

非霍奇金淋巴瘤患者外周血中 CD4⁺CD25⁺ 调节性 T 细胞 亚群变化初探

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摘要 目的 检测非霍奇金淋巴瘤 (non-Hodgkin's lymphoma, NHL) 患者外周血中 CD4⁺CD25⁺ 调节性 T 细胞 (CD4⁺CD25⁺ regulatory T cell, Treg) 的改变,探讨 Treg 与 NHL 的相关性。方法 病例组 (n=60) 为本院收治的初诊 NHL 患者,对照组 (n=60) 为本院健康体检者,用流式细胞技术联合标记 CD4、CD25 检测对照组及病例组化疗前、化疗后的外周血中 CD4⁺CD25⁺ 调节性 T 细胞的分布特点。结果 ①病例组化疗前外周血中 CD4⁺ 细胞比例显著低于对照组 ($P<0.05$),CD4⁺CD25⁺ 调节性 T 细胞比例显著高于对照组 ($P<0.05$) ②病例组化疗后,CD4⁺ 细胞比例明显高于化疗前 ($P<0.05$),CD4⁺CD25⁺ 调节性 T 细胞比例明显低于化疗前 ($P<0.05$) ③病例组化疗后 CD4⁺ 细胞比例与对照组无显著差异 ($P>0.05$),而 CD4⁺CD25⁺ 调节性 T 细胞比例显著高于对照组 ($P<0.05$)。结论 非霍奇金淋巴瘤患者外周血中 CD4⁺CD25⁺ 调节性 T 细胞比例升高,存在机体免疫抑制,化疗可降低 CD4⁺CD25⁺ 调节性 T 细胞比例。

关键词 非霍奇金淋巴瘤; CD4⁺CD25⁺ 调节性 T 细胞; 外周血; 变化

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The Exploration of Changes of CD4⁺CD25⁺ Regulatory T Cells in Peripheral Blood of Patients with Non-Hodgkin's Lymphoma

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ABSTRACT Objective: To detect the proportion of CD4⁺CD25⁺ regulatory T cells (Treg) in peripheral blood of patients with non-Hodgkin's lymphoma(NHL), and to explore the relationship between Treg and NHL. **Methods:** By using flow cytometry with surface staining fluorochrome-conjugated antibodies for CD4, CD25, the percentages of CD4⁺CD25⁺Treg in peripheral blood of 60 patients with NHL and 60 Healthy controls were detected and analyzed. **Results:** ①The average peripheral blood CD4⁺ levels of patients with NHL before chemotherapy was significant lower than that of the control group ($P<0.05$), while the percentages of CD4⁺CD25⁺Treg was significant higher than that of the control group ($P<0.05$). ②After chemotherapy, the percentages of CD4⁺CD25⁺Treg was decreased and CD4⁺ levels was increased than before chemotherapy. The difference between them was statistically significant ($P<0.05$). ③ There was no significant difference between the control group and patients with NHL after chemotherapy of peripheral blood CD4⁺ level ($P>0.05$). But the percentages of CD4⁺CD25⁺Treg of patients with NHL after chemotherapy was still higher than the control group($P<0.05$). **Conclusion:** The increase in Tregs in patients with NHL may induce immune suppression and chemotherapy may cause the decreasing of Tregs.

Key words: Non-Hodgkin's lymphoma; CD4⁺CD25⁺regulatory T cells; Peripheral blood; Changes

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非霍奇金淋巴瘤 (NHL) 是常见的淋巴结系统恶性增殖性疾病,其发生发展与免疫功能密切相关, NHL 患者常伴有免疫功能抑制、细胞免疫功能紊乱和体液免疫功能紊乱^[1]。报道指出,在肿瘤环境中,CD4⁺CD25⁺Treg 细胞比例增加,直接导致肿瘤免疫失调^[2-4]。本调查检测 NHL 初诊患者与健康者外周血中 Treg 水平变化,分析 NHL 患者化疗前后外周血中 CD4⁺CD25⁺ Treg 细胞的变化,以探讨外周血中 Treg 水平与 NHL 的关系,现报道如下。

1 资料与方法

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1.1 一般资料

①病例组 (n=60) 2007 年 6 月 -2010 年 10 月本院收治的 60 例初诊 NHL 患者,均经病理学确诊,其中男性 39 例、女性 21 例,中位年龄 46.5 岁;②对照组 (n=60) 本院健康体检者 60 例,其中男性 40 例、女性 20 例,中位年龄 48.0 岁,排除肿瘤性疾病及感染性疾病;两组受检对象的一般情况无显著差异 ($P>0.05$),具有调查可比性。

