



抵抗女性生殖道沙眼衣原体感染的免疫新策略

冯晓晶 唐玲丽*

中南大学湘雅二医院检验科 湖南 长沙 410011

摘要: 沙眼衣原体是引起沙眼和泌尿生殖道感染的主要病原体。据世界卫生组织 2015 年统计, 全球每年约有 1.3 亿沙眼衣原体感染新发病例。研究表明 CD4⁺Th1 型细胞免疫应答在抵抗沙眼衣原体感染中发挥着重要作用。因此, 研究者依照抗沙眼衣原体感染的免疫应答特点, 构建出许多候选疫苗, 但都没有成功地应用于临床。近年研究发现, 生殖道黏膜组织不仅存在体液免疫和细胞免疫, 还驻留着一些引人注目的免疫细胞, 提示增强黏膜免疫可作为预防沙眼衣原体感染的潜在途径, 是抵抗生殖道沙眼衣原体感染的免疫新策略。本文全面概述了黏膜免疫与女性生殖道沙眼衣原体感染的研究进展, 并为今后研制沙眼衣原体疫苗提供一些建议。

关键词: 沙眼衣原体, 疫苗, 黏膜免疫, 组织常驻记忆 T 细胞, 组织常驻 B 细胞, 调节 T 细胞, 天然淋巴细胞

A new immunization strategy against *Chlamydia trachomatis* infection in female genital tract

FENG Xiao-Jing TANG Ling-Li*

Department of Clinic Laboratory Medicine, Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China

Abstract: *Chlamydia trachomatis* is the main pathogen of trachoma and genitourinary infections. According to the World Health Organization (2015) statistics, there are about 130 million new cases of *Chlamydia trachomatis* infection every year in the world. Studies have shown that CD4⁺Th1 cellular immune response plays an important role in resistance to *Chlamydia trachomatis* infection. Therefore, based on the immune response characteristics of anti-*Chlamydia trachomatis* infection, the researchers constructed many candidate vaccines, but none of them were successfully applied in clinic. In recent years, it has been found that there are not only humoral immunity and cellular immunity, but also some noticeable immune cells resident in mucosa of reproductive tract, suggesting that enhancing mucosa immunity can be used as a potential way to prevent *Chlamydia trachomatis* infection. It is a new immunization strategy against *Chlamydia trachomatis* infection in reproductive tract. In this paper, the progress of mucosa immunization and *Chlamydia trachomatis* infection in female reproductive tract is reviewed, and some suggestions for the development of *Chlamydia trachomatis*

Foundation items: Hunan Provincial Health and Family Planning Commission Project (B2015-26); Natural Science Foundation of Hunan Province (2018JJ2559); National Natural Science Foundation of China (31670178)

*Corresponding author: Tel: 86-731-85292118; E-mail: linglitang@csu.edu.cn

Received: 10-03-2019; Accepted: 29-05-2019; Published online: 12-07-2019

基金项目: 湖南省卫生计生委计划项目(B2015-26); 湖南省自然科学基金(2018JJ2559); 国家自然科学基金(31670178)

*通信作者: Tel: 0731-85292118; E-mail: linglitang@csu.edu.cn

收稿日期: 2019-03-10; 接受日期: 2019-05-29; 网络首发日期: 2019-07-12

vaccine in the future are provided.

Keywords: *Chlamydia trachomatis*, Vaccine, Mucosal immunity, Tissue resident memory T cell, Tissue resident B cell, Regulatory T cell, Innate lymphoid cells

沙眼衣原体(*Chlamydia trachomatis*, Ct)是女性生殖道(female genital tract, FGT)感染性疾病最常见的性病病原体,专性寄生于黏膜上皮细胞^[1]。由于其感染的隐匿性,常常造成机体持续感染^[2],引起不孕不育等严重并发症^[3-4]。Ct感染还可增加艾滋病病毒(human immunodeficiency virus, HIV)感染率及宫颈癌和卵巢癌的发病率^[5-7],其所引起的生殖道感染已成为除艾滋病外耗资最大的性传播疾病。多年研究表明,接种疫苗是预防Ct感染最行之有效的措施^[2,8-9]。随着生物信息学、蛋白质组学和基因组学等快速发展,特别是Ct完整基因组序列的获得,大大地促进了Ct候选疫苗的研制^[10]。亚单位疫苗、DNA疫苗和树突状细胞疫苗等技术不断发展,给Ct疫苗的发展提供了很多思路,然而它们都没有成功地应用于临床^[2,11]。这可能与疫苗研制的着重点有关,研究者在构建疫苗时往往侧重于如何增强Ct抗原所诱导血液中的抗体和特异性T细胞反应强度,尽管血液中存在高浓度的抗体和特异性T细胞,它们却无法在感染早期快速清除Ct。

近期,研究者们陆续发现增强黏膜免疫更适合控制Ct感染^[9,12-13],增强黏膜免疫成为抵抗沙眼衣原体生殖道感染的新策略。生殖道黏膜免疫系统作为防御Ct感染的第一道防线,既存在体液免疫和细胞免疫,又驻留了一些特殊的免疫细胞^[14-16]。如果能增强其黏膜免疫效能,在感染早期提供强有力的保护,阻断Ct定殖,便能有效地防御Ct感染和传播。本文旨在阐述黏膜免疫在FGT Ct感染中的作用和在Ct疫苗研制中的应用现状,并归纳了一些增强Ct疫苗诱导黏膜免疫的策略。

