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# LIGHT 信号通路在鼠衣原体生殖道感染中的作用

陈丽丽[1,2],孙源彬[2],徐莎[2],符玺宗[2],周洲[1],陆春雪[1],杨菲[3],许桂莲[3*],吴移谋[1*]

南华大学,[1] 病原生物学研究所,[2] 公共卫生学院,湖南 衡阳 421001

[3] 第三军医大学免疫学研究所,重庆 400038

**摘要:**【目的】初步探讨与单纯疱疹病毒糖蛋白 D 竞争结合疱疹病毒侵入介体的淋巴毒素类似物(lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells,LIGHT)在抗衣原体感染免疫及介导衣原体生殖道病理损伤过程的作用。【方法】用 $1\times10^4$ IFUs 的 MoPn 经生殖道感染野生型(wild type,wt)、LIGHT KO 小鼠,每组一半小鼠于感染后 49d,再次感染相同剂量的 MoPn。每隔 3-4 d取生殖道分泌物,测定其中衣原体包涵体的数量。初次感染后 80d,处死小鼠,眼眶取血,分离血清,用间接免疫荧光法测定其中抗体类型及效价;同时分离生殖道,肉眼观察其输卵管、子宫角水肿程度,然后甲醛固定、切片,H&E 染色后,显微镜下观察各组织炎性浸润程度和管腔水肿程度。分离小鼠脾细胞,体外用衣原体 EB 刺激,测定上清中 IL-4、IL-5、IL-17 和 IFN-γ 等细胞因子水平。【结果】LIGHT KO 小鼠阴道带菌时间与 wt 组相当,大部分小鼠均在原发感染后 28d 左右完全清除感染,且均产生对再次感染的免疫力。LIGHT KO 和 wt 小鼠子宫角和输卵管均出现一定程度的病变,但差异无统计学意义。两组小鼠在原发和继发感染 MoPn 后,均产生高效价的特异性抗 MoPn IgG 抗体,总抗体及各 IgG 抗体亚类效价差异均无统计学意义($P>0.05$),且 IgG2a/IgG1 比值均大于 1。和 wt 小鼠一样,LIGHT KO 小鼠脾淋巴细胞经衣原体再次刺激后均可产生较高水平的 IFN-γ 和 IL-17,且未能检测到 IL-4 和 IL-5。【结论】小鼠抗 MoPn 生殖道感染及 MoPn 引起的生殖道病理损伤不依赖于 LIGHT 信号通路。

**关键词:**鼠衣原体,与单纯疱疹病毒糖蛋白 D 竞争结合疱疹病毒侵入介体的淋巴毒素类似物,泌尿生殖道感染,病理损伤

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沙眼衣原体(*Chlamydia trachomatis*,Ct)主要感染泌尿生殖道上皮细胞,引起性传播疾病,男性多表现为非淋球菌性尿道炎、附睾炎、前列腺炎;女性多表现为宫颈炎、子宫内膜炎、输卵管炎及盆腔炎,并可导致异位妊娠、不孕不育等严重并发症,是宫颈癌、卵巢癌的高危因素,还可促进人类免疫缺陷病毒(Human ImmunodeficiencyVirus,HIV)的感染[1-2]。Ct 根据其生物学特性和致病性分为鼠生物型、沙眼生物型和性病淋巴肉芽肿生物型 3 种生物型。Ct 鼠生物型主要感染小鼠引起小鼠肺炎,因而过去称为鼠肺炎型 Ct

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* 通信作者。吴移谋,Tel: +86-734-8280917, E-mail:yimouwu@sina.com; 许桂莲, E-mail: xuguilian@yahoo.com

**作者简介:**陈丽丽(1975-),女,湖南攸县人,教授,博士,主要从事衣原体致病机制研究。E-mail: chlili720612@163.com

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(*mouse pneumonitis agent*, MoPn),现将其划分为一新的衣原体种,即鼠衣原体(*Chlamydia muridarum*)。由于其引起的鼠泌尿生殖道病理反应与人类沙眼衣原体性泌尿生殖道感染极其相似,因此,目前常用 MoPn 鼠生殖道感染模型来研究沙眼衣原体的免疫保护和病理损伤机制[3-4]。

