研究进展

无菌猪的研究进展

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【摘要】 无菌猪是采用现有微生物检测技术,检测不出活的微生物(包括细菌、真菌、寄生虫和病毒等)的一种悉生物,在现代畜牧生产和医学生物学研究中具有重要的科学价值。无菌猪最早用于畜牧生产的疫病净化,研究肠道菌群、动物疫病之间的关系。无菌猪和人在解剖、生理及遗传上具相似性、无微生物背景干扰,目前在医学生物学研究肠道菌群与生长发育与疾病发生发展等方面发挥着重要作用,也作为以肠道菌群为靶点的预防、诊断及治疗新技术研究的特殊动物模型。本文主要综述无菌猪的特性、研究进展及未来的发展方向。

【关键词】 无菌猪;动物模型;研究进展
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Advances in research on germ-free pig models

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【Abstract】 Germ-free (GF) pigs are a special and adaptable experimental animal model for biomedical studies and animal productions, which are negative for bacteria, viruses, yeast and fungi tested by current microbiological examination. GF pigs were initially used in cleanse of epidemic diseases in animal production and in a bid to study the relationship between animal disease and intestinal flora. Because of the similarities to humans in anatomy, physiology and hematology, and the clear microbiological background, GF pigs have been playing an important role in detecting the relationship between intestinal flora with growth and the development of diseases in medical biology, and also providing a special medical animal model for intestinal flora targeted prevention, diagnosis and treatment for update technology research in the clinic. This paper reviews the characteristics, advancements and research tendency of GF piglets.

【Key words】 Germ-free pigs; Animal Model; Research advances

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1959 年,Trexler 等[1]最早开始研究软塑料无菌隔离装置,1960 年,Landy 等[2]在此基础上构建无菌实验室,通过子宫切除术获得第一头无菌猪(germ-free pigs,GF pigs)。最早的 GF 猪培育是为了净化畜牧生产过程中的重大疫病,研究肠道菌群与动物疫病之间的关系,用于提高畜牧生产成绩。由于 GF 猪的微生物背景清晰,保证了科学研究不受背景微生物的干扰;同时猪在解剖、生理、遗传学、营养代谢等[3-5]方面与人极其相似,结合异种菌群移植技术,可用于肠道菌群与人类健康关系的研究[6]。近年来,随着肠道菌群移植(fecal microbiota transplant, FMT)技术和宏基因组测序技术的进步,以 GF 猪为

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基础,基于肠道菌群与动物生长发育,疾病发生等已成为研究热点。无菌猪在现代畜牧生产和医学、生物学研究中显示出重要的科学价值和广泛的应用前景。本文就 GF 猪的特性、研究进展及未来发展做一综述。

1 无菌猪的特性

GF 猪是通过无菌剖腹产手术、饲养于无菌隔离器,经现有检测手段,检测不出任何微生物的特殊动物[7]。由于缺乏肠道菌群,GF 动物与普通级动物(conventional animals, CV animals)在血液、生长发育等方面存在多种差异[8]。血常规指标中,GF 猪的 WBC、MON 与 MON%、EOS 与 EOS%、BAS 与 BAS%、HGB、HCT、RBC、PCT、PLT,血清生化指标 TP、ALB、GLO、BUN、UA、CHOL、TG、HDL 含量均显著低于 CV 猪,且肠内未发现肠系膜淋巴结[9]。另有研究显示:GF 猪的小肠重量,小肠壁厚度和小肠/体重系数也均要小于 CV 猪[10]。GF 猪有小肠绒毛更长、隐窝更浅和固有层多孔性减少等特性[11]。肠道菌群与消化酶关系密切,GF 猪肠道内的氨肽酶 N 和乳糖酶的活性高于 CV 猪,这可能与微生物引起的酶灭活密切相关[12]。在免疫系统发育方面,GF 猪血液中免疫球蛋白浓度偏低,次级免疫器官发育受阻,在空肠的黏膜固有层中,树突状细胞和 T 细胞的数量较少,小肠中前炎症细胞因子 IL-1β 和 IL-6 的表达量高[11]。

尽管 GF 猪与 CV 猪在胃肠道结构和免疫系统方面有许多差异,但 GF 猪仍是肠道菌群功能研究的理想动物模型。与无菌啮齿类动物相比,猪的生理和遗传特性与人更为接近,特别是猪的肠道系统和杂食特性[4],与人高度相似。此外猪体型大、方便开展复杂的外科手术,可频繁地采集血液或其他体液[13];GF 猪微生物背景清晰,无多余微生物干扰[11];猪的胎盘结构决定 GF 猪出生时无母源抗体的干扰[14, 15],作为研究环境因素对免疫影响的特殊模型。特定菌群接种 GF 猪构建的悉生猪(gnotobiotic, GN)模型具有高度转化性[16],因此 GF 猪和悉生猪广泛应用于轮状病毒、出血性大肠杆菌、艰难梭菌和志贺氏痢疾菌的研究,以及益生菌和疫苗效果评价。是目前研究肠道菌群与器官发育、肠道形态、生理性能及免疫调节之间关系的独特动物模型。