1 Ct感染和FGT黏膜病理性免疫

Ct感染的主要黏膜病理改变过程为慢性炎症,造成输卵管损伤和瘢痕形成,引起并发症,其

中炎症细胞因子在疾病发生发展的过程中起了很大作用。Ct与靶细胞第一次接触时就会释放出大量的TNF- α 、IL-1、IL-8和GM-CSF等,从而造成子宫附件损伤^[17]。Agrawal等发现从Ct感染的FGT分离出的宫颈细胞经过体外培养后,会分泌大量的IL-1、IL-6、IL-8和IL-10,这些细胞因子预示着疾病恶化^[18]。TNF- α 由Ct特异性CD8⁺T细胞产生,会导致上生殖道病变^[19];IL-1能直接损伤女性输卵管上皮细胞^[20];高浓度的IFN- γ 或者IL-10与女性不孕相关^[21-22];IL-17与FGT炎症级联反应有关^[23]。Ct经典致病因子热休克蛋白60(heat-shock protein 60, HSP60)也是通过产生异常的细胞因子导致机体处于慢性炎症状态,引起盆腔炎和输卵管炎^[24-25]。HSP-60还能激活细胞毒性T淋巴细胞(cytotoxic T lymphocyte, CTL),从而破坏宿主细胞和诱导迟发型变态反应^[24]。

Ct为了确保自身生存和繁殖充分利用本身和宿主细胞的蛋白、脂类和核酸等物质在下生殖道上皮细胞内建立感染,然后通过上行感染途径感染至上生殖道,造成不孕不育等严重疾病。Ct原体(elementary body, EB)通过性交进入FGT(图1),首先黏附于由杯状细胞分泌的黏液组成的黏液层,黏液层含有多种可以产生吡啶的微生物,Ct(D-K型血清型)作为一种色氨酸营养缺陷体,能从生殖道正常微生物群中获取吡啶,再利用色氨酸合成酶TrpBA将吡啶转化为色氨酸,促进Ct感染存活^[26]。穿过黏液层后,Ct需要粘附于上皮细胞来诱导主动吞入EB,并在细胞内形成包涵体便于Ct发育和繁殖。大多研究表明Ct主要通过脂多糖(lipopolysaccharide, LPS)、主要外膜蛋白(major outer membrane protein, MOMP)和多形态膜蛋白(polymorphic membrane proteins, Pmps)等多种粘附蛋白与黏膜上皮细胞表面的硫酸乙酰肝素蛋白聚

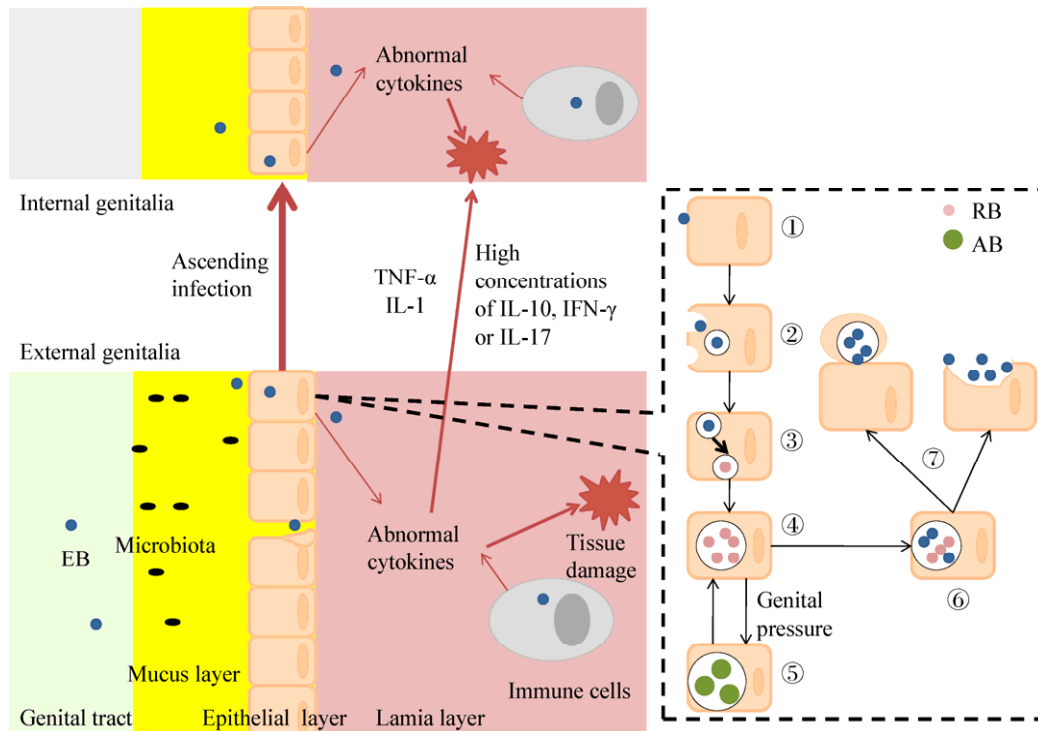


图1 女性生殖道 Ct 感染和致病途径

Figure 1 The infection and pathogenesis of Ct in female genital tract

注: Ct 通过性传播途径进入女性下生殖道, 首先必须与黏液层的多种抗菌物质和正常微生物群相互作用, 才能穿过黏液进入上皮层. 大部分 Ct 侵犯上皮细胞, 通过: ① EB 粘附与入侵宿主细胞; ② 形成包涵体; ③ EB 转化为 RB; ④ RB 复制; ⑤ RB 转化为 AB 或 AB 再活化; ⑥ RB 转化为子代 EB; ⑦ 子代 EB 释放出来继续感染邻近上皮细胞, 经上行感染造成生殖道病变, 少部分可以通过上皮层的裂缝或者释放出的子代 Ct 进入固有层, 入侵免疫细胞, 诱导异常炎症因子产生, 引发损伤.

