

rhG-CSF 动员对供者 CD4⁺ T 细胞免疫表型和功能影响 *汪菲 高春记[△] 黄文荣 李晓红 李猛

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摘要 目的 研究重组人粒细胞集落刺激因子 (rhG-CSF) 动员对供者 CD4⁺ T 细胞表面分子淋巴细胞功能相关抗原-1 (LFA-1)、细胞间黏附分子-1 (ICAM-1)、L-选择素 (LAM-1) 和人整合素-4 (VLA-4) 的表达及其介导的 CD4⁺ T 细胞功能的影响,探讨外周血干细胞移植过程中 CD4⁺ T 细胞免疫耐受机制。方法:使用三色荧光标记检测动员前及动员后第 5 天供者外周血 LFA-1、ICAM-1、LAM-1 和 VLA-4 的表达率,ELISA 方法检测动员前后 CD4⁺ T 细胞分泌 IFN- γ 和 IL-4 能力,免疫磁性分选法分离纯化 CD4⁺ T 细胞,检测动员前后 CD4⁺ T 细胞对基质细胞衍生因子-1 α (SDF-1 α) 的迁移能力和对 ICAM-1 的黏附能力。结果 动员前后 CD4⁺ T 细胞 LFA-1 (CD11a) 和 VLA-4 (CD49d) 表达率差异无统计学意义 ($P>0.01$),动员前后 CD4⁺ T 细胞 LAM-1 (CD62L) 和 ICAM-1 (CD54) 的表达率差异均有统计学意义,动员前显著高于动员后 ($P<0.01$)。动员前后 CD4⁺ T 淋巴细胞向 SDF-1 α 的迁移率差异无统计学意义 ($P>0.01$)。动员后 CD4⁺ T 细胞对 ICAM-1 的黏附率降低 ($P<0.01$)。动员后 IL-4 和 IFN- γ 两个细胞因子在外周血血清的浓度均降低 ($P<0.01$)。结论 rhG-CSF 动员不影响 CD4⁺ T 细胞 LFA-1 和 VLA-4 表达及 CD4⁺ T 细胞迁移,但影响 CD4⁺ T 细胞 ICAM-1 和 LAM-1 表达以及 CD4⁺ T 细胞通过 LFA-1 对 ICAM-1 的黏附能力影响,并可能影响 CD4⁺ T 细胞分泌细胞因子 IL-4 及 IFN- γ 的功能。

关键词 CD4⁺ T 细胞; rhG-CSF 动员; LFA-1; ICAM-1

中图分类号 R55 **文献标识码** A **文章编号** 1673-6273(2012)04-631-04

Impact on Immunophenotypes and Functions of CD4⁺ T Cell During Mobilization in Donors with rhG-CSF*WANG Fei, GAO Chun-ji[△], HUANG Wen-rong, LI Xiao-hong, LI Meng

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ABSTRACT Objective: To investigate the mechanism of CD4⁺ T cells immunotolerance during allogeneic peripheral blood stem cell transplantation by studying the impact of recombinant human granulocyte colony stimulating factor (rhG-CSF) on expression of lymphocyte function antigen-1 (LFA-1), intercellular adhesion molecule (ICAM-1), L-Selectin (LAM-1) and very late antigen 4 α (VLA-4) on CD4⁺ T cells, and functions of CD4⁺ T cells through these molecules during mobilization. Methods: The expression of LFA-1, ICAM-1, LAM-1 and VLA-4 of CD4⁺ T cells were determined by FACS before rhG-CSF mobilization and the fifth day of mobilization. The expression of IFN- γ and IL-4 by CD4⁺ T cells were examined with ELISA. At the same time, the migration and adhesion activity of CD4⁺ T cells to SDF-1 α and ICAM-1 were detected. Results: The expression of LFA-1 on CD4⁺ T lymphocytes were both 100%. The expression of VLA-4 didn't change markedly before and the fifth day of mobilization ($P>0.01$). The expression of LAM-1 (CD62L) and ICAM-1 (CD54) on CD4⁺ T lymphocytes decreased during mobilization ($P<0.01$). SDF-1 α induced CD4⁺ T lymphocytes migration for 4 hours didn't change markedly during mobilization ($P>0.10$). The adhesion activity of CD4⁺ T lymphocytes to ICAM-1 decreased markedly during mobilization ($P>0.10$). Conclusion: The concentration of IL-4 and IFN- γ in peripheral blood serum decreased markedly during mobilization ($P<0.01$). In conclusions, rhG-CSF does not have effect on the expression of LFA-1 and VLA-4 on CD4⁺ T cell, and the migration activity of CD4⁺ T cell during mobilization, but can affect the expression of ICAM-1 and LAM-1 on CD4⁺ T cell, and the adhesion activity of CD4⁺ T cell to ICAM-1 through LFA-1.