动物实验已证实,衣原体感染后在局部黏膜免疫和机体系统免疫中都有高水平活化的 Th1 细胞参与免疫应答,说明 T 细胞的活化在抗衣原体免疫中至关重要。正常 T 细胞的活化至少需要 2 个信号,即抗原信号和协同刺激信号。缺乏协同刺激信号的抗原信号不能活化 T 细胞而导致 T 细胞耐受。与单纯疱疹病毒糖蛋白 D 竞争结合疱疹病毒侵入介体的淋巴毒素类似物(lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells, LIGHT),即肿瘤坏死因子配体超家族成员 14(tumor necrosis factor superfamily, TNFSF14),是一种非 CD28 依赖的协同刺激分子。目前已经证实 LIGHT 可以与三种膜结合型的 TNF 受体家族成员结合,即疱疹病毒侵入介体(herpes virus entry mediator, HVEM)、淋巴毒素 β 受体(lymphotoxinβ receptor, LTβR)和诱饵受体 3(Decoy receptor 3, DcR3)/TR6[5]。现有研究发现,LIGHT 信号通路通过刺激 T 细胞增殖、分化和诱导靶细胞凋亡在抗感染免疫中发挥重要作用[6-9]。而 LIGHT 信号通路在衣原体感染与免疫中的作用,国内外尚未见报道。本文以 LIGHT KO 小鼠为模型,经生殖道感染 MoPn 后,比较其与 wt 小鼠的衣原体清除速度、生殖道组织的病变及 T、B 细胞免疫反应,分析 LIGHT 信号通路在鼠衣原体生殖道感染中的作用。

# 1 材料和方法

## 1.1 材料

**1.1.1 实验动物**:雌性 C57BL/6J 野生型(wild type, wt)小鼠 20 只,购自湖南斯莱克景达实验动物有限公司,LIGHT 基因敲除(knock out, KO)小鼠 19 只,由第三军医大学全军免疫学研究所许桂莲教授惠赠,均为 5~6 周龄。

**1.1.2 菌株和试剂**: *C. muridarum* (MoPn, Nigg 株),由实验室传代保存。HeLa 细胞,购自美国标准菌株保存中心(ATCC, cat# CCL2)。Cy3 标记的羊抗鼠 IgG(H+L)、IgG1、IgG2a、IgG2b 和 IgG3 均购自 Jackson ImmunoResearch 公司; IL-4、IL-5、IFN-γ、IL17 等细胞因子检测试剂盒购自 R&D 公司。

## 1.2 鼠生殖道感染模型制备

20 只 C57BL/6J wt 和 19 只 LIGHT KO 小鼠经阴道接种含 $1\times10^4$ IFUs MoPn 的 20 μL SPG,其中一半小鼠(LIGHT KO 小鼠 9 只, wt 小鼠 10 只)在初次感染后 49 d,再次感染相同剂量的 MoPn。小鼠初次和再次感染前 5d 均皮下注射 2.5 mg Depo-provera 以增加小鼠对衣原体感染的敏感性。初次感染后 80 d 处死小鼠,收集血清,分离生殖道并收集脾细胞。

## 1.3 染菌小鼠生殖道带菌量检测

小鼠经生殖道感染 MoPn 后,每隔 3-4 d 取生殖道分泌物于 500 μL SPG 中,然后超声、系列稀释,取合适稀释度的稀释液感染 HeLa 单层细胞,于 $CO_2$ 培养箱中培养 24 h 后,进行荧光抗体染色。用荧光显微镜计数其中衣原体包涵体数量。

## 1.4 染菌小鼠生殖道病理检测

初次感染后 80 d 处死小鼠,分离生殖道(包括子宫、输卵管和卵巢),肉眼观察并照像记录其输卵管水肿情况;然后用 10% 甲醛固定,切片,做 H&E 染色,再用盲法分别给子宫角及输卵管的炎症反应和水肿程度评分[10]。

## 1.5 血清抗体类型检测

用 MoPn 感染 HeLa 细胞单层 24h,经固定、透膜、封闭后,加入系列稀释的感染 80d 后的小鼠血清,37℃孵育 1h;充分洗涤,再分别加入 1∶100 稀释的 Cy3 标记的羊抗鼠 IgG、IgG1、IgG2a、IgG2b、IgG3 和 1∶1000 稀释的细胞核染色试剂 hoechst,37℃孵育1 h;用不加一抗只加二抗的细胞孔为对照。充分洗涤,封片,用 Olympus AX-70 荧光显微镜观察,以能明显观察到包涵体的血清最高稀释度定义为该血清中相应抗体的效价。

## 1.6 脾细胞上清中细胞因子水平测定

感染 80d 后处死小鼠,无菌取脾脏,按常规方法[3-4]制备脾细胞悬液,接种于 24 孔板中,每个样品接种 2 孔,每孔 1 mL,于其中一孔中加入 $1\times10^6$ IFU UV 灭活的 MoPn EB,另一孔不刺激做对照。$CO_2$ 培养箱中培养 72h 后,收集上清,用 ELISA 方法测定上清中 IL-4、IL-5、IFN-γ、IL-17 等细胞因子水平,严格按试剂盒说明书操作。