Note: Ct has to interact with a variety of antimicrobial substances and normal microbiota in the mucus layer before it can pass through the mucus and enter the epidermis when it enters the female lower reproductive tract through sexual transmission. Then Ct invades epithelial cells and finishes its life cycle (① Extracellular EB binds to and invades host cells; ② Establishing an intracellular niche; ③ Transition of elementary to reticulate body; ④ Replication; ⑤ Transition of reticulate body to aberrant body and reactivation; ⑥ Transition of reticulate to elementary body; ⑦ Exiting the host cell), and their offspring continue to infect adjacent epithelial cells, make genital tract lesions by ascending infection; a few can enter lamina propria by cracks in the epidermis or released from the host cells, then they mainly infect immune cells and induce abnormal inflammatory cytokines to cause damage.

糖、甘露糖受体和表皮生长因子受体结合^[27-28], 再利用 III 型分泌系统(the type III secretion system, T3SS)效应蛋白诱导细胞骨架重排, 促进 Ct 入侵并形成包涵体^[27,29]。为了保持靶细胞的完整性以完成 Ct 在细胞内的发育周期, Ct 通过抑制 caspase8 活化等途径来阻止宿主细胞发生凋亡, 并以包涵体形式再通过表面蛋白和转运体从宿主细胞高尔基体、线粒体和溶酶体中获取营养物质和能量, 促进包涵体内的网状体(reticulate body, RB)复制^[30]。Ct 在

生殖道压力(抗生素的使用, 铁缺乏或者合并感染等)的作用下会进入隐匿感染状态, RBs 改变 HSP60、外膜蛋白和 LPS 的表达量, 转化为异型小体(aberrant body, AB)以逃避宿主抗感染免疫反应, 引发 Ct 持续感染^[25,31]; 当外界压力减弱或者消失时, ABs 又可以恢复为活跃状态的 RBs, RBs 再转化为感染型 EBs 并从靶细胞中释放, 这种释放机制与 Ct 蛋白酶样活性因子(chlamydial protease-like activity factor, CPAF)等的酶解作用有关^[32]。释放出的子

代 EB 逐渐移行感染至子宫和输卵管上皮细胞, 引起盆腔炎和输卵管损伤, 还能进入黏膜下层侵犯免疫细胞, 干扰细胞信号转导途径, 释放出异常炎症因子, 引起 FGT 异常的炎症反应, 最终导致不孕不育等疾病^[3]。

2 Ct 感染和 FGT 黏膜保护性免疫

Ct 感染 FGT 后激活黏膜免疫系统, 刺激弥散免疫细胞和黏膜相关淋巴组织产生免疫分子(以 IFN- γ 为主)来抵抗 Ct 感染(图 2)。女性上/下生殖道黏膜分别由柱状上皮和复层鳞状上皮细胞覆盖, 在先天性和适应性免疫反应中具有不同的保护机

制^[15]。Ct 进入 FGT 后, 首先遇到的屏障为黏液层, FGT 黏液的厚度、pH 值和成分会随着月经周期的不同而变化, 这些变化也会影响 Ct 的易感性^[33]。穿过黏液层后, Ct 可以利用自身的外膜蛋白、肽聚糖和质粒编码基因分别与上皮细胞的 Toll 样受体、核苷酸结合寡聚化结构域样受体和胞质传感器 STING 结合, 使细胞发生主动免疫反应^[27]; 另外, Ct 也可以通过上皮屏障的裂口进入黏膜下层, 并利用病原体相关分子模式与 FGT 黏膜固有免疫细胞模式识别受体结合, 释放出多种细胞因子, 激活宿主体液和细胞免疫^[20]。值得注意的是, 中性粒

