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过敏性疾病研究专题 论 文
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外周血单核细胞及肺巨噬细胞TLR9在过敏性鼻炎及哮喘发作时的表达变化

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摘要 近年来, 单核细胞-巨噬细胞系统及Toll样受体9(Toll-like receptor 9, TLR9)在过敏性疾病中的作用引起了关注. 本文采用流式细胞术, 分析了过敏性鼻炎(allergic rhinitis, AR)和过敏性哮喘(allergic asthma, AA)及过敏性鼻炎合并哮喘(allergic rhinitis with allergic asthma, ARA)外周血TLR9$^+$单核细胞的表达变化, 并应用小鼠的AR和AA模型对过敏原激发后小鼠血液单核细胞、肺组织中巨噬细胞TLR9的表达变化进行了探讨, 发现AR、AA及ARA外周血单核细胞群中TLR9$^+$细胞比例显著升高, 代表每个细胞内TLR9分子表达数量的MFI表达增加. 大籽蒿过敏原提取物(artemisia sieversiana wild allergen extract, ASWE)、屋尘螨过敏原提取物(house dust mite allergen extract, HDME)、梧桐花粉过敏原提取物(platanus pollen allergen extract, PPE)刺激AR、AA模型小鼠血液TLR9$^+$单核细胞、肺组织巨噬细胞比例及TLR9 MFI的表达水平增高. 高亲和力IgE受体基因敲除(FcεR Ⅰ KO)的AR和AA模型小鼠较野生型(wild-type, WT)小鼠的TLR9$^+$单核细胞及巨噬细胞比例显著减少, 提示过敏原提取物诱导的TLR9$^+$细胞表达上调是由FcεR Ⅰ依赖和非依赖性途径共同实现的.

关键词 过敏性鼻炎, 过敏性哮喘, 单核细胞, 巨噬细胞, Toll样受体9

过敏性鼻炎(allergic rhinitis, AR)和过敏性哮喘(allergic asthma, AA)是常见的慢性炎症性疾病, 患病率高达全球人口的10%~30%, 为社会造成巨大的医疗负担[1]. 但是, 迄今为止, 人们对这两种疾病发病机制的研究主要集中在肥大细胞、嗜酸性粒细胞、II型炎症及经典的IgE依赖途径上, 对单核细胞-巨噬细胞系统在过敏性疾病发病机制中的作用尚缺乏深入的了解.

近年来, 研究发现IgE非依赖途径参与过敏性疾病的发展过程[2]. 已经发现单核细胞尤其是CD14$^+$CD16$^+$中间型单核细胞亚群在AR和AA的发病中均可能扮演着重要角色. 该亚群在AA患者的外周血中比例显著升高, 且与气道高反应性的严重程度呈正相关[3]; 通过促进Th2型炎症发展、破坏上皮屏障功能以及与嗜酸性粒细胞协同作用参与过敏性疾病的进程[4].

巨噬细胞在过敏性哮喘中通过多重机制调控气道炎症与重塑, 肺泡巨噬细胞在稳态时维持免疫平衡, 但在过敏原刺激后极化为促炎表型, 分泌IL-4、IL-13等Th2细胞因子, 募集嗜酸性粒细胞并促进黏液高分泌[5,6]. 近年研究表明, 肺巨噬细胞由外周血单核细胞分化或驻留巨噬细胞增殖形成, 具有促炎和抗炎双重作用[7,8].

Toll样受体9(Toll-like receptor 9, TLR9)作为模式识

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别受体家族的重要成员，主要识别细菌DNA中的非甲基化CpG序列, 在抗感染免疫中发挥关键作用[9]. TLR9在AR和AA中的作用存在争议. 部分研究显示TLR9激动剂在动物模型中减轻过敏症状[10]，而另一些研究则发现AR和AA患者局部组织(如鼻黏膜、气道)TLR9表达上调且与疾病严重程度正相关[11]. 有研究报道TLR9参与调控Th2型免疫反应[12], 但关于单核细胞及巨噬细胞TLR9表达变化与AR和AA患者的关系研究及其临床意义所知甚少.

为进一步理解TLR9在AR、AA发病机制中的作用，本文探讨了在过敏原刺激时外周血单核细胞及肺组织中巨噬细胞中TLR9的表达变化规律，为理解AR、AA的发病机制提供了新的视角.

1 材料和方法

1.1 仪器与试剂

(i) 仪器. FACSVerse流式细胞仪(BD Biosciences, 美国); 低温高速离心机(Thermo Fisher, 美国); DM500倒置显微镜(Leica, 德国); MPS52生物显微镜(Leica, 德国); XSE105DU万分之一电子天平(梅特勒, 瑞士); Hera cell150i CO_2培养箱(Thermo, 美国); MSC生物安全柜(Thermo, 美国).

(ii) 试剂. 红细胞裂解液、Human TruStain FcX™ (Fc受体阻断液)、亮紫510^{T}mol/L Zombie Aqua™活细胞鉴定液、PE/Cy7标记抗人CD14抗体(clone HCD14)、FITC标记抗人TLR9抗体(clone S16013D)、TruStain FcX™抗鼠CD16/32抗体(clone 93)、PerCP标记抗鼠CD11b抗体(clone M1/70)、亮紫510^{TM}标记抗鼠Ly-6G/Ly-6C(Gr-1)抗体(clone RB6-8C5)、PE/Cy7抗鼠CD11c抗体(clone N418)、亮紫510^{TM}标记抗鼠F4/80抗体均购于Biolegend公司(美国), 均为100 tests. Cytofix/Cytoperm™细胞固定/透膜试剂盒购于Biosciences Pharmingen公司(美国). 大籽蒿过敏原提取物(*Artemisia sieversiana* wild allergen extract, ASWE)、屋尘螨过敏原提取物(house dust mite allergen extract, HDME)、梧桐花粉过敏原提取物(platanus pollen allergen extract, PPE)购于新华联协和药业公司(中国).

1.2 研究对象

(1) 患者. 本研究纳入2021年1月~2022年6月期间于锦州医科大学附属第一医院变态反应门诊的患者(表1), AR组符合《中国变应性鼻炎诊断和治疗指南(2015天津)》[13])标准，AA组符合《中国支气管哮喘防治指南(第一版，2019)》[14])标准，过敏性鼻炎合并哮喘(ARA)组(同时满足上述两项标准), 健康对照(HC)组: 无过敏性疾病史、无近期感染及免疫相关疾病. 本实验已获得锦州医科大学附属第一医院临床试验伦理委员会的批准(KY201801), 本研究符合《郝尔辛基宣言》.

