍。目前其病因

[基金项目]国家自然科学基金面上项目(No. 81072921)。
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和发病机制尚不明确,遗传背景和环境因素在 RA 发病中共同发挥作用。研究表明,肠道微生物作为重要的环境因素之一可能参与 RA 的发病,但其因果关系未明。

无菌动物是探讨肠道菌群与类风湿关节炎发病相关性研究良好的实验工具。无菌(germ-free, GF)动物是指在整个生命周期中不含任何微生物的动物,即不可检出一切细菌、病毒、真菌、原生动物和寄生虫[1],包括悉生(gnotobiotic)动物[2,3]。无菌动物模型的应用能够严格地排除其他微生物的干扰,为实验提供良好的同质性基础。无菌动物具有特定的免疫系统及免疫功能,利用无菌动物模型证实肠道微生物群对宿主免疫系统的发育和成熟起着至关重要的作用,同时亦可验证肠道菌群是否参与类风湿关节炎发病及其潜在机制,进一步为 RA 早期诊断和治疗提供新的方向。

1 基于无菌小鼠/大鼠探讨肠道菌群对宿主免疫系统发育和成熟的作用

无菌小鼠由于缺乏肠道微生物刺激故其免疫系统未发育完全。与普通小鼠相比,无菌小鼠具有更小的派伊尔氏小结(Peyer patches, PP),更少的肠系膜淋巴结(mesenteric lymph nodes, MLN)、肠相关淋巴组织及杯状细胞[4-6],小肠发育未完全,小肠表面积减少、绒毛细小、小肠固有层薄、细胞较少、上皮细胞更新较慢以及较大的盲肠与较薄的肠壁等特点。

肠道微生物对宿主免疫系统的发育起着至关重要的作用。与普通小鼠相比,GF 小鼠肠上皮细胞(intestinal epithelial cells, IECs)表面 Toll 样受体(Toll-like receptors, TLR)和主要组织相容性复合体(major histocompatibility complex, MHC)表达减少且分泌较少分泌型 IgA(SIgA)[7]。无菌小鼠较无特殊病原体(special pathogens free, SPF)小鼠相比,肠上皮淋巴细胞(intestinal epithelial lymphocytes, IEL)表达 αβ T 淋巴细胞受体(T lymphocyte receptor, TCR)减少以及 T 细胞免疫偏向 TH2 型免疫反应,研究发现将普通小鼠的肠道菌群移植至 GF 小鼠,αβTCR 表达和 Th1 亚群细胞数量增加,同时出现上皮内淋巴细胞中表型 $CD8\alpha\beta^+$ 细胞增多,$CD4^-CD8^-$ 相对减少[8-10]。相比与 SPF 小鼠,GF 小鼠的脾脏 $CD4^+$ T 细胞及脾内生发中心减少导致抗体产生减少[11],同时发现此种小鼠体内免疫球蛋白 IgA、IgG、IgM 的含量亦显著降低[4]。

无菌动物具有特定的免疫系统和免疫功能,肠道微生物促进无菌小鼠免疫系统的发育和成熟,无菌动物模型为进一步探讨肠道菌群是否参与 RA 发病及其潜在机制提供良好的实验工具。

2 利用无菌动物模型探讨肠道菌群与 RA 发病相关性

2.1 常用自发性关节炎动物模型

2.1.1 IL-1Ra$^{-/-}$ 自发性关节炎模型

IL-1 受体拮抗剂基因敲除小鼠(IL-1Ra$^{-/-}$)是一种由 T 细胞介导 IL-1 信号传导过表达而引发的自发性关节炎模型[12]。该模型的发生 T 细胞起关键作用,T 细胞缺乏者不发生关节炎。

2.1.2 K/BxN 自发性炎性关节炎模型

葡萄糖-6 磷酸异构酶(glucose-6 phosphate-isomerase, GPI)T 细胞受体转基因小鼠(K / BxN)是一种由自身反应性 T 细胞诱导浆细胞分泌大量抗 GPI 自身抗体而引发的自发性关节炎模型。该小鼠血清中含有大量高亲和力 IgG1 抗 GPI 抗体。

2.1.3 SKG 小鼠自发性关节炎模型

SKG 小鼠编码 ZAP-70(T 细胞中的信号转导分子)区域 SH2 的基因发生突变,异常 ZAP-70 改变了 T 细胞胸腺选择阈值,导致自身免疫性 T 细胞的阳性选择减少[13]。SKG 小鼠也是一种 Th17 细胞依赖性的炎性关节炎模型。