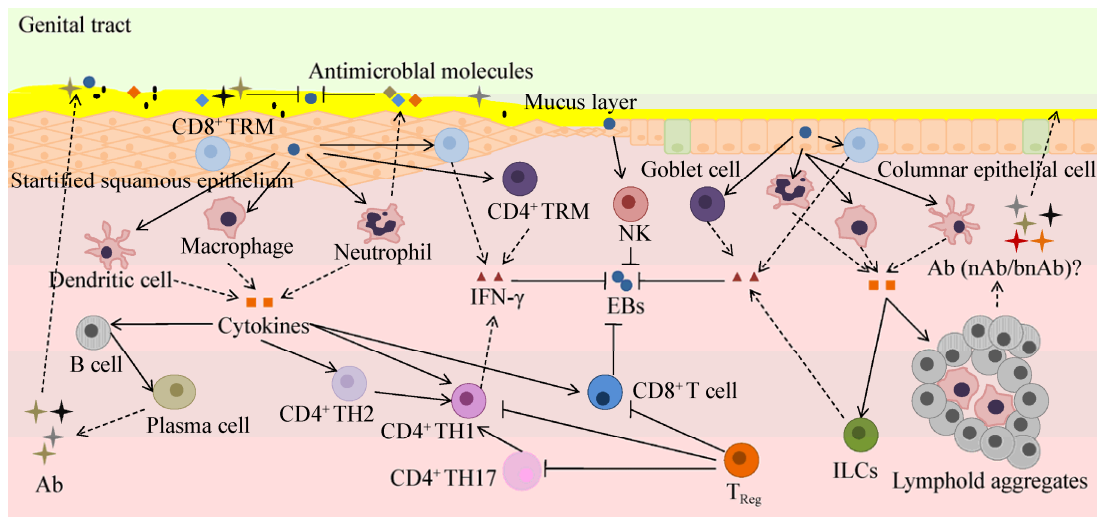


图 2 女性生殖道抗 Ct 感染的黏膜免疫保护机制

Figure 2 The mucosal immunity against Ct infection in female genital tract

注: 生殖道黏液与阴道微生物菌群(主要为乳酸杆菌)维持着生殖道黏膜的稳态。当 EB 通过性交进入生殖道, 与黏液层阴道微生物菌群、多种抗菌微粒和抗体相互作用后入侵上皮层, 感染上皮细胞, 释放出感染信号, 固有免疫细胞(中性粒细胞、树突状细胞和单核-巨噬细胞等)接收到信号, 释放出细胞因子, 招募固有层免疫细胞参与反应。生殖道黏膜 CD4⁺ 和 CD8⁺T 细胞通过各种免疫保护机制抵抗 Ct 感染; B 细胞分化为浆细胞, 浆细胞通过分泌 IgG、IgA 和 IgM 抵抗 Ct 感染。另外, FGT 还驻留一些特殊的免疫细胞, CD4⁺ 和 CD8⁺TRM 细胞在 Ct 入侵的第一时间可快速产生 IFN- γ 清除 Ct; ILCs 也能释放细胞因子增强子宫防御功能; 巨噬细胞和 B 细胞参与淋巴滤泡的形成, 可能参与抗原呈递并分泌多种细胞因子和抗体(nAb/bnAb); 而 T_{Reg} 主要负性调节其他免疫细胞, 维持免疫系统的稳态。

Note: The genital tract mucosa maintains homeostasis with the vaginal microbiome, comprising primarily *Lactobacillus*. When the EB enters into the reproductive tract through sexual intercourse, and fights with antibacterial particles, antibodies and vaginal mucous layer microbial flora, then they infect epithelial cells and release signals to make innate immune cells (neutrophils, dendritic cells and monocyte-macrophages) to release cytokines that recruiting lamina propria immune cells. In female genital tract mucosa, CD4⁺ and CD8⁺T cells provide various immune protections against Ct infection; B cells differentiate into plasma cells, which resist Ct by secreting IgG, IgA and IgM. More strikingly, a number of special immune cells reside in female genital tract mucosa, CD4⁺ and CD8⁺TRM can rapidly generate IFN- γ once Ct invades; ILCs also release cytokines that enhance uterine defense. Macrophages and B cells are involved in the formation of lymphoid follicles and may be involved in antigen presentation and secrete a variety of cytokines and antibodies (nAb/bnAb); however T_{Reg} cells mainly negatively regulate others and maintain the homeostasis of the immune system.

细胞还能在机体初次感染 12 h 内接收到上皮细胞发出的感染信号^[34], 通过变形从外周血大规模地迁移至感染部位, 随后直接吞噬病原体、形成中性粒细胞胞外诱捕网再释放富含蛋白酶的颗粒和活性氧物质^[35]或者辅助抗体发挥中和作用^[36]来清除 Ct。体液免疫主要是 B 细胞产生的 IgG 和分泌型 IgA (secretory IgA, sIgA) 主导的, 这些抗体也能被转运到感染部位并停留于黏液层^[20]。同时 CD4⁺Th1 细胞会进入防御状态, 释放以 IFN- γ 为主的细胞因子, 抵御 Ct 感染^[21]; 其他的活化 CD4⁺Th2 和 Th17 细胞能够产生 IL-13 和 IL-17, 辅助 Th1 细胞抵抗 Ct^[31]。CD8⁺T 细胞活化后形成的 CTL 能够诱导 Ct 感染细胞发生细胞凋亡, 阻碍 Ct 生长^[20]。最新研究显示, FGT 黏膜组织还驻留着一些特殊的免疫细胞, 它们在抵抗 Ct 感染时具有很大的潜能, 能在感染早期发挥有效的灭菌免疫, 逐渐成为衣原体的研究热点^[29,35,37-38]。

3 黏膜常驻免疫细胞介导的免疫反应

3.1 组织常驻记忆 T 细胞

组织常驻记忆 T 细胞(tissue resident memory T cell, TRM)是近期发现的一种 T 细胞谱系, 驻留于局部组织, 不参与血液和淋巴循环^[39], 对局部 Ct 感染具有高效的清除作用。TRM 细胞来源于血液中的效应 T 细胞, 通过调节 KLF2、鞘氨醇-1-磷酸酯受体 1、CD69 和 CCR7 的表达长期驻留于组织中^[40]。FGT CD8⁺TRM 细胞表达 CD103, 与上皮细胞表达的 E 型钙黏素结合驻留于上皮层, 而 CD4⁺TRM 细胞不表达 CD103, 停留在阴道固有层^[39], 由于其优越的地理位置, TRM 细胞能够第一时间接触入侵的 Ct 并清除感染。Johnson 等^[16]对 TRM 细胞在 Ct 感染中的作用进行了系统性的总结, 认为 TRM 细胞在 Ct 感染的过程中能提供快速有效的保护, 但似乎只能通过黏膜免疫接种途径和黏膜佐剂才能诱导 FGT 产生 TRM 细胞。Stary 等^[12]研制出一种新型疫苗——紫外线灭活 Ct 结合电荷交换粒子合成佐剂, 这种疫苗能诱导出系统记

忆性 T 细胞, 但仅通过黏膜免疫接种时才产生 TRM 细胞, TRM 细胞在无 Ct 抗原存在的情况下至少能存活 6 个月。当 Ct 再次感染机体时, 上皮层 CD8⁺TRM 以最快的速度合成并分泌颗粒酶 B 和 IFN- γ , 固有层 CD4⁺TRM 细胞快速产生 IFN- γ 和 IL-13^[15,38], 在最短时间内破坏感染细胞并招募其他免疫细胞抵抗 Ct 感染。