(2) 小鼠. 本研究选用C57 BL/6背景的野生型(WT)小鼠(辽宁长生生物)和高亲和力IgE受体(FcεRⅠ)基因敲除(KO)小鼠(江苏集萃药康生物). 所有实验动物均为6~8周龄，体重18~22 g, 饲养于锦州医科大学SPF级动物实验中心. 实验方案经锦州医科大学动物伦理委员会审批(20180916002).

1.3 流式细胞术检测人单核细胞内TLR9 的表达

(1) 人外周血标本处理: 外周血样本经1500×*g*离心10 min分离血浆和血细胞. 其中血细胞用含2% FBS的RPMI 1640培养液重悬后，分别用1.0 μg/mL ASWE、

表 1 志愿者一般资料[a)]

Table 1 General information of volunteers

	HC(*n*=18)	AR(*n*=36)	AA(*n*=24)	ARA(*n*=21)
年龄(岁)	23(20~45)	34(6~52)	47(11~58)	32(17~60)
男性/女性	8/10	16/20	13/11	12/9
发病年龄中位数(岁)		25(6~47)	35(7~50)	27(9~49)
病程中位数(年)		4(1~40)	5(1~45)	7(1~38)
大籽蒿花粉(SPT^{+})		22	6	12
屋尘螨(SPT^{+})		18	12	8
梧桐花粉(SPT^{+})		6	6	6

a) *n*为总数，表中年龄、病程和发病年龄的描述均为中位数(极小值~极大值). SPT为皮肤点刺试验. HC(healthy control), 健康对照组; AR(allergic rhinitis), 过敏性鼻炎组; AA(allergic asthma), 哮喘组; ARA(allergic rhinitis combined with asthma), 过敏性鼻炎合并哮喘组

HDME或PPE在CO_2培养箱中37°C温度条件下刺激1 h. 用含0.5% FBS的RPMI 1640培养液重悬细胞.

(2) 流式细胞术检测: 将Human TruStain FcX™, 亮紫510^{T}mol/L Zombie Aqua™活细胞鉴定液与外周血细胞悬液在室温下避光孵育15 min. 室温下加入PE/Cy7标记抗人CD14抗体, 避光孵育15 min. 加入红细胞裂解液室温避光12 min, 离心(1200×*g*, 6 min, 20°C)弃上清. 加入细胞固定/透膜液, 4°C孵育20 min后加入5 μL FITC标记抗人TLR9抗体4°C孵育30 min. 加入1 mL透膜洗液, 离心(2500×*g*, 10 min), 弃上清, 细胞用磷酸盐缓冲液(PBS)重悬后在FACSVerse流式细胞仪上检测, 观察TLR9^{+}细胞比例在单核细胞群中的变化和TLR9在单核细胞群内表达的平均荧光强度(MFI)变化.

1.4 建造小鼠模型

(1) AR模型: 将SPF级环境中饲养的WT小鼠和FcεR Ⅰ KO小鼠按随机数字表法分为三组: 第一组为过敏原实验组(过敏原提取液免疫+过敏原提取液激发), 第二组为过敏原对照组(过敏原提取液免疫+生理盐水(normal saline, NS)激发), 第三组为空白对照组(NS免疫+NS激发), 每组6只鼠. 参照既往文献方法建立AR[15,16]小鼠模型, 具体操作流程如图1所示.

(2) AA模型: 参照既往文献方法建立AA[17,18]小鼠模型, 具体操作流程如图2所示.

1.5 小鼠标本采集及处理

(1) 血液. AR模型小鼠最后一次滴鼻24 h后取血, AA模型小鼠最后一次雾化吸入24 h后取血, 抗凝处理, 每只小鼠150 μL.

(2) 肺组织. AA模型小鼠摘取肺组织. 新鲜肺组织经生理盐水灌洗后, 剔除大血管, 剪碎至0.5 mm^{3}大小, 用0.5% FBS的RPMI 1640培养液洗涤(500×*g*, 20°C, 6 min), 重复2次. 用1:10(w/v)加入含Ⅰ型胶原酶(2 mg/mL)和透明质酸酶(1 mg/mL)的DMEM培养液, 在37°C下与肺组织共同孵育65 min, 消化后的组织悬液用100 μm滤网过滤后离心(500×*g*, 20°C, 6 min). 收集细胞, 用牛鲍计数板计数, 用0.5% FBS的RPMI 1640培养液重悬细胞, 调整至1×10^{6} cells/mL.

1.6 流式细胞术检测鼠TLR9 的表达

(1) 血液. 将BV510 Zombie Aqua™(1 μL)+TruStain FcX™(3 μL)活细胞鉴定液与外周血细胞悬液在室温下

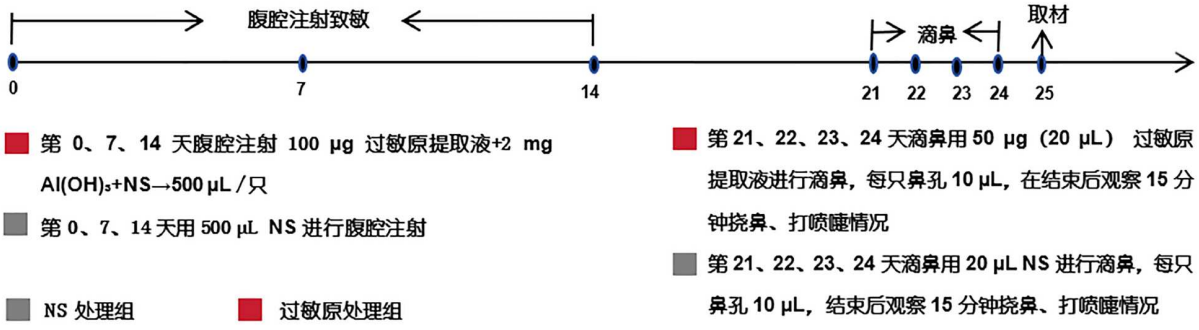

图 1 (网络版彩色)建立AR小鼠模型
Figure 1 (Color online) Establishment of AR mouse model

图 2 (网络版彩色)建立AA小鼠模型
Figure 2 (Color online) Establishment of AA mouse model

避光孵育15 min. 室温下加入BV510^{TM}-Gr-1+PerCP-CD11b(各5 μL)室温避光孵育15 min. 加入红细胞裂解液, 室温避光12 min, 离心(1200×*g*, 6 min, 20°C)弃上清. 加入细胞固定/透膜液, 4°C孵育20 min后加入5 μL APC-抗鼠TLR9 (CD289)抗体, 4°C避光孵育30 min.