Key words: CD4⁺ T lymphocyte; rhG-CSF; Mobilization; LFA-1; ICAM-1

Chinese Library Classification (CLC): R55 **Document code:** A

Article ID: 1673-6273(2012)04-631-04

前言

重组人粒细胞集落刺激因子 (recombinant human granulocyte-colony-stimulating factor, rhG-CSF) 是异基因外周血造血

干细胞移植 (allogeneic peripheral blood stem-cells transplantation, allo-PBSCT) 中获得造血干/祖细胞最常用并且安全有效的动员剂^[1]。由于 PBSCTs 中 T 细胞及其他免疫活性细胞含量显著高于骨髓细胞^[2], 因此 allo-PBSCT 后移植抗宿主病

* 基金项目 国家自然科学基金项目 (30570776)

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(收稿日期 2012-01-07 接受日期 2012-01-30)

(graft-versus-host disease, GVHD) 发生率会显著高于异基因骨髓移植 (allo-BMT)。但已有许多临床研究和循证医学研究证实^[3]。allo-PBSCT 与 allo-BMT 的 aGVHD 发生率无显著性差异。CD4⁺ T 细胞是 GVHD 发生过程中产生免疫反应的重要细胞, 迁移和黏附以及分泌细胞因子能力是 CD4⁺ T 细胞执行其免疫功能的基础, 本研究通过观察动员前后 CD4⁺ T 细胞迁移和黏附能力以及分泌细胞因子功能的变化, 初步了解 rhG-CSF 对 CD4⁺ T 细胞表面 LFA-1 和 ICAM-1 等分子所介导功能和相关信号机制的影响, 探讨外周血干细胞移植过程中 CD4⁺ T 细胞免疫耐受机制和异基因外周血造血干细胞移植后 aGVHD 的发生率和严重程度并不高于异基因骨髓移植的原因。

1 材料和方法

1.1 标本来源

正常健康供者 12 例, 男 7 例, 女 5 例, 中位年龄 31.5 (21~51) 岁。

1.2 实验方法

1.2.1 动员方案 全面查体完毕后给予正常供者 rhG-CSF (惠尔血, KIRIN 公司) 10 μg/kg/d 进行动员, 连续进行 6 天。

1.2.2 动员前后细胞 LFA-1、ICAM-1、LAM-1 和 VLA-4 的表达检测 分别在 rhG-CSF 动员前 1 天和动员第 5 天取外周血 0.5 ml, 以 100 μl 全血制作 1 个标本, 以 Cy-CD4 结合 FITC-IgG1、PE-IgG1、FITC-CD3、FITC-CD11a、FITC-CD62L、PE-CD8、PE-CD49d、PE-54 (BD 公司) 进行三色荧光标记 30 min, 向每份标本加入 1.5 ml 红细胞裂解液裂解红细胞 10 min 后, 应用流式细胞术进行检测。

1.2.3 免疫磁性分离动员前后 CD4⁺ T 淋巴细胞 rhG-CSF 动员前 1 天和动员第 5 天各取肝素抗凝外周血 10 ml, 分离出单个核细胞后使用 PBS 洗涤两次。将洗涤后细胞悬于 300 μl PBS 中, 加入抗 CD4 免疫磁珠试剂 (Miltenyi Biotec 公司) 200 μl, 打匀, 4℃ 放置 30~45 min, 用 PBS 洗涤 2 次, 然后将细胞悬于 500 μl PBS 中, 将分离柱在磁铁中安放后, 把细胞悬液加到润湿好的分离柱上, 用 PBS 洗柱 3 遍。将从磁铁中取出分离柱, 从分离柱中洗脱细胞, 计数备用, 通过流式细胞术测定 CD4⁺ T 细胞纯度。