3.2 组织常驻 B 细胞

黏膜组织体液免疫主要由 B 细胞产生的 IgG 和 sIgA 组成, 而且受激素的影响, FGT B 细胞分泌的 IgG 含量大于 sIgA^[15], sIgA 参与抗体依赖细胞介导的吞噬作用或者细胞毒性作用, IgG 通过激活补体或者与巨噬细胞和自然杀伤细胞(natural killer cell, NK)表达的 FcRs 结合诱导机体免疫反应消灭病原体^[14]。近期研究表明, FGT 黏膜组织存在一种与循环 B 细胞功能相似但能常驻于组织的 B 细胞, 能在病原体感染早期提供比循环 B 细胞更有效的保护^[16]。组织常驻 B 细胞似乎存在于 Ct 感染人类生殖道所诱导的具有选择性记忆淋巴细胞滤泡中^[16], 其寿命长达 10 个月^[14], 能较为长期地控制继发性 Ct 感染。组织常驻 B 细胞分泌的 IgG 和 sIgA 还能分别与 FGT 上皮细胞表达的新生儿 Fc 受体和多聚免疫球蛋白受体结合而聚集在上皮层或者转运至黏液层, 能够抵抗高浓度病原体入侵黏膜组织^[41]。另外, 由 B 细胞分泌的 nAbs 能在 Ct 入侵靶细胞之前破坏 Ct 抗原, 阻止该病原体粘附靶细胞受体^[42], 并在组织受损伤轻微的情况下激活补体清除 Ct, 为机体提供杀菌效应^[2,14]。Bulir 等^[43]使用 T3SS 抗原通过滴鼻免疫接种小鼠, 能在血清中产生高浓度中和抗体(neutralizing antibody, nAb), 尽管只有部分血清 nAbs 被运送到 FGT 中, 但仍能有效地清除 CM 感染和预防输卵管病变。Bøje 等^[44]应用多亚单位疫苗通过阴道接种哥根廷小型猪, 这种新型疫苗诱导机体产生 TH1 细胞和中和抗体, 抵抗 Ct 血清型 D 和 F 并建立长期保护。近几年还出现一种广谱中和抗体(broadly neutralizing

antibody, bnAb), 这种抗体几乎能消灭每一个病原体变种^[45-46]。因此, 如果能诱导组织常驻 B 细胞的合成和分泌 nAbs/bnAb, 便能在第一时间抵抗不同血清型 Ct 感染。

3.3 调节 T 细胞

调节 T 细胞(regulatory T, T_{Reg})是一类负性调节机体免疫反应的 T 细胞亚群, 常驻于黏膜组织, 可分为自然 T_{Reg} 和适应性 T_{Reg}, 能与靶细胞直接接触或者分泌 TGF- β 和 IL-10 来维持机体免疫系统的稳态^[47]。Marks 等研究通过比较已感染和未感染 Ct 的 C57BL/6 小鼠上下生殖道中 T_{Reg} 分子标记物 FoxP3 mRNA 的表达量, 结果显示上生殖道 FoxP3 mRNA 的增长量是下生殖道的 6 倍, 并且在感染 Ct 后的第 10 天其表达量就开始增长^[48]。这个结果与我们之前的研究相吻合, 由于具有免疫抑制功能的 T_{Reg} 主要存在于上生殖道, 我们发现只有通过下生殖道接种提供的保护性免疫才能完全清除 Ct^[49]。然而, 最新研究表明 CM 感染颠覆了 CD4⁺CD25⁺ FoxP3⁺T_{Reg} 的免疫抑制功能, 其分泌的 TGF- β 能促进 Th17 细胞分化和成熟, 而对 Th1 细胞丧失了调控能力, 机体内 T_{Reg} 的含量与 CM 感染诱导的输卵管病变程度成正相关^[50]。目前关于 T_{Reg} 与 FGT 衣原体感染的研究仍处于初级阶段, 其具体的抗衣原体感染的免疫调节机制还有待研究。

3.4 天然淋巴细胞

天然淋巴细胞(innate lymphoid cells, ILCs)常驻于黏膜组织中^[15], 起源于普通淋巴祖细胞, 但缺乏 T、B 细胞抗原受体, 在病原体的刺激下可以快速活化参与免疫应答^[37]。ILCs 分为杀伤性和辅助性两类细胞, 杀伤性 ILCs 主要为 NK 细胞, 可以释放颗粒酶、穿孔素和 IFN- γ 对 Ct 感染发挥免疫抵抗作用^[51]; 辅助性 ILCs 根据其特征性的表面标志分为 ILC1、ILC2 和 ILC3, 其中 ILC1 和 ILC3 均可以分泌 IFN- γ ^[52], 另外 ILC3 还能表达 IL-22, IL-22 可以协助上皮细胞产生抗菌肽^[53], ILC2 与

Th2 相似, 主要分泌 IL-5 和 IL-13^[52], 这些细胞因子或肽类在病原体感染初期就会被释放出来参与免疫应答^[37]。然而当 ILCs 异常时也会加重机体的炎症反应, 引发特异性皮炎、炎症性肠病和牛皮癣等^[54]。虽然目前关于 ILCs 与衣原体感染的研究很少, 但是 ILCs 在黏膜组织中抗病原体感染的免疫反应和修复组织损伤的作用不容忽视。

4 阻碍 Ct 疫苗研制的因素和潜在的解决方案

Ct 独特的双相发育周期和拥有多种血清型是其疫苗研发的主要阻碍^[20,25], 在其生长繁殖过程中, Ct 可以在 EB、RB 和 AB 三种存在形式间相互转化; 另外 Ct 有 15 种血清型, 不同血清型其致病机制存在差异。因此, Ct 利用其不同状态下的存在形式和血清型进化出多种机制以逃避宿主的免疫清除作用, 从而制约了人们对 Ct 疫苗的研制。