(2) 肺组织细胞悬液. 将BV510 Zombie Aqua™ (1 μL)+TruStain FcX™(3 μL)活细胞鉴定液与肺组织细胞悬液在室温下避光孵育15 min. 室温下加入PE/Cy7-CD11c+BV510^{TM}-F4/80(各5 μL), 室温避光孵育15 min. 加入1 mL洗液, 离心(2500×*g*, 10 min), 弃上清, 细胞用PBS重悬后在FACSVerse流式细胞仪上检测.

1.7 流式细胞仪分析

一级设门: 通过前向散射/侧向散射(FSC/SSC)参数圈定单个核细胞群. 二级设门: 在活细胞(Zombie Aqua™阴性)区域内, 人血液标本以CD14^{+}细胞作为单核细胞群; 小鼠血液以Gr-1^{lowmid}, CD11b^{mid}细胞作为单核细胞群[19]; 小鼠肺组织细胞悬液以F4/80^{+}、CD11c^{+}细胞作为巨噬细胞群[20]. 在圈定单核细胞群后, 检测TLR9在细胞内的表达.

1.8 数据处理和统计学分析

使用SPSS 13.0进行统计学分析, 采用Kruskal-Wallis秩和检验分析各组间数据, 如果组间差异有统计学意义, 用Mann-Whitney U检验进一步比较组内各组数据是否有统计学意义. 人源样本分析: AR、AA和ARA患者与健康对照组单核细胞TLR9表达情况的数据采用散点图展示; 小鼠模型分析: 血液单核及肺组织巨噬细胞TLR9表达数据采用箱式图呈现; 所有分析结果P<0.05表示具有显著性差异.

2 结果

2.1 人外周血单核细胞TLR9 表达变化

流式细胞分析人外周血CD14^{+}单核细胞群(图3(a))发现TLR9^{+}细胞比例在AR组、AA组及ARA组均显著高于HC组, 分别增加2.64、2.05和2.32倍(P<0.05)(图3(b, c)). ASWE刺激使TLR9^{+}细胞比例在AA组有显著增加(P<0.05). 图3(d, e)显示, TLR9在单核细胞内表达的MFI在AR组、AA组及ARA组均显著高于HC组, 分别上调1.27、1.45和2.14倍(P<0.05). HDME刺激使AR组TLR9 MFI提升1.15倍(P<0.05); AA组和ARA组在过敏原刺激后TLR9 MFI无显著变化.

2.2 AR和AA模型小鼠血液单核细胞中TLR9表达特征

2.2.1 AR模型小鼠

本实验用Gr-1^{lowmid}和CD11b^{mid}双标记策略鉴定小鼠血液单核细胞(图4(a)). 流式细胞分析结果显示, WT小鼠中: 过敏原ASWE和HDME激发组TLR9^{+}细胞百分比与NS组相比均显著升高(P<0.05); ASWE-ASWE与ASWE-NS相比, HDME-HDME与HDME-NS相比TLR9^{+}细胞百分比均显著升高(P<0.05)(图4(b, d)). FcεR Ⅰ KO小鼠中, ASWE-ASWE与ASWE-NS或NS-NS组相比TLR9^{+}细胞百分比显著升高(P<0.05), HDME-HDME、HDME-NS与NS-NS组相比TLR9^{+}细胞百分比均显著升高(P<0.05). 除ASWE-ASWE组外, FcεR Ⅰ KO鼠其他各组TLR9^{+}细胞比例较WT小鼠显著减少(P<0.05)(图4(b, d)).

WT小鼠中, 与NS-NS组相比, 各过敏原刺激组单核细胞中TLR9 MFI均明显上调(P<0.05)(图4(c, e)). FcεR Ⅰ KO小鼠中, ASWE-ASWE组与ASWE-NS或NS-NS组相比, TLR9 MFI均显著升高(P<0.05); 相似地, HDME-HDME组与HDME-NS与NS-NS组相比, TLR9 MFI均显著升高(P<0.05)(图4(c, e)). 除NS-NS组外, FcεR Ⅰ KO鼠其他各组TLR9 MFI表达水平较WT小鼠对应的各组的TLR9 MFI表达水平均显著减少(P<0.05)(图4(c, e)).

2.2.2 AA模型小鼠

流式检测分析结果显示: WT小鼠中, ASWE-ASWE、HDME-HDME、HDME-NS组的单核细胞群中TLR9^{+}细胞百分比与NS组相比均显著升高(P<0.05)(图5(a, c)); ASWE-ASWE组TLR9^{+}细胞百分比显著高于ASWE-NS组(P<0.05); FcεR Ⅰ KO小鼠中, HDME-NS和HDME-HDME组TLR9^{+}细胞百分比显著高于NS组(P<0.05)(图5(a, c)). FcεR Ⅰ KO鼠ASWE-ASWE组和HDME-NS组TLR9^{+}细胞比例较WT小鼠相对应的各组显著减少(P<0.05)(图5(a, c)).

WT小鼠中, 与NS-NS组相比, ASWE-ASWE、HDME-HDME、HDME-NS组的单核细胞群中TLR9表达强度(MFI)均明显上调(P<0.05); ASWE-ASWE与ASWE-NS、HDME-HDME与HDME-NS组相比, 单核细胞群中MFI均明显上调(P<0.05)(图5(b, d)). FcεR Ⅰ KO小鼠中, 与NS-NS组相比, ASWE-ASWE、HDME-