2.2 无菌动物实验证实肠道菌群参与关节炎发病

研究表明,IL-1Ra$^{-/-}$ 小鼠饲养在无菌条件下并不能自发关节炎,然而单定植乳酸菌、双歧杆菌后可快速发生关节炎,且关节炎的发生率和严重程度与常规饲养的小鼠相似[14]。无菌状态下的 IL-1Ra$^{-/-}$ 小鼠,在 CD 3 以及 TLR2 和 TLR4 刺激下,脾脏 Th17 细胞产生 IL-17 和 IL-1β 水平明显降低,可能的原因是激活 TLR2 / TLR4 信号通路引起 Th17/ Treg 免疫反应失衡[14]。(见表 1)

Wu 等[15]发现 K/BxN 无菌小鼠较普通小鼠关节炎症状明显减轻,同时伴有血清抗 GPI 抗体滴度降低,肠道固有层及脾脏 Th17 细胞的数量减少;然而在定植(segmented filamentous bacteria, SFB)分节丝状菌后,小鼠肠道固有层中 Th17 细胞数量增加,血清中自身抗体水平升高,小鼠亦出现明显关节炎症状。此外,K / BxN 小鼠定植 SFB 后,小肠派氏集合淋巴结内滤泡辅助性 T 细胞(follicular helper T cells, TFH)数量增多,且自身抗体产生增多[16]。(见表 1)

表 1 常用自发性关节炎动物模型肠道细菌定植实验

Tab. 1 Animal models of spontaneous arthritis known to be correlated with intestinal bacteria colonization

小鼠品系 Mouse strains	饲养条件 Environmental conditions	关节炎发病机制 Mechanism of involvement of arthritis	肠道细菌 Intestinal bacteria
K/BxN	GF:无关节炎 No arthritis SPF:关节炎 arthritis	大量抗 GPI 抗体及 TH17 细胞增多 Production of GPI-antibody Th17 cell expansion in the intestine	分节丝状菌 *SFB*
IL-1Ra$^{-/-}$	GF:无关节炎 No arthritis CV:关节炎 arthritis	激活 TLR 2、TLR 4 TH17 细胞增多及 Treg 细胞减少 Activation of TLR2 and TLR4 Th17 cells increased, Treg cells decreased	乳酸菌 *Lactobacillus* 双歧杆菌 *Bifidobacterium*
SKG	GF、SPF:无关节炎 No arthritis CV:关节炎 arthritis	自身反应性 T 细胞活化 真菌刺激下的先天免疫反应激活 Production of auto-reactive T cells Activation of innate immunity by fungi	普氏菌 *Prevotella-dominated microbiota*

注:GF:无菌动物;SPF:无特殊病原体动物;CV:普通动物;GPI:葡萄糖-6 磷酸异构酶;SFB:分节丝状菌;TLR:Toll 样受体;Treg cells:调节性 T 细胞;K/BxN:葡萄糖-6 磷酸异构酶(GPI)T 细胞受体转基因小鼠;IL-1Ra$^{-/-}$:IL-1 受体拮抗剂基因敲除小鼠。

Note. GF: germ-free; SPF: specific pathogen free; CV: Conventional; GPI: glucose-6-phosphate isomerase; SFB: segmented filamentous bacteria; TLR: Toll-like receptor; Treg cells: regulatory T cells; K/BxN: Glucose-6 phosphate-isomerase (GPI) T-cell receptor transgenic mice; IL-1Ra$^{-/-}$: IL-1 receptor antagonist gene knockout mice.

SKG 小鼠在普通饲养条件下,可出现自发性关节炎,同时伴有血清中高水平 IL-6(促进 Th17 分化),但在无菌条件下此种小鼠则不能发生关节炎,血清中亦检测不到 IL-6[17]。另有研究发现,将早期 RA 患者肠道菌群(具有高丰度普氏菌)移植或将 *P. copri* 单菌定植至 GF-SKG 小鼠后,可诱导小鼠出现严重关节炎[18]。(见表 1)