2 无菌猪目前的研究应用进展

2.1 畜牧生产方面

无菌猪最早用于重大疾病净化,以降低家畜生产的疾病风险[17]。产肠毒素性大肠杆菌病(ETEC)是幼龄小猪最易患的疾病[18],极易引起仔猪腹泻甚至导致死亡。Kohler 等利用 GF 猪成功鉴定造成仔猪腹泻的产肠毒素性大肠杆菌病致病亚型[18],Lin[19]利用断奶 GF 猪构建 ETEC 腹泻仔猪模型,验证了 K88 疫苗对 ETEC 病的防治效果。Yamada 等[20]利用 GF 猪模型观察猪传染性脑膜炎(PTV)的临床症状,发现其病理机制。为了验证猪新型冠状病毒的致病性,Jung 等用两株冠状病毒 OH-FD22 和 OH-FD100 接种 GF 猪,发现这两株病毒均会导致猪传染性腹泻(PEDV)[21]。随着研究的发展,GF 猪为模型用于研究饲料的转化和营养吸收,研发或评估新型饲料产品,益生素等饲料添加剂。

2.2 医学生物学研究

利用 GF 猪构建人源菌群猪模型,重现供体肠道微生物特性,已用于肠道微生物与人类健康及疾病发生的研究。Shen 等[22]构建人源菌群悉生猪模型研究短链低聚果糖的益生作用,向接种成年粪便悬液的悉生猪连续饲喂每千克体重 0.5 g 的低聚果糖 37 d 之后,能有效增加肠道双歧杆菌的数量。A 组轮状病毒(HRV)是全世界婴幼儿感染脱水性腹泻的主要原因[23],鉴于猪对 HRV 病毒的长期敏感性,Saif 等[24]利用 GF 猪构建人轮状病毒的猪模型,进行免疫动态分析。Hulst 等[25]利用微阵列分析轮状病毒进入肠道后的转录反应,发现鸟苷结合蛋白 2(GBP2)利于机体形成对抗轮状病毒等肠道性疾病的先天性屏障。Yang 等[16]进一步通过 GF 猪模型研究发现米糠可以促进益生菌增长、加强免疫屏障功能、激发先天性免疫,进而对抗 HRV 引起的腹泻。

2.3 免疫机制相关研究

无菌动物的肠道相关淋巴组织(GALT)缺乏,肠内的淋巴细胞数量、分泌性 IgA 浆细胞[26]、上皮内淋巴细胞[27]或固有层的 $CD4^+$ T 淋巴细胞均低于 CV 猪[28]。猪回肠黏膜由共生菌群的胞外信号控制形成大量 B 细胞,影响肠道免疫蛋白的形成[29]。Potockova 等[30]发现 B 细胞虽未在回肠内发育但却大量存在,表明菌群定植能促进回肠内 B 细胞的存在以及小肠淋巴细胞表达。共生菌对宿主免疫结构有深远影响,定植将导致固有层的 DC 细胞和 T 细胞广泛增加[31]。免疫细胞表面的一些受体能限制其杀伤自体组织,肿瘤组织同样能够激活这些受体,导致特异性免疫细胞无法对其进行识别与杀伤。此外,广泛认为是肠道微生物种群的差异影响肿瘤生长,微生物能够影响药物治疗效果[32, 33]。Sinkora 等[34]发现回肠派尔淋巴结(IPP)是小肠内 B 细胞

优先积累的区域,猜测 B 细胞的积累可能是为使细菌在无菌动物上定植,而不是通过积累细菌定植而促进 B 细胞增殖。Haverson 等[15]让 GF 猪分别定植血清型 O83 和 O86 的两株大肠杆菌菌株 20 d 后,研究两株大肠杆菌对免疫结构发育的影响,发现固有层的 DC 细胞、上皮组织和固有层的 T 细胞广泛增加,最早迁移的细胞是单核细胞,T 细胞迁移的速度稍慢。

2.4 食品科学及营养学

无菌动物模型适用于研究和评价功能性食品对肠道微生物群落代谢的影响,在功能性食物消化率、生物利用度等研究上提供更可靠的反馈[35]。研究表明,补充益生元或益生菌可有效改善肠道微生物紊乱造成的哮喘[36]、湿疹[37]、炎症性疾病[38]、坏死性小肠结肠炎[39]和肥胖[40]等。Veiga 等[41]研究发现益生菌产生大量的丁酸盐,减少沃氏嗜胆菌(*Bilophila wadsworthia*)的数量,对肠道健康有益。Liu 等[42]发现益生菌鼠李糖乳杆菌 GG 株(*Lactobacillus rhamnosus* strain GG, LGG)对于治疗轮状病毒引起的回肠上皮损伤有效。营养代谢方面,Thompson 等[43]发现新霉素可以在不破坏肠黏膜的情况下,增加 GF 猪粪便中的中性固醇类物质和脂肪酸的排泄。