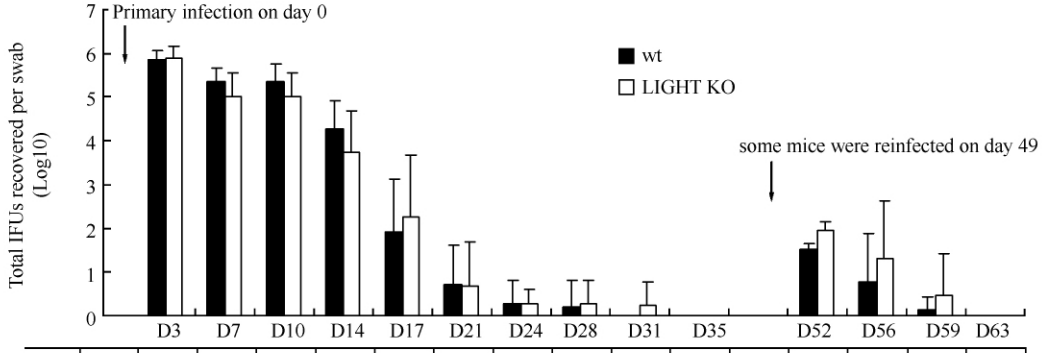
### 1.7 统计学分析

用 *t* 检验(two-tailed Student's *t* test, Microsoft Eccel)比较两组间 IFU、细胞因子水平、抗体亚类比值及病理评分结果的差异;子宫角和输卵管积水肉眼统计数据(表 1)用 Fisher's exact test(Microsoft Excel)比较。$P<0.05$ 为差异有统计学意义。

## 2 结果

### 2.1 LIGHT 缺失对生殖道鼠衣原体清除速度的影响

LIGHT KO 小鼠和 wt 鼠经阴道感染 MoPn,每隔 3–4 d 取生殖道分泌物,检测其中衣原体数量。结果显示,不管是初次感染还是再次感染后,LIGHT KO 小鼠清除速度几乎与 wt 小鼠一致(图 1)。大部分 LIGHT KO 小鼠在感染后 28 d 生殖道中已清除衣原体,只有 2 只(共 19 只)小鼠低水平带菌至31 d。在初次感染后 49 d,每组一半小鼠再次感染相同剂量的衣原体,3 d 后,每组小鼠相对初次感染生殖道带菌量均降低 1000 倍左右,所有小鼠均在再次感染后 10 d 内转为阴性,表明 LIGHT KO 和 wt 小鼠初次感染后均获得了对再次感染的免疫力。




| | | D3 | D7 | D10 | D14 | D17 | D21 | D24 | D28 | D31 | D35 | | D52 | D56 | D59 | D63 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Wild type | IFU+ | 20 | 20 | 20 | 20 | 16 | 6 | 4 | 2 | 0 | 0 | | 10 | 4 | 1 | 0 |
| | Total | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | | 10 | 10 | 10 | 10 |
| LIGHT KO | IFU+ | 19 | 19 | 19 | 19 | 19 | 6 | 4 | 3 | 2 | 0 | | 9 | 3 | 1 | 0 |
| | Total | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 0 | | 9 | 9 | 9 | 9 |

**图 1. LIGHT 缺失对衣原体清除速度的影响**

Figure 1. Effect of LIGHT deficiency on live organism shedding following chlamydial infection. IFU +: The number of mice with detectable IFUs. Total: The number of mice infected with *C. muridarum*.

### 2.2 LIGHT 缺失对衣原体引起的小鼠生殖道病变的影响

原发感染后 80 d 处死小鼠,分离生殖道组织进行病理检测。肉眼观察,大部分小鼠出现单侧或双侧子宫角和输卵管炎性积水/水肿,但 LIGHT KO 和 wt 小鼠子宫角和输卵管积水发生率差异无统计学意义($P>0.05$,图 2-A,表 1)。

**表 1. 肉眼观输卵管和子宫角积水发生率**

Table 1. Incidence of gross pathologies of uterine horn and fallopian tube

| Primer | Group | Mouse No. | Uterine horn dilatation | | | Hydrosalpinx | | |
|---|---|---|---|---|---|---|---|---|
| | | | Normal | Unilateral | Bilateral | Normal | Unilateral | Bilateral |
| 1 | wt | 10 | 4 | 3 | 3 | 5 | 4 | 1 |
| | LIGHT KO | 10 | 6 | 3 | 1 | 5 | 3 | 2 |
| 2 | wt | 10 | 2 | 4 | 4 | 4 | 3 | 3 |
| | LIGHT KO | 9 | 5 | 0 | 4 | 3 | 2 | 4 |

Number of mice showing pathology on single or both sides of the reproductive tissues were recorded and tabulated. There was no significant difference between LIGHT KO and wt mice ($P>0.05$, two-tailed Fisher's exact test).