自 1950 年以来, Ct 疫苗的研制在候选抗原、动物模型、免疫途径、佐剂和传送系统这些方面都在不断地创新, 但都未能达到理想的结果^[8,21]。然而 Ct 黏膜免疫疫苗的研制和发展充满前景, 它们主要是通过黏膜免疫接种途径和黏膜佐剂激活机体黏膜免疫和系统免疫来清除和控制 Ct 感染; 再加上共同黏膜免疫系统的存在, 使黏膜免疫活化的淋巴细胞归巢到黏膜效应部位, 打破了接种方式的局限性^[55-57]。Wang 等^[58]研究表明胃肠道 CM 是非致病的菌株, 通过口服接种胃肠道 CM 疫苗能在生殖道感染部位引起 CD4⁺T 细胞和 B 细胞的协同作用, 而且不会产生任何有意义的病理损害。类似的研究也表明胃肠道 CM 能在气道中诱导以 Th1 细胞为主的黏膜免疫效应, 预防同类型衣原体的再感染^[55]。Olsen 等^[59]研究表明滴鼻免疫接种 Hirep1 疫苗促使 Hirep1 在生殖道产生中和抗体和 CD4⁺T 细胞。以上研究均表明胃肠道和鼻内免疫接种衣原体可使生殖道产生对衣原体的免疫保护作用。

从近几年的文献中我们归纳出 2 条针对解决 Ct 疫苗发展阻碍的方案(图 3)。如果能够利用现代

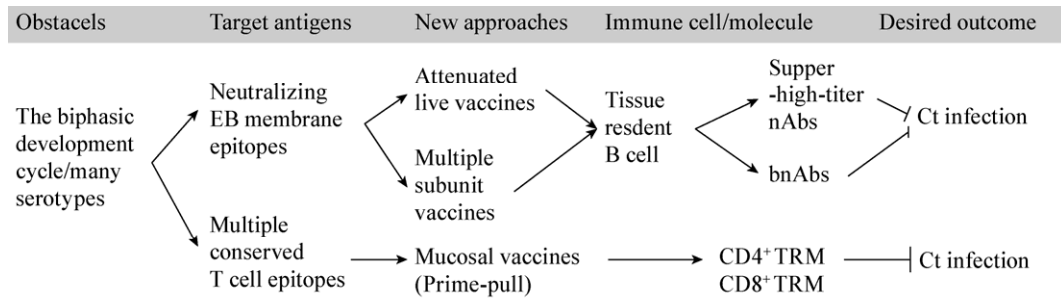


图3 Ct疫苗发展的阻碍和潜在的策略

Figure 3 The obstacles of development and potential strategies in Ct vaccine

生物学技术和逆向疫苗学寻找到中和 EB 外膜蛋白的抗原表位,设计出减毒活疫苗或多亚单位疫苗,结合黏膜佐剂,通过易于接受的黏膜免疫接种途径在局部黏膜组织分别产生超高滴度的 nAbs 和 bnAbs,抵抗任何 Ct 血清型的感染;还可以利用多个保守的 T 细胞表位,通过“Prime-pull”策略能使黏膜免疫疫苗在局部黏膜组织诱导 CD4⁺和 CD8⁺TRM,产生强大的灭菌免疫,预防 Ct 感染。因此,一个真正可用于临床的疫苗需要在黏膜组织中诱导高浓度的 TRM 细胞、nAbs 和 bnAbs,切断 Ct 入侵途径,保护其他细胞或器官免受 Ct 感染。

5 展望

FGT 黏膜免疫系统由黏膜上皮和广泛存在于固有层的免疫细胞组成,是宿主接触并摄取抗原和最初产生免疫应答的场所,其免疫状态是决定 Ct 是否成功感染宿主细胞的关键。在未来,如果能够将 Ct 疫苗研制的侧重点转移至黏膜免疫,设计出新型黏膜免疫疫苗,诱导机体在生殖道产生大量 TRM 细胞和分泌 nAbs/bnAbs 的组织常驻 B 细胞,从而增强生殖道黏膜组织的免疫防御功能,阻碍 Ct 定殖,有效地抵抗 Ct 感染。另外,随着单细胞 RNA 测序、质谱流式细胞术和逆向疫苗学的发展,将会大大促进我们对生殖道黏膜组织免疫细胞的理解,为寻找理想的 Ct 疫苗靶抗原奠定基础。

REFERENCES

- [1] WHO. Progress report of the implementation of the global strategy for prevention and control of sexually transmitted infections: 2006–2015[R]. World Health Organization, 2015
- [2] Hafner LM, Timms P. Development of a *Chlamydia trachomatis* vaccine for urogenital infections: novel tools and new strategies point to bright future prospects[J]. Expert Review of Vaccines, 2018, 17(1): 57-69
- [3] Sahu R, Verma R, Dixit S, et al. Future of human *Chlamydia* vaccine: potential of self-adjuncting biodegradable nanoparticles as safe vaccine delivery vehicles[J]. Expert Review of Vaccines, 2018, 17(3): 217-227
- [4] Mackern-Oberti JP, Motrich RD, Damiani MT, et al. Male genital tract immune response against *Chlamydia trachomatis* infection[J]. Reproduction, 2017, 154(4): R99-R110
- [5] Travassos AG, Xavier-Souza E, Netto E, et al. Anogenital infection by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in HIV-infected men and women in Salvador, Brazil[J]. The Brazilian Journal of Infectious Diseases, 2016, 20(6): 569-575
- [6] Trabert B, Waterboer T, Idahl A, et al. Antibodies against *Chlamydia trachomatis* and ovarian cancer risk in two independent populations[J]. Journal of the National Cancer Institute, 2019, 111(2): 129-136
- [7] Di Pietro M, Filardo S, Porpora MG, et al. HPV/*Chlamydia trachomatis* co-infection: metagenomic analysis of cervical microbiota in asymptomatic women[J]. New Microbiologica, 2018, 41(1): 34-41
- [8] Fan HZ, Zhong GM. 2017: beginning of a new era for *Chlamydia* research in China and the rest of the world[J]. Microbes and Infection, 2018, 20(1): 5-8
- [9] Stambach MN. Action needed on *Chlamydia* vaccines[J]. Trends in Microbiology, 2018, 26(8): 639-640
- [10] Nunes A, Gomes JP, Karunakaran KP, et al. Bioinformatic analysis of *Chlamydia trachomatis* polymorphic membrane proteins PmpE, PmpF, PmpG and PmpH as potential vaccine antigens[J]. PLoS One, 2015, 10(7): e0131695
- [11] Gottlieb SL, Johnston C. Future prospects for new vaccines against sexually transmitted infections[J]. Current Opinion in