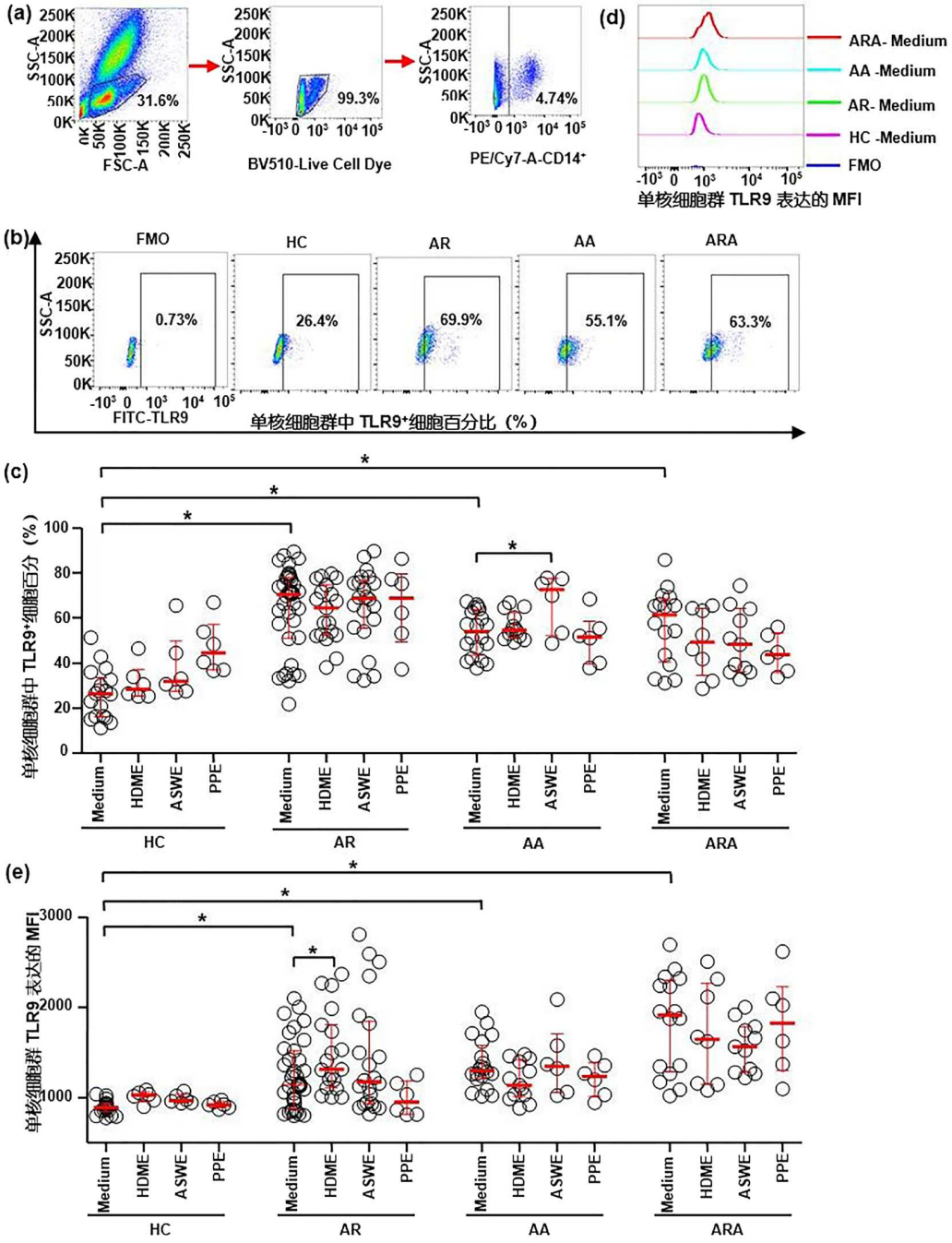

图 3 (网络版彩色)流式细胞术分析人外周血CD14$^+$单核细胞群中TLR9表达百分比和平均荧光强度(mean fluorescence intensity, MFI)的变化. (a) 单核细胞的设门策略图; (b) 单核细胞群中TLR9$^+$细胞百分比代表图; (c) 单核细胞群中TLR9$^+$细胞百分比散点图; (d) 单核细胞群表达TLR9的MFI代表图; (e) 单核细胞群表达TLR9的MFI散点图. *, P<0.05. FMO: 荧光减一对照. 试验对象为过敏原激发前后健康受试者(HC)、过敏性鼻炎(AR)、过敏性哮喘(AA)、过敏性鼻炎合并哮喘(ARA)患者. 屋尘螨过敏原提取物(HDME)、大籽蒿过敏原提取物(ASWE)及梧桐花粉过敏原提取物(PPE)浓度均为1 μg/mL. 每个符号代表一个标本, 中位数由中间的一条红色水平线表示

Figure 3 (Color online) Flow cytometry analyses of changes in percentage and mean fluorescence intensity (MFI) of TLR9 expression in human peripheral blood CD14$^+$ monocyte population. (a) Gated strategy diagram for monocytes; (b) representative graph of percentage of TLR9$^+$ cells in monocyte population; (c) scatter plot of percentage of TLR9$^+$ cells in monocyte population; (d) representative graph of TLR9 MFI in monocyte population; (e) MFI scatter plot of TLR9 expression in monocyte population. *, P<0.05. FMO: fluorescence minus one. The test subjects are healthy subjects (HC), patients with allergic rhinitis (AR), allergic asthma (AA), and allergic rhinitis combined with asthma (ARA) before and after the allergen challenge. The concentration of house dust mite allergen extract (HDME), artemisia sieversiana wild allergen extract (ASWE) and platanus pollen allergen extract (PPE) was 1 μg/mL. Each symbol represents a specimen, with the median value represented by a red horizontal line in the middle