显微镜下可见，病变子宫和输卵管粘膜柱状上皮细胞呈矮柱状，顶部纤毛明显减少或消失，管壁纤维细胞或纤维母细胞增生，导致管壁变厚，严重者管腔狭窄甚至阻塞，部分管腔扩大，浆膜层毛细血管扩张，大量淋巴细胞和浆细胞浸润（图 2-B）。

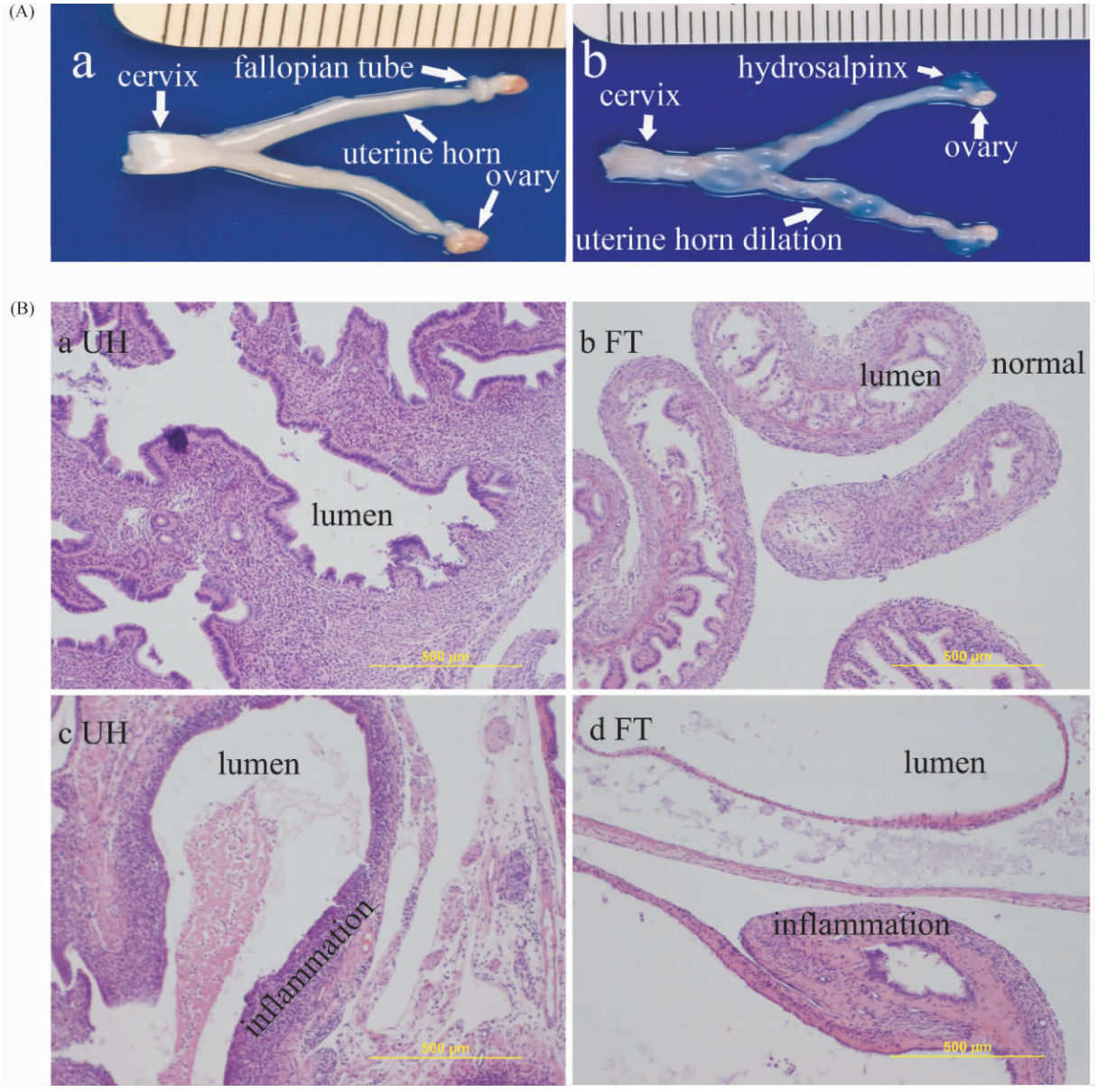



**图 2. LIGHT 缺失对生殖道病理反应的影响**

Figure 2. Effect of LIGHT-deficiency on the development of inflammatory pathologies in the mouse urogenital tract following chlamydial infection. (A) The gross appearance of urogenital tract tissues. a and b show the normal and abnormal urogenital tract tissues. The pathologies were recorded as hydrosalpinx & uterine horn dilatation observable with naked eye. (B) The urogenial tract tissues were examined under microscope after H&E staining. a and b show the normal uterine horns or fallopian tubes. c and d show the pathologies which