HLA-B27 转基因大鼠为自发脊柱关节炎和炎性肠病模型,然而在无菌环境中并不发生炎性肠病或外周关节疾病[19]。链球菌细胞壁(streptoccocal cell wall, SCW)诱导的大鼠关节炎模型研究表明,普通条件饲养的大鼠对关节炎抵抗,而无菌大鼠对关节炎易感,主要是由于打破 T 细胞免疫耐受[20]。在佐剂诱导的关节炎(adjuvant arthritis, AA)模型中,无菌饲养条件下的大鼠出现严重关节炎症,而普通饲养条件下仅出现中度关节炎症状且关节炎发病率较低。说明肠道细菌可调控免疫反应而抑制炎症。但具体机制仍不清楚[21]。研究发现,无菌 ROS 缺陷型 Ncf 1 突变小鼠,与野生型小鼠相比对胶原诱导性关节炎(collagen-induced arthritis model, CIA)易感性增加,其发生机制有待进一步研究[22]。

本课题组[23]研究发现 CIA 易感小鼠与 CIA 抵抗小鼠肠道菌群不同;分别移植 CIA 易感小鼠与 CIA 抵抗小鼠肠道菌群至无菌小鼠,前者能够使无菌小鼠 CIA 发病率增高、关节炎严重程度加重、血清 IL-17 含量及脾脏 Th17 细胞的比例增高($P < 0.05$),然而,其中作用机制并不清楚,有待进一步研究。

以上研究结果表明某些特定肠道微生物能够触发 T 细胞反应失衡,导致遗传易感性宿主发生自身免疫性关节炎。无菌动物的使用为研究肠道微生物在类风湿关节炎发病中的作用及机制研究提供了重要工具。

3 基于无菌小鼠/大鼠探讨肠道菌群参与 RA 发病的潜在机制

RA 是由一种由 T 淋巴细胞介导为主的自身免疫性疾病。Th17/Treg 细胞失衡在 RA 发病中起到重要作用且与疾病活动度等因素相关[24],肠道菌群在调节 Th17/Treg 细胞平衡、维持免疫耐受及免疫应答中必不可少[25]。研究发现 TLR2/TLR4 在 RA 发病中具有重要作用[26],其中 TLR4 尤为重要[27]。

肠道菌群可诱导初始 $CD4^+$ T 细胞的分化。GF 小鼠肠道固有层有较少的 $CD4^+$ T 细胞,肠道共生菌促进肠道 Th17 细胞及调节性 T 细胞(regulatory T cells, Treg)的分化[28]。研究发现 GF 小鼠免疫耐受丧失,表现为体内 $CD4^+$ $CD25^+$ Foxp3T 细胞减少、Th17 细胞增加及 IL-10 分泌减少[25,29];脆弱拟杆菌的胞外多糖 A(polysaccharide A, PSA)可以上调 CD39 表达以促进 Treg 细胞的分化,进而诱导白介素-10 (IL-10)产生[30,31]。GF 小鼠 TLR 表达减少,定植乳杆菌后 TLR4 的 mRNA 表达及蛋白的含量显著增加[32]。肠道共生菌诱导 T 细胞分化、免疫反应发生,同时在维持免疫稳态、免疫耐受中具有重要的作用[33]。

利用无菌动物模型探讨肠道菌群参与 RA 发病的潜在机制,为 RA 早期诊断和治疗提供新的方向。

4 无菌动物模型在探讨肠道微生物与类风湿关节炎发病相关性研究中的意义及不足

无菌动物模型已经广泛用于研究宿主-微生物相互作用的各个领域,包括神经消化内科、心脏病学、生殖生物学、脂质代谢、骨稳态和自身免疫性疾病等方面。近年来,越来越多的研究表明,将特定肠道细菌定植于无菌小鼠,可证实肠道菌群参与关节炎的发病,将人体肠道微生物群移植至 GF 小鼠,建立人源化的类风湿关节炎动物模型,为进一步深入探讨肠道微生物与类风湿关节炎发病之间的关系提供重要实验工具。

然而,肠道微生物与类风湿关节炎发病之间的因果关系还未明确,通过比较 GF 和 SPF 小鼠研究结果尚不能直接应用于人体疾病的诊断、治疗和预防,在无菌动物实验中观察到的肠道菌群与类风湿关节炎联系的具体机制尚未完全了解,故仍需要进一步深入研究。

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(下转第 675 页)

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[收稿日期] 2017 – 10 – 09

(上接第 670 页)

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[收稿日期] 2017 – 09 – 10