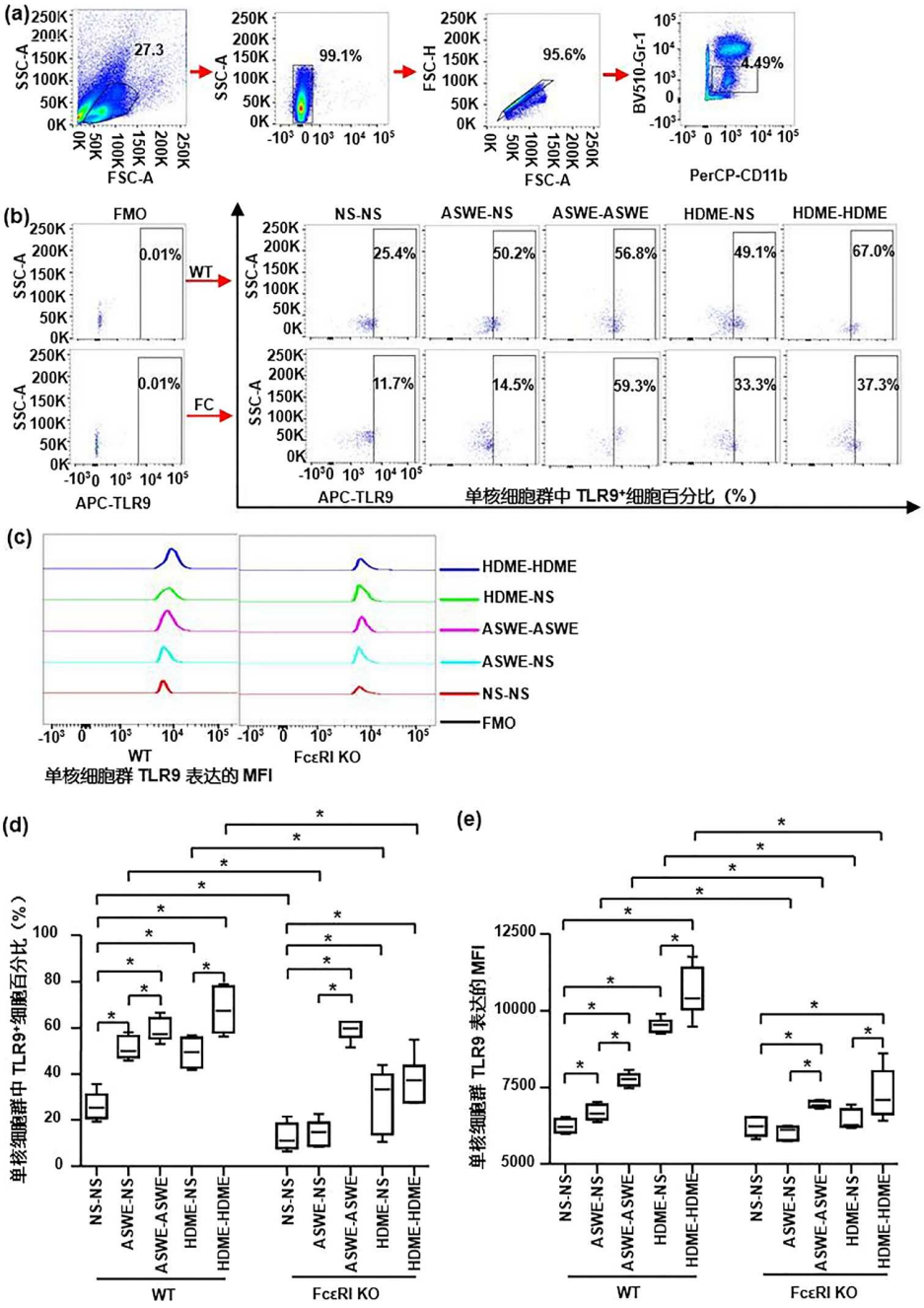

图 4 (网络版彩色)流式细胞术分析过敏性鼻炎(AR)模型小鼠血液单核细胞群TLR9表达的百分比和平均荧光强度(MFI)的变化. (a) 小鼠血液单核细胞设门, 以Gr-1^{lowmid}和CD11b^{mid}双标记策略鉴定小鼠血液单核细胞; (b) TLR9^{+}细胞占单核细胞的百分比(%)代表图; (c) 单核细胞TLR9表达的MFI代表图; (d) TLR9^{+}细胞占单核细胞百分比(%); (e) 单核细胞TLR9表达的MFI. *, $P<0.05$. FMO为荧光减一对照. NS-NS, 生理盐水免疫-生理盐水激发组; ASWE-ASWE, 大籽蒿过敏原提取物免疫-大籽蒿过敏原提取物激发组; ASWE-NS, 大籽蒿过敏原提取物免疫-生理盐水激发组; HDME-HDME, 屋尘螨过敏原提取物免疫-屋尘螨过敏原提取物激发组; HDME-NS, 屋尘螨过敏原提取物免疫-生理盐水激发组; WT, 野生型小鼠; FcεR Ⅰ KO, *FcεR Ⅰ*基因敲除小鼠

Figure 4 (Color online) Flow cytometry analyses of changes in percentage and mean fluorescence intensity (MFI) of TLR9 expression in the blood mononuclear cell population of allergic rhinitis (AR) model mice. (a) Mouse blood mononuclear cells were identified using a dual labeling strategy of Gr-1lowmid and CD11bmid; (b) representative graph of the percentage (%) of TLR9^{+} cells in the monocyte population; (c) representative graph of MFI of TLR9 expression in monocytes; (d) percentage of TLR9^{+} cells in monocytes (%); (e) MFI of TLR9 expression in monocytes. *, $P<0.05$. FMO is a fluorescence minus one. NS-NS, the NS immunization and NS challenge group; ASWE-ASWE, the ASWE immunization and ASWE challenge group; ASWE-NS, ASWE immunization and NS challenge group; HDME-HDME, the HDME immunization and HDME challenge group; HDME-NS, the HDME immunization and NS challenge group; WT, wild-type mouse; FcεR Ⅰ KO, *FcεR Ⅰ* gene knockout mouse