were recorded as extensive infiltration of mononuclear cells and fallopian tubes or uterine horns luminal dilation.

盲法对小鼠生殖道组织的炎性浸润和管腔扩张程度进行半定量分析。结果显示，LIGHT KO 和 wt 小鼠输卵管和子宫角的炎症浸润程度（炎症评分）或管腔积水/水肿程度差异无统计学意义（$P > 0.05$，图 3）。

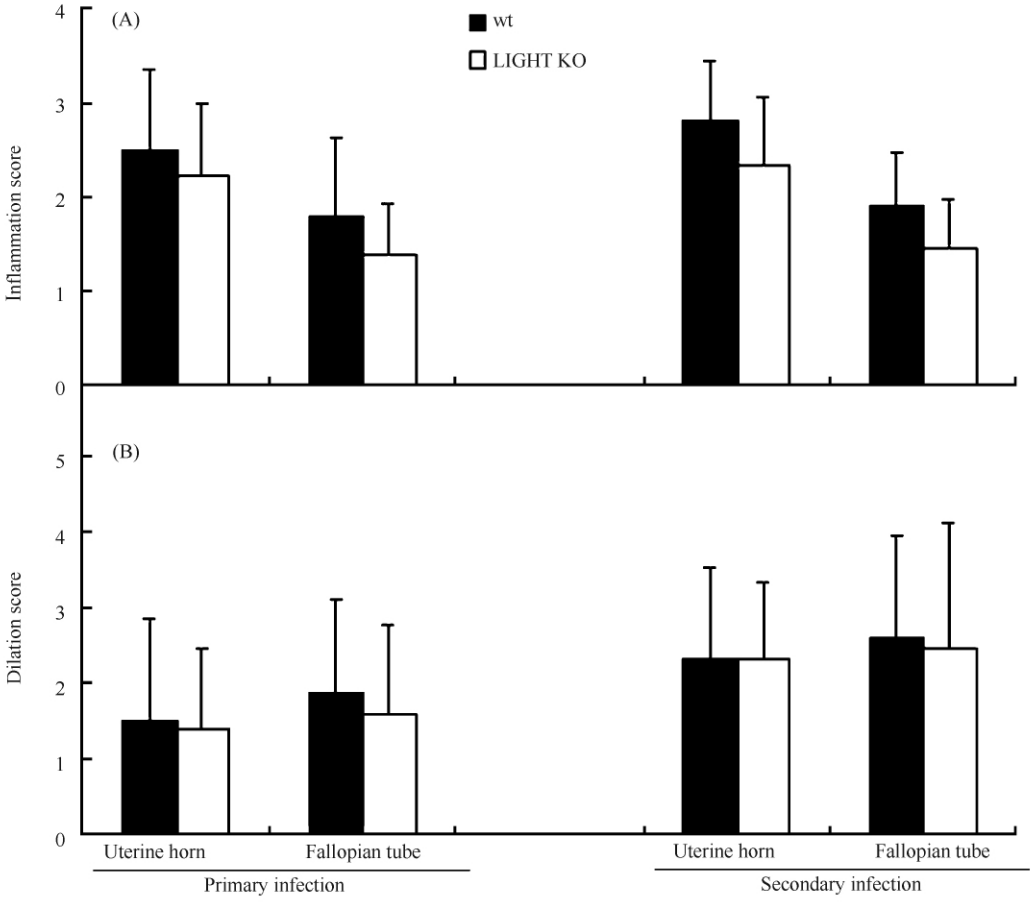



**图 3. 小鼠子宫角和输卵管炎性浸润和积水扩张程度评分**

Figure 3. Comparation of inflammation and dilation of mice uterine horn and fallopian tube following chlamydial infection. Inflammation and lumen dilatation of both uterine horns and fallopian tubes were semiquantitatively scored under microscope and the scores were used to calculate the means and standard errors for each group as shown along the Y-axis. The various tissue and mouse groups were indicated along the X-axis.