1.2 检测方法

1.2.1 仪器与试剂 FC500 流式细胞仪 (美国 Beckman Coulter 公司);抗 CD4 单克隆抗体为异硫氰酸 (fluorescein isothiocyanate, FITC) 标记,抗 CD25 抗体为藻红蛋白 (R-phycoerythrin, R-PE) 标记,抗体均为美国 eBioscience 公司生产。

1.2.2 试剂配制 细胞固定剂及破膜液 (1× fixation/permeabi-

lization buffer) 由 4× fixation/permeabilization concentrate 和 fixation/permeabilization 按照 1:3 体积比配制而成; 1× permeabilization buffer 由 10× permeabilization buffer 和去离子蒸馏水按 1:9 体积比配制而成, 每一份血液样本需要 8mL 的 1× permeabilization buffer。

1.2.3 分组检测 本次调查检测健康对照组及病例组化疗前、病例组患者经 6~8 个周期 CHOP 或 CHOP-E 或 R-CHOP 方案化疗。

1.2.4 外周血中 CD4⁺CD25⁺Treg 的检测 清晨空腹抽取受检者肘静脉血 3mL,室温下经 EDTA 抗凝保存,4 小时内完成检测;采用流式细胞仪及荧光直接标记法检测外周血中单个核细胞膜表面 CD4⁺CD25⁺Treg,取 100 mL 细胞加入 1 mg CD4 和 0.125 mgCD25 混合抗体 20 mL,置 4℃ 中避光孵育 20 min,用冷 PBS 洗涤后弃去上清液,加入 1mL 的 1× fixation/permeabilization buffer,混匀后置 4℃ 中避光孵育 60min,用 1× fixa-

tion/permeabilization buffer 洗涤 2 次,用流式细胞仪进行检测,采用 Cellquest 软件进行分析,观察并记录阳性细胞百分比,减去非特异对照值。

1.3 统计学分析

采用 SAS 软件作单因素方差分析,以 P<0.05 为差异有显著性。

2 结果

2.1 临床资料

60 例 NHL 的病理类型以弥漫大 B 细胞型占多数 (30 例,占 50.0%), 其余依次为黏膜相关淋巴细胞型 (17 例,占 28.3%)、脾边缘区 B 细胞型 (8 例,占 13.3%)、滤泡细胞型 (3 例,占 5.0%)、套细胞型 (2 例,占 3.3%),各种病理类型的性别、年龄、分期情况见表 1。

表 1 60 例 NHL 的临床资料

Table 1 Clinical data of 60 patients with NHL

Dathologica typesl	Case Number	Sex		Age		Stage			
		Male	Female	<60 years old	≥ 60 year old	I	II	III	IV
Diffuse large B-cell lymphoma	30	19	11	19	11	4	11	11	4
Splenic marginal zone B cell type	8	6	2	4	4	1	4	3	0
Mucosa-associated lymphoid cell type	17	10	7	9	8	3	5	1	8
Follicle cell type	3	2	1	1	2	0	0	2	1
Mantle cell type	2	2	0	1	1	0	0	1	1
Figure up	60	39	21	34	26	8	20	18	14

2.2 外周血测定结果

(1) 病例组化疗前外周血中 CD4⁺ 细胞比例显著低于对照组 (P<0.05),CD4⁺CD25⁺ 调节性 T 细胞比例显著高于对照组 (P<0.05);(2) 病例组化疗后,CD4⁺ 细胞比例明显高于化疗前

(P<0.05),CD4⁺CD25⁺ 调节性 T 细胞比例明显低于化疗前 (P<0.05);(3) 病例组化疗后 CD4⁺ 细胞比例与对照组无显著差异(P>0.05),而 CD4⁺CD25⁺ 调节性 T 细胞比例显著高于对照组 (P<0.05);见表 2。

表 2 病例组化疗前、化疗后与对照组外周血中单个核细胞膜表面 CD4⁺CD25⁺ 调节性 T 细胞的对比

Table 2 Comparion of the CD4⁺CD25⁺ regulatory T cell between the healthy control group and the group of before and after chemotherapy($\bar{x} \pm s, \%$)

Group	n	CD4 ⁺	CD25 ⁺	CD4 ⁺ CD25 ⁺ Treg
Patients with NHL				
Before chemotherapy	60	26.13± 8.50*⊙	7.66± 7.45*	6.12± 4.02*⊙
After chemotherapy	60	34.59± 6.59	7.40± 6.22*	3.25± 2.90*
Healthy controls	60	37.38± 14.30	2.18± 0.98	1.86± 0.88

Note: *comparison with control group ,the difference was significant (P<0.05); ⊙comparison with group after chemotherapy, the difference was significant(P<0.05).