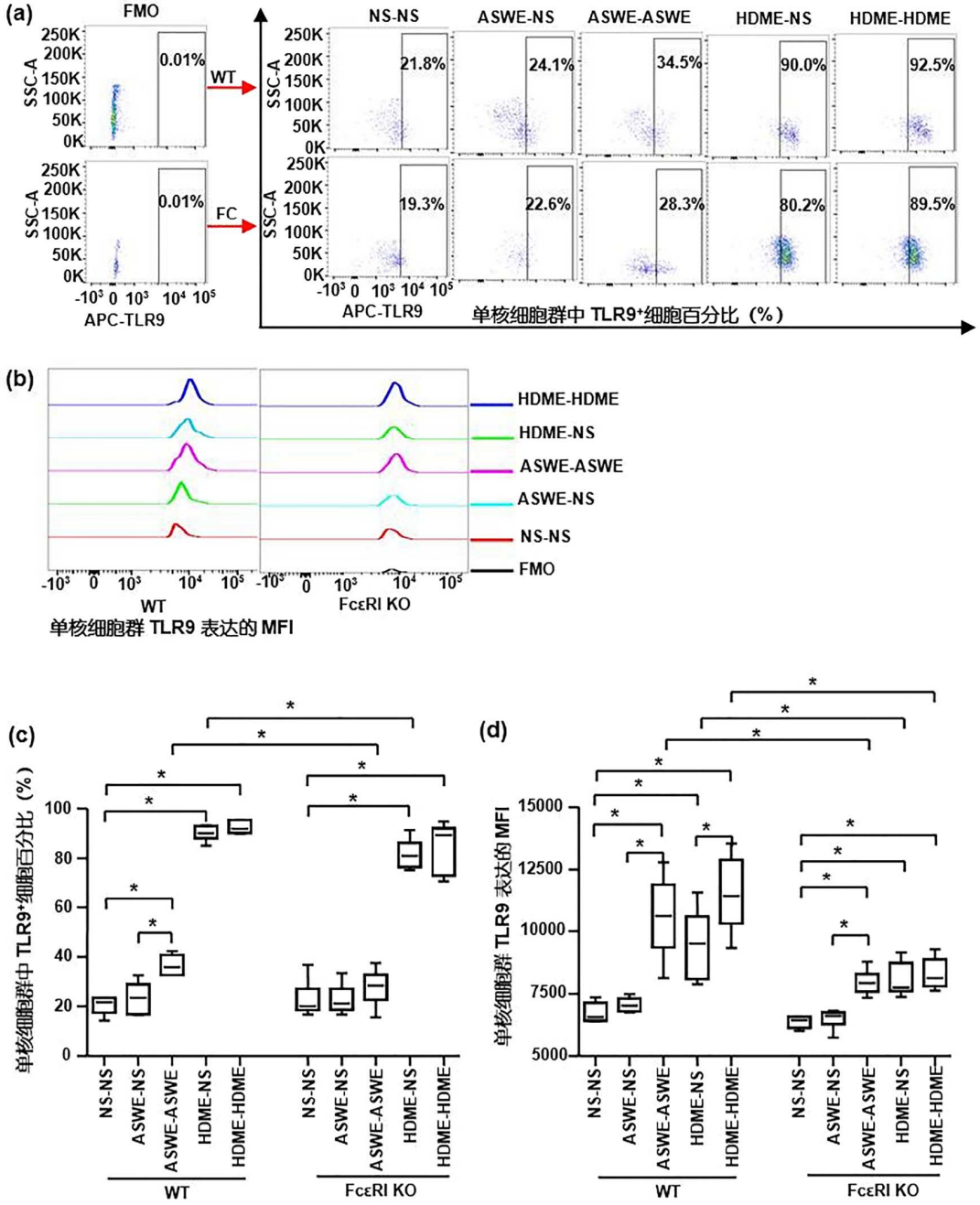

图 5 (网络版彩色)流式细胞术分析过敏性哮喘(AA)模型小鼠血液单核细胞群TLR9表达的百分比和平均荧光强度(MFI)的变化. (a) TLR9^{+}细胞占单核细胞的百分比(%)代表图; (b) 单核细胞TLR9表达的MFI代表图; (c) TLR9^{+}细胞占单核细胞百分比(%); (d) 单核细胞TLR9表达的MFI. *, P<0.05. FMO为荧光减一对照. NS-NS, 生理盐水免疫-生理盐水激发组; ASWE-ASWE, 大籽蒿过敏原提取物免疫-大籽蒿过敏原提取物激发组; ASWE-NS, 大籽蒿过敏原提取物免疫-生理盐水激发组; HDME-HDME, 屋尘螨过敏原提取物免疫-屋尘螨过敏原提取物激发组; HDME-NS, 屋尘螨过敏原提取物免疫-生理盐水激发组; WT, 野生型小鼠; FcεR Ⅰ KO, *FcεR Ⅰ*基因敲除小鼠

Figure 5 (Color online) Flow cytometry analyses of changes in percentage and mean fluorescence intensity (MFI) of TLR9 expression in the blood mononuclear cell population of allergic asthma (AA) model mice. (a) Mouse blood mononuclear cells were identified using a dual labeling strategy of Gr-1lowmid and CD11bmid; (b) representative graph of the percentage (%) of TLR9+cells in monocyte population; (c) representative graph of MFI of TLR9 expression in monocytes; (d) percentage of TLR9+cells in monocytes (%); (e) MFI of TLR9 expression in monocytes. *P<0.05 indicates a significant difference. FMO is a fluorescence minus one. NS-NS, the NS immunization and NS challenge group; ASWE-ASWE, the ASWE immunization and ASWE challenge group; ASWE-NS, ASWE immunization and NS challenge group; HDME-HDME, the HDME immunization and HDME challenge group; HDME-NS, the HDME immunization and NS challenge group; WT, wild-type mouse; FcεR Ⅰ KO, *FcεR Ⅰ* gene knockout mouse

HDME、HDME-NS组的单核细胞群中TLR9 MFI均明显上调($P<0.05$); ASWE-ASWE组与ASWE-NS相比, TLR9 MFI显著升高($P<0.05$)(图5(b, d)). FcεR Ⅰ KO鼠ASWE-ASWE、HDME-NS和HDME-HDME组TLR9 MFI表达水平较WT小鼠相对应的各组的TLR9 MFI表达水平均显著减少($P<0.05$)(图5(b, d)).

2.3 AA模型小鼠肺组织巨噬细胞中TLR9 表达特征

本实验采用CD11c、F4/80双标记策略鉴定肺组织巨噬细胞(图6(a)). 流式细胞分析结果显示: WT小鼠中, 过敏原ASWE-ASWE、ASWE-NS、HDME-HDME组与NS-NS组相比巨噬细胞群TLR9$^+$细胞百分比均显著升高($P<0.05$); ASWE-ASWE与ASWE-NS相比, HDME-HDME与HDME-NS相比TLR9$^+$细胞百分比均显著升高($P<0.05$)(图6(b, d)). FcεR Ⅰ KO小鼠中, ASWE-ASWE与NS-NS组相比TLR9$^+$细胞百分比显著升高($P<0.05$), HDME-HDME与HDME-NS组相比TLR9$^+$细胞百分比均显著升高($P<0.05$)(图6(b, d)). FcεR Ⅰ KO鼠各组TLR9$^+$细胞比例较WT小鼠相对应的各组显著减少($P<0.05$)(图6(b, d)).

WT小鼠中, 与NS-NS组相比, 除ASWE-NS外, 其他各过敏原刺激组巨噬细胞中TLR9 MFI均明显上调($P<0.05$), ASWE-ASWE与ASWE-NS组相比巨噬细胞中TLR9 MFI明显上调($P<0.05$)(图6(c, e)). FcεR Ⅰ KO小鼠中, ASWE-ASWE、HDME-HDME、HDME-NS组与NS-NS组相比, TLR9 MFI均显著升高($P<0.05$); ASWE-ASWE与ASWE-NS相比, 巨噬细胞中TLR9 MFI明显上调($P<0.05$)(图6(c, e)). FcεR Ⅰ KO鼠NS-NS、ASWE-ASWE、ASWE-NS组TLR9 MFI表达水平较WT小鼠相对应的各组的TLR9 MFI表达水平均显著减少($P<0.05$)(图6(c, e)).