### 2.3 LIGHT 缺失对衣原体感染后特异性抗体和脾细胞因子产生的影响

**2.3.1 LIGHT 缺失对 MoPn 特异性抗体产生的影响**：LIGHT KO 和 wt 小鼠在原发和继发感染 MoPn 后，均产生高效价的特异性抗 MoPn IgG 抗体，抗体亚类检测显示两组小鼠均产生较高水平的 IgG2a 和 IgG1 且 IgG2a/IgG1 比值均大于 1（图 4），两组小鼠血清抗体效价及 IgG2a/IgG1 比值差异无统计学意义。

**2.3.2 LIGHT 缺失对小鼠脾淋巴细胞因子产生的影响**：通过体外脾淋巴细胞再刺激试验，比较 LIGHT KO 和 wt 小鼠 T 细胞应答能力。和 wt 小鼠一样，LIGHT KO 小鼠脾淋巴细胞经衣原体再次刺激后产生较高水平的 IFN-γ 和 IL-17，经双侧 t 检验，两组间两细胞因子水平差异无统计学意义（$P > 0.05$，图 5）。同时，两组小鼠脾淋巴细胞上清中均未检测到 IL-4 和 IL-5。

## 3 讨论

作为一个多功能多效应分子，LIGHT 兼具共刺激和细胞毒两方面的活性，根据靶细胞上表达的受体与 T 细胞所分泌的细胞因子的不同，可启动不同的生物学效应。我们以 LIGHT 基因敲除小鼠为研究对象，建立 MoPn 生殖道感染模型，研究 LIGHT 信号通路在衣原体感染中的作用。

实验结果显示，LIGHT KO 小鼠不管是生殖道衣原体清除速度还是 MoPn 所致的上生殖道病理损伤均与 wt 小鼠无明显区别，说明 LIGHT 在抗衣原体生殖道感染免疫中的作用不明显。这和 Ehlers 等[11]将结核分枝杆菌滴鼻感染 LIGHT KO 和 wt 小鼠，发现 LIGHT KO 和 wt 小鼠均能有效控制结核分