- Infectious Diseases, 2017, 30(1): 77-86
- [12] Stary G, Olive A, Radovicmoreno AF, et al. A mucosal vaccine against *Chlamydia trachomatis* generates two waves of protective memory T cells[J]. Science, 2015, 348(6241): aaa8205
- [13] Agrawal T, Vats V, Salhan S, et al. The mucosal immune response to *Chlamydia trachomatis* infection of the reproductive tract in women[J]. Journal of Reproductive Immunology, 2009, 83(1/2): 173-178
- [14] Iwasaki A. Exploiting mucosal immunity for antiviral vaccines[J]. Annual Review of Immunology, 2016, 34(1): 575-608
- [15] Zhou JZ, Way SS, Kang C. Immunology of the uterine and vaginal mucosae[J]. Trends in Immunology, 2018, 39(4): 302-314
- [16] Johnson RM, Brunham RC. Tissue-resident T cells as the central paradigm of *Chlamydia* immunity[J]. Infection and Immunity, 2016, 84(4): 868-873
- [17] Rey-Ladino J, Ross AGP, Cripps AW. Immunity, immunopathology, and human vaccine development against sexually transmitted *Chlamydia trachomatis*[J]. Human Vaccines & Immunotherapeutics, 2014, 10(9): 2664-2673
- [18] Agrawal T, Gupta R, Dutta R, et al. Protective or pathogenic immune response to genital chlamydial infection in women — a possible role of cytokine secretion profile of cervical mucosal cells[J]. Clinical Immunology, 2009, 130(3): 347-354
- [19] Vlcek KR, Li WD, Manam S, et al. The contribution of *Chlamydia*-specific CD8⁺ T cells to upper genital tract pathology[J]. Immunology & Cell Biology, 2016, 94(2): 208-212
- [20] Poston TB, Darville T. *Chlamydia trachomatis*: protective adaptive responses and prospects for a vaccine[A]/Häcker G. Biology of *Chlamydia*. Current Topics in Microbiology and Immunology[M]. Springer, 2016, 412: 217-237
- [21] Zhong GM, Brunham RC, de la Maza LM, et al. National institute of allergy and infectious diseases workshop report: “*Chlamydia* vaccines: the way forward”[J]. Vaccine, 2017. DOI: 10.1016/j.vaccine.2017.10.075
- [22] Knudsen NPH, Olsen A, Buonsanti C, et al. Different human vaccine adjuvants promote distinct antigen-independent immunological signatures tailored to different pathogens[J]. Scientific Reports, 2016, 6: 19570
- [23] Masson L, Salkinder AL, Olivier AJ, et al. Relationship between female genital tract infections, mucosal interleukin-17 production and local T helper type 17 cells[J]. Immunology, 2015, 146(4): 557-567
- [24] Cheong HC, Lee CYQ, Cheok YY, et al. CPAF, HSP60 and MOMP antigens elicit pro-inflammatory cytokines production in the peripheral blood mononuclear cells from genital *Chlamydia trachomatis*-infected patients[J]. Immunobiology, 2019, 224(1): 34-41
- [25] Witkin SS, Minis E, Athanasiou A, et al. *Chlamydia trachomatis*: the persistent pathogen[J]. Clinical and Vaccine Immunology, 2017, 24(10): e00203-17
- [26] Ziklo N, Huston WM, Hocking JS, et al. *Chlamydia trachomatis* genital tract infections: when host immune response and the microbiome collide[J]. Trends in Microbiology, 2016, 24(9): 750-765
- [27] Elwell C, Mirrashidi K, Engel J. Chlamydia cell biology and pathogenesis[J]. Nature Reviews Microbiology, 2016, 14(6): 385-400
- [28] Qu YY, Frazer LC, O’Connell CM, et al. Comparable genital tract infection, pathology, and immunity in rhesus macaques inoculated with wild-type or plasmid-deficient *Chlamydia trachomatis* Serovar D[J]. Infection and Immunity, 2015, 83(10): 4056-4067
- [29] Moore ER, Ouellette SP. Reconceptualizing the chlamydial inclusion as a pathogen-specified parasitic organelle: an expanded role for Inc proteins[J]. Frontiers in Cellular & Infection Microbiology, 2014, 4(10): 157
- [30] Damiani MT, Gambarte TJ, Capmany A. Targeting eukaryotic Rab proteins: a smart strategy for chlamydial survival and replication[J]. Cellular Microbiology, 2014, 16(9): 1329-1338
- [31] Hafner LM, Wilson DP, Timms P. Development status and future prospects for a vaccine against *Chlamydia trachomatis* infection[J]. Vaccine, 2014, 32(14): 1563-1571
- [32] Yang ZS, Tang LL, Sun X, et al. Characterization of CPAF critical residues and secretion during *Chlamydia trachomatis* infection[J]. Infection and Immunity, 2015, 83(6): 2234-2241
- [33] Gregorczyk K, Krzyżowska M. Innate immunity to infection in the lower female genital tract[J]. Postępy Higieny I Medycyny Doświadczalnej, 2013, 67: 388-401
- [34] Lehr S, Vier J, Häcker G, et al. Activation of neutrophils by *Chlamydia trachomatis*-infected epithelial cells is modulated by the chlamydial plasmid[J]. Microbes and Infection, 2018, 20(5): 284-292
- [35] Rajeev K, Das S, Prusty BK, et al. *Chlamydia trachomatis* paralyzes neutrophils to evade the host innate immune response[J]. Nature Microbiology, 2018, 3(7): 824-835
- [36] Naglak EK, Morrison SG, Morrison RP. Neutrophils are central to antibody-mediated protection against genital *Chlamydia*[J]. Infection and Immunity, 2017, 85(10): e00409-17
- [37] Ebbo M, Crinier A, Vély F, et al. Innate lymphoid cells: major players in inflammatory diseases[J]. Nature Reviews Immunology, 2017, 17(11): 665-678
- [38] Rosato PC, Beura LK, Masopust D. Tissue resident memory T cells and viral immunity[J]. Current Opinion in Virology, 2017, 22: 44-50
- [39] Mueller SN, Mackay LK. Tissue-resident memory T cells: local specialists in immune defence[J]. Nature Reviews Immunology, 2016, 16(2): 79-89
- [40] Schenkel JM, Masopust D. Tissue-resident memory T cells[J]. Immunity, 2014, 41(6): 886-897