2.5 疫苗及生物制剂

肠道菌群与免疫系统发育密切相关,因此无菌动物是进行疫苗和生物制剂评价的理想动物模型。Jeong 等[44]对宋内志贺菌(*Shigella sonnei*)活菌疫苗的毒性进行评估,发现初生 GF 猪对不同的口服志贺菌菌株毒性敏感。Foster [45]发现 GF 猪口服去毒的婴儿沙门氏菌(*Salmonella infantis*)突变体后,动物断奶前体重明显升高,且能阻止鼠伤寒沙门氏菌(*Salmonella typhimurium*)的感染。Wen 等[46]研究了 LGG 的不同剂量对接种 HRV 疫苗免疫效果的影响,发现 LGG 接种 6 次到 14 次,每次浓度 $10^6 \sim 10^9$ CFU/mL 之间时可诱导 HRV 特异性 IFN-γ 因子产生 T 细胞,而口服 LGG 也能阻止 HRV 感染引起的肠道微生物组成的改变[47]。此外,无菌动物也已用于治疗类风湿性关节炎[48]、抗乙肝新途径[49]等的研究。

3 未来的研究趋势

3.1 肠道菌群相关的健康研究

肠道微生物的数量是宿主细胞数量的十倍以上,所含基因是宿主基因的数百倍,被称为机体的最大免疫器官[50]。与宿主免疫、营养和发病机理等健康相关研究息息相关[51]。Man 等[52]利用化合物处理小鼠模拟结肠癌的发生,发现 AIM2 的缺失会导致小肠干细胞异常增殖,改变肠道菌群的组成,进而导致结肠癌的发生。克利夫兰诊所研究人员首次在肠道中发现一种在动物脂肪消化过程中产生的物质—氧化三甲胺(TMAO),它与动脉粥样硬化及心脏疾病的发生直接相关,靶向抑制 TMAO 作用肠道微生物可以帮助抑制某些心脏疾病的发生[53]。Suárez 等[54]研究发现,无菌小鼠机体中存在高水平的特定细胞因子,而抑制这种特殊信号或许会损伤抗生素诱导的皮下脂肪褐变过程、抑制肠道菌群剔除小鼠的葡萄糖表型,有助于开发治疗肥胖等疾病的新型疗法。还有众多疾病如:坏死性结肠炎(NEC)[39]、炎症性肠病(IBD)[38],心脏疾病[53]、糖尿病[55]、肥胖[56],帕金森[57]、儿童自闭症[58]、风湿性关节炎[59]和自身免疫疾病、癌症发病机理[60]等均与肠道菌群相关。因此,可预见未来 GF 猪和悉生猪模型将在动物健康养殖和人类健康研究中发挥重要作用。

3.2 人类器官的潜在供体

利用无菌猪作为自体器官体外培养工厂将是未来器官移植供体的重要研究方向。2010 年 Kobayashi 等[61]通过种间囊胚注射多能干细胞,在胰腺缺陷型小鼠体内形成胰腺器官,并能成功传代表达。2013 年 Matsunari 等[62]开始利用体细胞克隆技术形成的囊胚导入肾脏或胰腺缺陷型猪上,获得缺陷型器官并稳定传代。利用 GF 猪与上述器官缺陷猪技术相结合的技术,将病人组织、细胞或体外构建的组织工程器官导入缺陷型 GF 猪,经培养后移植,可有效解决器官移植供体不足的问题,并有效降低移植的免疫排斥[63]。

3.3 微生物遗传学

特殊微生物的存在或缺乏则对宿主的特征影响显著,而无菌动物则是研究微生物遗传学的独特模型。Bercik 等[64]研究发现携带更富冒险精神的小鼠肠道内的微生物后,原来“相对害羞”的小鼠表现出更具探索性的行为,说明肠道微生物能驱动着宿主行为,并表现出明显差异;Yano 等[65]发现一个由 20 种产芽孢细菌组成的微生物组可以增加无菌小鼠机体血清素的产生水平,而这种外周血清素的水平和多种疾病的发生有关,如肠易激综合征、心血管疾病及骨质疏松症;微生物可实现物种间的 DNA 转移,并入宿主基因组中,Turnbaugh 等[66]发现家庭成员有相似的肠道菌群结构,同卵比异卵双胞胎粪便菌群差异小,说明微生物可实现相互转移。利用 GF

猪进行微生物遗传学研究,将丰富传统遗传学的内容,有利于促进遗传学中一些基本理论的阐明。

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