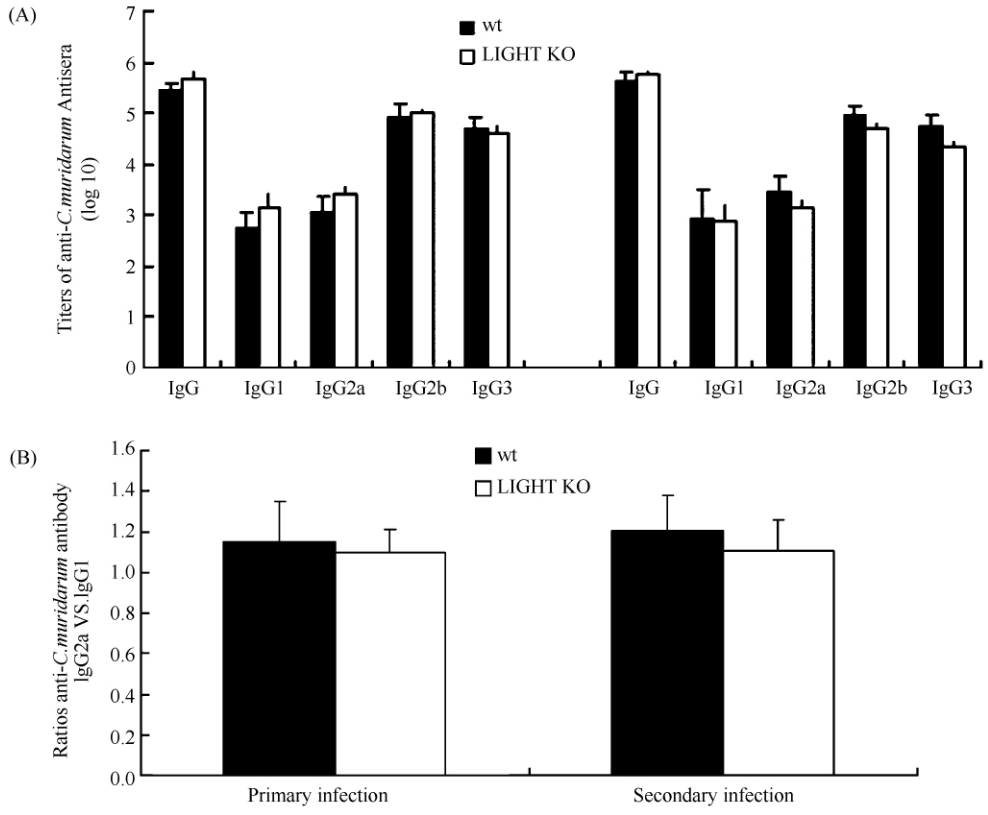



**图 4. LIGHT 缺失对衣原体感染后抗体产生的影响**

Figure 4. Effect of LIGHT deficiency on mouse antibody responses after chlamydial infection. A. The titers of Chlamydia-specific total IgG Abs and IgG in different isotypes (including g1, g2a, g2b, and g3). The Ab titers were expressed as log10 dilution. B. The ratio of IgG2a versus IgG1 was calculated for each mouse group. Note that the LIGHT KO mice almost produced same levels of IgG2a and IgG1.

枝杆菌感染,肺部病理损伤也无明显差异报道一致。而 Stanley 等[12-13]发现,LIGHT KO 小鼠在感染硕大利什曼原虫、伯氏疟原虫和巨细胞病毒后,带菌量显著高于 wt 小鼠。这说明 LIGHT 信号通路发挥的抗感染作用与病原体种类有关。当然,如果加大 MoPn 感染剂量或改变实验小鼠遗传背景,实验结果可能会不一样。本文所用的感染剂量 $1\times10^4$ IFUs 是在前期实验基础上确立的衣原体生殖道感染模型的最佳剂量[3-4],此种强度的衣原体感染可能不需要活化 LIGHT 协调刺激信号,就可活化 T 细胞应答。

大量文献报道 LIGHT 与 HVEM 结合,可共刺激 T 细胞增殖、分化及 Th1 型细胞因子 IFN-γ 的产生[14-15]。我们用 UV-MoPn EB 体外再刺激感染小鼠脾淋巴细胞,发现 LIGHT KO 和 wt 小鼠均产生较高水平的 Th1 型细胞因子 IFN-γ 和 Th17 型细胞因子 IL-17,而未检出 Th2 型细胞因子 IL-4 和 IL-5,表明 LIGHT 缺失并不影响衣原体感染后 Th1 型细胞应答。Liu 等[16]也证实,LIGHT 缺失可影响 T 细胞分裂增殖,但不影响其功能。而尚宇航等[17]用抗 CD3 和抗 CD28 mAb 刺激 LIGHT KO 小鼠脾淋巴细胞,发现 LIGHT KO 比 wt 小鼠 IFN-γ 产生明显减少,而 IL-10 水平明显增高;而且进一步检测发现,LIGHT KO 小鼠 IFN-γ 产生减少表现为 $CD8^+$ T 细胞中 IFN-γ 产生缺陷,而 $CD4^+$ T 细胞中 IFN-γ 产生正常。机体抗衣原体感染免疫是以 $CD4^+$ T 细胞为主,这也和本文中衣原体感染后 LIGHT KO 和 wt 小鼠 IFN-γ 水平无差异是一致的。

有文献报道,在外界刺激因素作用下,B 细胞表面 LIGHT 表达增高,并且可促进 B 细胞分化及抗体产生[18]。但本文结果显示,LIGHT 缺失并不影响抗 MoPn 特异性抗体的产生及抗体亚类,与 Sedgmen 等[19]报道的腹腔注射或滴鼻感染流行性感冒病毒后,LIGHT KO 小鼠产生的血清特异性抗体效价及抗体亚类与 wt 小鼠也没有区别结果一致,说明 LIGHT 信号途径对 B 细胞的活化作用随抗原不同而有所差异。