3 讨论

本研究发现AR、AA及ARA患者外周血单核细胞群中TLR9$^+$细胞比例显著升高, 而且代表每个单核细胞内TLR9分子表达数量的MFI表达增加, 表明单核细胞可能通过TLR9表达上调参与上述过敏性疾病的病理生理过程. 体外试验结果, 过敏原提取物ASWE刺激增加AA患者TLR9$^+$细胞比例, HDME刺激上调TLR9 MFI进一步表明单核细胞通过TLR9参与气道过敏性疾病的可能性. 这一发现和近期研究报道的TLR9在过敏性疾病中被活化的结论保持一致[21]. 其机理可能基于持续抗原暴露: 环境过敏原和微生物通过呼吸道黏膜进入循环系统, 其DNA(CpG序列)持续刺激单核细胞TLR9, 使其通过TLR9-MYD88-IRF7通路诱导I型干扰素和促炎因子产生加重过敏反应[22]. 近期研究表明, 尘螨提取物中的细菌DNA污染是TLR9激活的重要来源[23]. ARA患者血单核细胞上TLR9表达升高, 提示TLR9$^+$单核细胞可能同时在鼻黏膜和支气管黏膜活化并向肺部迁移, 而TLR9激活会增强巨噬细胞MHC-II和共刺激分子的表达, 过度提呈抗原给Th2类细胞, 放大特异性免疫应答[24].

WT小鼠试验发现过敏原提取物ASWE和HDME激发AR和AA模型小鼠导致血液单核细胞群TLR9$^+$细胞百分比显著升高, 单核细胞中TLR9 MFI明显上调, 进一步表明过敏原提取物能够上调单核细胞中TLR9的表达水平, 支持上述AR、AA及ARA患者外周血单核细胞中TLR9的表达水平升高的发现. 本试验还发现FcεR Ⅰ KO的AR和AA模型小鼠, ASWE尤其是HDME也能诱导TLR9$^+$细胞百分比和单核细胞中TLR9 MFI上调. 结合FcεR Ⅰ KO的AR和AA小鼠TLR9$^+$细胞比例和TLR9 MFI表达水平较WT小鼠对应的各组百分比及表达水平显著减少的发现, 可以得出过敏原提取物诱导的血单核细胞比例增加和单核细胞中TLR9表达水平的上调是由FcεR Ⅰ依赖和非依赖性途径共同实现的. FcεR Ⅰ非依赖性途径可能涉及: 提取物中的细菌DNA持续刺激单核细胞TLR9表达, 尘螨蛋白酶通过蛋白酶激活受体(PAR2)直接激活TLR9信号[25], 补体C5a受体通过PI3K-Akt通路增强TLR9的信号转导[26]等机制.

WT小鼠试验还发现过敏原提取物ASWE和HDME激发可以诱发AA模型小鼠肺组织巨噬细胞群TLR9$^+$细胞百分比显著升高, 巨噬细胞中TLR9 MFI明显上调, 提示单核-巨噬细胞转化的可能性: 可能的机制为外周血中TLR9$^+$单核细胞通过CCL2-CCR2轴迁移至肺部, 分化为巨噬细胞[27]并维持TLR9高表达. 在过敏原的刺激之下单核细胞具有更强的组织迁移能力[28], 进而在局部组织形成一个正反馈循环: 肺组织中的TLR9$^+$巨噬细胞释放IL-6和IFN[29], 这些细胞因子进入血液, 又可进一步上调血液中单核细胞TLR9表达, 形成一种新的局部炎症放大环路.

FcεR Ⅰ KO小鼠试验发现过敏原提取物ASWE和HDME可以诱发AA模型小鼠肺组织巨噬细胞群TLR9$^+$细胞百分比显著升高, 巨噬细胞中TLR9 MFI明