- [41] Li QS, Zeng M, Duan LJ, et al. Live simian immunodeficiency virus vaccine correlate of protection: local antibody production and concentration on the path of virus entry[J]. *Journal of Immunology*, 2014, 193(6): 3113-3125
- [42] Olsen AW, Follmann F, Erneholm K, et al. Protection against *Chlamydia trachomatis* infection and upper genital tract pathological changes by vaccine-promoted neutralizing antibodies directed to the VD4 of the major outer membrane protein[J]. *Journal of Infectious Diseases*, 2015, 212(6): 978-989
- [43] Bulir DC, Liang S, Lee A, et al. Immunization with chlamydial type III secretion antigens reduces vaginal shedding and prevents fallopian tube pathology following live *C. muridarum* challenge[J]. *Vaccine*, 2016, 34(34): 3979-3985
- [44] Bøje S, Olsen AW, Erneholm K, et al. A multi-subunit *Chlamydia* vaccine inducing neutralizing antibodies and strong IFN- γ^+ CMI responses protects against a genital infection in minipigs[J]. *Immunology and Cell Biology*, 2016, 94(2): 185-195
- [45] Xu L, Pegu A, Rao E, et al. Trispecific broadly neutralizing HIV antibodies mediate potent SHIV protection in macaques[J]. *Science*, 2017, 358(6359): 85-90
- [46] Caskey M, Klein F, Nussenzweig MC. Broadly neutralizing antibodies for HIV-1 prevention or immunotherapy[J]. *New England Journal of Medicine*, 2016, 375(21): 2019-2021
- [47] Rowe JH, Ertelt JM, Way SS. Foxp3⁺ regulatory T cells, immune stimulation and host defence against infection[J]. *Immunology*, 2012, 136(1): 1-10
- [48] Marks E, Tam MA, Lycke NY. The female lower genital tract is a privileged compartment with IL-10 producing dendritic cells and poor Th1 immunity following *Chlamydia trachomatis* infection[J]. *PLoS Pathogens*, 2010, 6(11): e1001179
- [49] Tang LL, Yang ZS, Zhang HB, et al. Induction of protective immunity against *Chlamydia muridarum* intracervical infection in DBA/1j mice[J]. *Vaccine*, 2014, 32(12): 1407-1413
- [50] Moore-Connors JM, Fraser R, Halperin SA, et al. CD4⁺CD25⁺FoxP3⁺ regulatory T cells promote Th17 responses and genital tract inflammation upon intracellular *Chlamydia muridarum* infection[J]. *Journal of Immunology*, 2013, 191(6): 3430-3439
- [51] Hook CE, Telyatnikova N, Goodall JC, et al. Effects of *Chlamydia trachomatis* infection on the expression of natural killer (NK) cell ligands and susceptibility to NK cell lysis[J]. *Clinical & Experimental Immunology*, 2004, 138(1): 54-60
- [52] Zook EC, Kee BL. Development of innate lymphoid cells[J]. *Nature Immunology*, 2016, 17(7): 775-782
- [53] Zheng Y, Valdez PA, Danilenko DM, et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens[J]. *Nature Medicine*, 2008, 14: 282-289
- [54] Montaldo E, Vacca P, Vitale C, et al. Human innate lymphoid cells[J]. *Immunology Letters*, 2016, 179: 2-8
- [55] Zhu CM, Lin H, Tang LL, et al. Oral *Chlamydia* vaccination induces transmucosal protection in the airway[J]. *Vaccine*, 2018, 36(16): 2061-2068
- [56] Date Y, Ebisawa M, Fukuda S, et al. NALT M cells are important for immune induction for the common mucosal immune system[J]. *International Immunology*, 2017, 29(10): 471-478
- [57] Feller L, Altini M, Khammissa RAG, et al. Oral mucosal immunity[J]. *Oral Surgery, Oral Medicine, Oral Pathology & Oral Radiology*, 2013, 116(5): 576-583
- [58] Wang LY, Zhu CM, Zhang TY, et al. Nonpathogenic colonization with *Chlamydia* in the gastrointestinal tract as oral vaccination for inducing transmucosal protection[J]. *Infection and Immunity*, 2018, 86(2): e00630-17
- [59] Olsen AW, Lorenzen EK, Rosenkrands I, et al. Protective effect of vaccine promoted neutralizing antibodies against the intracellular pathogen *Chlamydia trachomatis*[J]. *Frontiers in Immunology*, 2017, 8(12): 1652