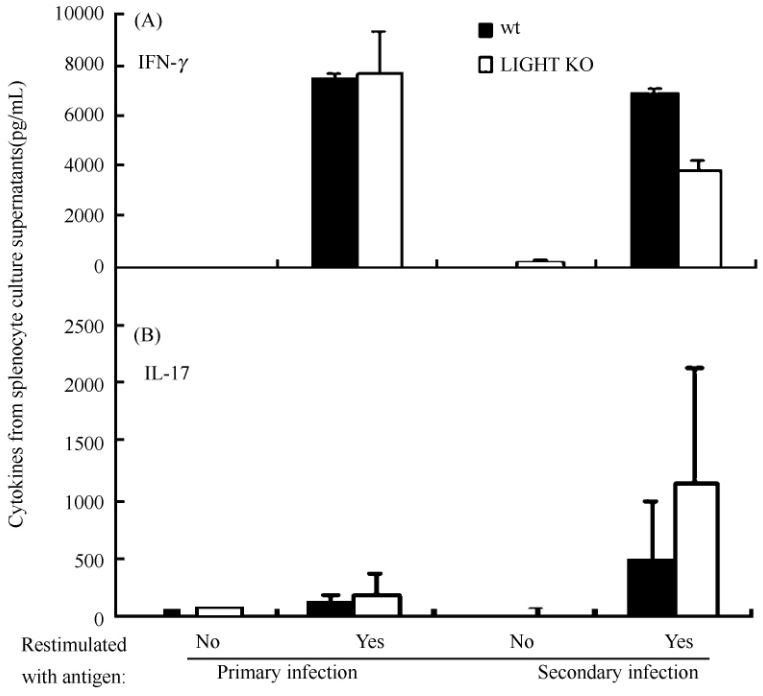



**图 5. LIGHT 缺失对衣原体感染后小鼠脾淋巴细胞因子产生的影响**

Figure 5. Effect of LIGHT deficiency on cytokine production in splenocytes after in vitro restimulated with chlamydial organisms. Splenocytes were harvested from wt and LIGHT KO mice on day 80 after intravaginal infection with *C. muridarum*. The splenocytes were restimulated with UV-inactivated chlamydial organism Ags for 72 h. The culture supernatants were measured for IFN-γ (A), IL-17 (B) using ELISA. IL-4 and IL-5 could not be detected.

总之，本实验结果初步表明，小鼠抗 MoPn 生殖道感染及 MoPn 引起的生殖道病理损伤不依赖于 LIGHT 信号通路。因为 LIGHT 可分别与 HVEM 和 LTβR 结合，启动不同的生物学效应，使用 LIGHT KO 小鼠使 LIGHT 信号通路完全缺失，可能会掩盖 HVEM 和 LTβR 在衣原体感染中的作用，因此下一步可通过特异性抗体分别阻断 LIGHT 与 HVEM 或 LTβR 途径，或使用 LTα KO、LTβ KO 和 LTβR KO 小鼠进行研究，进一步明确 LIGHT 分子在衣原体感染中的作用。

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# Role of LIGHT signal pathway in *Chlamydia muridarum* urogenital infection in mice

Lili Chen[1,2], Yuanbin Sun[2], Sha Xu[2], Xizong Fu[2], Zhou Zhou[1], Chunxue Lu[1], Fei Yang[2], Guilian Xu[2*], Yimou Wu[1*]

[1] Institute of Pathogenic Biology, Medical College; [2] School of Public Health, University of South China, Hengyang 421001, Hunan Province, China

[3] Institute of Immunology, the Third Military Medical University, Chongqing 400038, China

**Abstract**: [**Objective**] To study the role of lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells (LIGHT) in the development of protective immunity and pathology during *Chlamydia Muridarum* urogenital infection in mice. [**Methods**] C57BL/6J wild type (wt) and mice deficient in LIGHT (LIGHT KO) were inoculated intravaginally with $1 \times 10^4$ IFUs of live *C. muridarum* organisms. Half mice of each group were reinfected on day 49 after primary infection. We took mice vaginal swabs every 3 or 4 days to monitor live organism shedding. On day 80 after the primary infection, mice were sacrificed, the vaginal tract was isolated for pathology analysis. The spleen cells were collected and IL-4, IL-5, IL-17 and IFN-γ were detected by ELISA in the spleen cells culture supernatant after restimulated by UV-MoPn EB. The titers of different Ab isotypes were measured in mice serum by Indirect Immunofluorescence Assay. [**Results**] The chlamydia shedding time of LIGHT KO mice was similar to wild type mice, which cleared the organisms within 28 days after primary infection, and acquired protective immunity against *C. muridarum* reinfection. All mice regardless of genotypes developed severe upper genital tract pathology and showed no significant difference between LIGHT KO and wild type mice. All mice developed robust anti-C. *muridarum* organism IgG antibody responses and the ratios of IgG2a versus IgG1 showed no significant difference between LIGHT KO and wild type mice. Splenocytes from MoPn-infected LIGHT KO and wild type mice produced high levels of IFN-γ and IL-17, but IL-4 and IL-5 couldn't be detected. [Conclusions] LIGHT signal pathway may not correlated with protection against *C. muridarum* urogenital tract infection and urogenital tract pathology induced by *C. muridarum*.

**Keywords**: *Chlamydia muridarum* (MoPn), lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells (LIGHT), urogenital tract infection, pathology

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* Corresponding author. Yimou Wu, Tel: +86-734-8280917, E-mail: yimouwu@sina.com; Guilian Xu, E-mail: xuguilian@yahoo.com

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