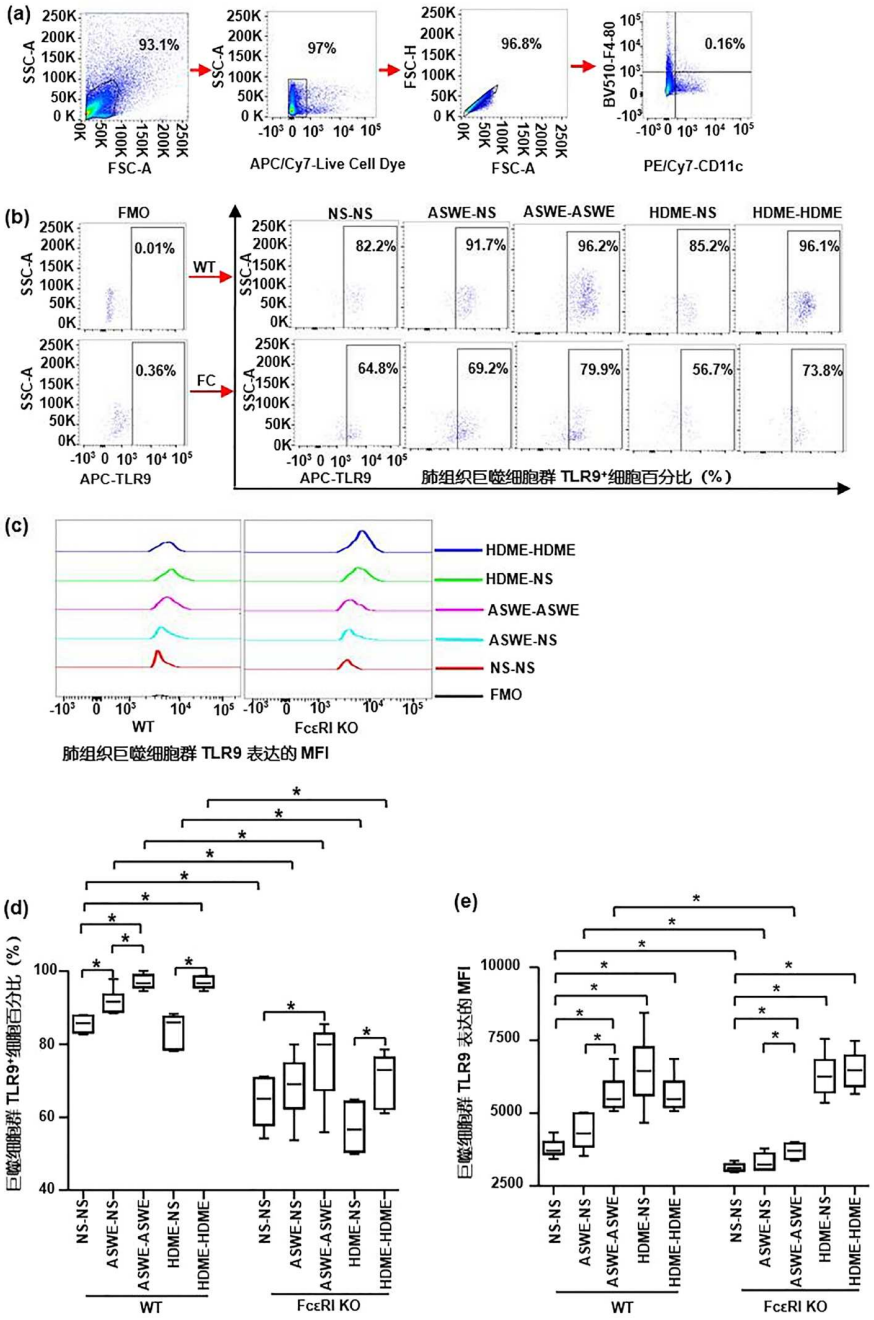

图 6 (网络版彩色)流式细胞术分析过敏性哮喘(AA)模型小鼠肺组织巨噬细胞群TLR9表达的百分比和平均荧光强度(MFI)的变化. (a) 小鼠肺组织巨噬细胞设门, 以CD11c$^+$、F4/80$^+$双标记策略鉴定小鼠肺组织巨噬细胞; (b) TLR9$^+$细胞占巨噬细胞的百分比(%)代表图; (c) 巨噬细胞TLR9表达的MFI代表图; (d) TLR9$^+$细胞占巨噬细胞百分比(%); (e) 巨噬细胞TLR9表达的MFI. *, P<0.05. FMO为荧光减一对照. NS-NS, 生理盐水免疫-生理盐水激发组; ASWE-ASWE, 大籽蒿过敏原提取物免疫-大籽蒿过敏原提取物激发组; ASWE-NS, 大籽蒿过敏原提取物免疫-生理盐水激发组; HDME-HDME, 屋尘螨过敏原提取物免疫-屋尘螨过敏原提取物激发组; HDME-NS, 屋尘螨过敏原提取物免疫-生理盐水激发组; WT, 野生型小鼠; FcεR Ⅰ KO, *FcεR Ⅰ*基因敲除小鼠

Figure 6 (Color online) Flow cytometry analyses of changes in percentage and mean fluorescence intensity (MFI) of TLR9 expression in the macrophage populations obtained from the lung tissue of allergic asthma (AA) model mice. (a) Mouse lung tissue macrophages were identified using a CD11c$^+$ and F4/80$^+$ dual labeling strategy; (b) representative graph of the percentage (%) of TLR9$^+$ cells in macrophages; (c) representative graph of TLR9 MFI expression in macrophages; (d) percentage of TLR9$^+$ cells in macrophages (%); (e) MFI of TLR9 expression in macrophages. *, P<0.05. FMO is fluorescence minus one. NS-NS, the NS immunization and NS challenge group; ASWE-ASWE, the ASWE immunization and ASWE challenge group; ASWE-NS, ASWE immunization and NS challenge group; HDME-HDME, the HDME immunization and HDME challenge group; HDME-NS, the HDME immunization and NS challenge group; WT, wild-type mouse; FcεR Ⅰ KO, *FcεR Ⅰ* gene knockout mouse

显上调；结合FcεR Ⅰ KO小鼠TLR9^{+}细胞比例较WT小鼠相对应的各组显著减少，ASWE-ASWE、ASWE-NS组TLR9 MFI表达水平较WT小鼠相对应的各组的TLR9 MFI表达水平均显著减少的试验结果分析，也可以得出过敏原提取物诱导的肺组织巨噬细胞比例增加和巨噬细胞中TLR9表达水平的上调是由FcεR Ⅰ依赖和非依赖性途径共同实现的．这一点与上述血液单核细胞的分析结果一致．单核细胞可能通过TLR9表达上调参与AR、AA及ARA的病理生理过程，其机制可能是由FcεR Ⅰ依赖和非依赖性途径共同实现的．肺组织巨噬细胞中TLR9表达上调提示在过敏原的刺激之下单核细胞与巨噬细胞可能通过高表达TLR9在局部组织形成一种新的炎症信号放大环路，为理解过敏性气道疾病的发病机制提供了新的思路．

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Summary for “外周血单核细胞及肺巨噬细胞TLR9在过敏性鼻炎及哮喘发作时的表达变化”

Changes in the expression of TLR9 in PBMCs and pulmonary macrophages during allergic rhinitis and asthma attacks

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In recent years, the role of the monocyte macrophage system and Toll like receptor 9 (TLR9) in the pathogenesis of allergic diseases has attracted attention, but little is known about the relationship between changes in TLR9 expression on monocytes and macrophages and allergic rhinitis (AR) and allergic asthma (AA). This article uses flow cytometry to analyze the expression changes of $TLR9^+$ monocytes in peripheral blood of patients with AR, AA, and allergic rhinitis combined with asthma (ARA). And the AR and AA models of wild-type (WT) and *FcεR Ⅰ* gene knockout (FcεR Ⅰ KO) mice were used to investigate the expression changes of TLR9 in monocytes and macrophages in lung tissue of mice after allergen stimulation. It was found that the proportion of $TLR9^+$ cells in the peripheral blood mononuclear cell population of AR, AA, and ARA patients was significantly increased, indicating an increase in MFI expression representing the number of TLR9 molecules expressed in each mononuclear cell. Artemisia selengensis allergen extract (ASWE), house dust mite allergen extract (HDME), wutong pollen allergen extract (PPE) stimulate to increase the proportion of $TLR9^+$ monocytes in the blood of AR and AA model mice, upregulate the expression level of TLR9 MFI in monocytes, and make the expression level of TLR9 in macrophages in lung tissue higher than that of control mice. In AR and AA mouse models with high-affinity IgE receptor (FcεR Ⅰ) KO, ASWE, especially HDME, can also induce upregulation of $TLR9^+$ cell percentage and TLR9 MFI in monocytes. Based on the significant reduction in the proportion of $TLR9^+$ cells and TLR9 MFI expression levels in AR and AA mice compared to wild-type (WT) mice, it can be concluded that the increase in the proportion of blood monocytes induced by allergen extract and the upregulation of TLR9 expression levels in monocytes are achieved through both FcεR Ⅰ dependent and non-dependent pathways. Similarly, compared with WT mice, the AA model mice of FcεR Ⅰ KO showed a significant increase in the proportion of lung tissue macrophages induced by allergen extract and a significant decrease in the upregulation of TLR9 expression level. In summary, monocytes may participate in the pathophysiological processes of AR, AA, and ARA through upregulation of TLR9 expression. The upregulation of TLR9 expression in lung tissue macrophages further suggests that monocytes and macrophages may form a new inflammatory signal amplification loop in local tissues through high expression of TLR9 under allergen stimulation, providing new ideas for understanding the pathogenesis of allergic airway diseases.

allergic rhinitis, allergic asthma, monocyte, macrophage, Toll-like receptor 9

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