3 讨论

NHL 发生于淋巴结或 (和) 淋巴结外组织的一种恶性肿

瘤,国外报道指出^[5],其发病率有逐年稳定增长的趋势,已经成为发病率第七位的恶性肿瘤。近年来,许多文献报道^[6-7]揭示了调节性 T 细胞与实体瘤之间的关系,但有关调节性 T 细胞与

淋巴瘤的关系仍然存在争议^[8-10]。NHL 的病因复杂,其发病还可能与病毒感染、细菌感染、环境污染、家族遗传等有关^[11]。

随着肿瘤免疫逃逸方面的科学研究的深入,发现在肺癌、卵巢癌等实体肿瘤病人的瘤体组织中有 CD4⁺CD25⁺ Treg 浸润,而浸润程度与患者的预后呈负相关^[12]。文献报道提出^[13],慢性淋巴细胞白血病患者治疗前外周血中 CD4⁺CD25^{high}Treg 含量显著高于正常值,而治疗后有所降低。T 细胞免疫应答具有重要的抗肿瘤效应,临床应用单克隆抗体及流式细胞技术可以观察机体 T 细胞亚群变化,有利于研究 NHL 和人体细胞免疫功能状态的关系。CD4⁺T 细胞亚群是免疫应答中的主要反应细胞,可影响抑制性 T 淋巴细胞(T_s)、辅助性 T 淋巴细胞(T_H)、B 细胞抗体等表达及成熟,CD4⁺活化释放大量细胞因子可促进 CD8⁺细胞群及吞噬细胞的活性,从而增强肿瘤效应^[14]。CD4⁺CD25⁺调节性 T 细胞是一种免疫抑制细胞,1995 年由 Sakaguchi 等首次报道^[15],其免疫抑制记录尚未完全阐明。

本次调查结果显示,对照组健康体检者的外周血中 CD4⁺CD25⁺调节性 T 细胞比例为(1.86±0.88),与临床相关报道^[16-18]结果一致。以往临床多采用 CD4、CD25 标记 Treg^[19],2007 年开始有报道^[20-21]采用 CD4、CD25、CD127 联合标记外周血中 Treg 水平,也有报道^[22]提出 CD25 除了在 Treg 有表达,还在激活的效应 T 细胞有表达,目前联合各种标记检测外周血 Treg 水平仍无统一的标准方案。通过化疗能够改变肿瘤患者 Treg 比例,也有研究提示,手术切除肿瘤也能降低肺癌患者的 Treg 比例^[23]。

通过调查发现,CD4⁺CD25⁺调节性 T 细胞比例在 NHL 患者中显著升高,而化疗可通过一定的机制影响患者的免疫机能,显著提高 NHL 患者外周血中 CD4⁺T 细胞亚群的水平,但是无法降低 CD4⁺CD25⁺调节性 T 细胞比例至正常水平。化疗对免疫系统有明显的影 响,化疗后 NHL 患者外周血中 CD4⁺CD25⁺Treg 显著降低,机体免疫机制部分得到解除,可能是化疗活化了机体部分抗肿瘤免疫作用,进而清除微小残留病灶,但是仍然存在一定程度的免疫抑制,化疗能够起到适当的免疫干预作用。外周血中 CD4⁺CD25⁺Treg 比例变化在一定程度上反应了疾病的缓解情况,其诱导的抗肿瘤免疫反应具有抑制肿瘤生长或减少肿瘤发生的作用,如果临床能将特异性阻断外周血中 CD4⁺CD25⁺Treg 比例变化的治疗方法与增强机体特异性抗肿瘤免疫反应的手段有效结合起来,将有可能提高非霍奇金淋巴瘤的治疗效果。故此认为,外周血中 Treg 增加导致患者机体免疫受到抑制,与 NHL 存在密切关联,有望通过动态监测 NHL 患者外周血中 CD4⁺CD25⁺调节性 T 细胞水平来观察疾病发展及化疗的效果,指导临床治疗。

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