1.2.4 基质细胞衍生因子-1α 对动员前后 CD4⁺ T 淋巴细胞的迁移作用 在作为迁移下室的 24 孔组织培养板孔中加入含 80 ng/ml SDF-1α (Cytolab 公司) 和 0.1% BSA 的 RPMI-1640 液 600 μl, 将 Transwells (Costar 公司) 迁移杯 (直径 5 μm) 没入其中作为上室; 使用 0.1% BSA 的 RPMI-1640 液将分离所的 CD4⁺ T 细胞调整细胞密度为 5 × 10⁶/ml, 把 200 μl 细胞悬液加到迁移杯中, 培养 4 h 后, 通过计数下室细胞数计算出 SDF-1α 对 CD4⁺ T 细胞的迁移率。

1.2.5 动员前后 CD4⁺ T 淋巴细胞对 ICAM-1 的黏附能力的影响 以 100 μl/孔加入 3 μg/ml 的 ICAM-1 (Bender Medsystems 公司) 溶液, 包被 96 孔平底培养板孔, 4℃ 过夜。然后吸去 ICAM-1 溶液, 每孔加入 100 μl 含 1% BSA 的 PBS 封闭 2 h, 吸除后, 用无血清 RPMI-1640 洗涤 2 次, 然后再向每孔加入 100 μl 无血清 RPMI-1640 备用。使用 PBS-BSA 液将 CD4⁺ T 细胞密度调整为

2 × 10⁶/ml, 将 100 μl 细胞悬液加到前述处理的培养孔中, 然后每孔加入终浓度为 500 ng/ml 的 CD3 单克隆抗体 (Neo Markers 公司), 在 37℃、5% CO₂ 条件下, 孵箱内培养 90 min。吸去未黏附的细胞, PBS 洗涤培养板 3 遍。细胞定量分析通过 cytotox 96R non-radioactive cytotoxicity assay kit (Promega 公司) 进行: 每孔加入 90 μl PBS 以及 10 μl 裂解液, 4℃ 裂解 30 min 500 × g 离心 10 分钟; 各取每一样品 50 μl 加到另一 96 孔板后再向每孔加入 50 μl 的底物, 室温避光 30 分钟后, 每孔加入 50 μl 终止反应液以终止反应; 在检测波长 490 nm 的酶联仪上测定 A 值。细胞黏附率(%) = 各孔黏附细胞的 A 值 / 总体细胞的 A 值。

1.2.6 动员前后外周血血清 IFN-γ 和 IL-4 浓度 动员前后供者外周血离心后取血清 400 μl; 用人 IL-4 和 IFN-γ 定量 EIA 试剂盒 (森雄公司进口分装) 设标准孔, 建立标准曲线。待测品孔加入血清 100 ml/孔, 混匀后置 37℃ 120 min, 洗涤反应板; 加第一抗体工作液 50 μl/孔, 混匀后置 37℃ 120 min, 洗板同前; 加酶标抗体工作液 100 ml/孔, 混匀后置 37℃ 120 min, 洗板同前; 加入底物工作液 100 ml/孔, 置 37℃ 暗处反应 10 min, 加 1 滴终止液混匀后在 492 nm 测吸光值; 以标准品 1000、500、250、125、62.5、31.25、15.625、7.8125、3.90625、1.953125、0.9765625、0.48828125、0.244140625、0.1220703125、0.06103515625、0.030517578125、0.0152587890625、0.00762939453125、0.003814697265625、0.0019073486328125、0.00095367431640625、0.000476837158203125、0.0002384185791015625、0.00011920928955078125、0.000059604644775390625、0.0000298023223876953125、0.00001490116119384765625、0.000007450580596923828125、0.0000037252902984619140625、0.00000186264514923095703125、0.000000931322574615478515625、0.0000004656612873077392578125、0.00000023283064365386962890625、0.000000116415321826934814453125、0.0000000582076609134674072265625、0.00000002910383045673370361328125、0.000000014551915228366851806640625、0.0000000072759576141834259033203125、0.00000000363797880709171295166015625、0.000000001818989403545856475830078125、0.0000000009094947017729282379150390625、0.00000000045474735088646411895751953125、0.000000000227373675443232059478759765625、0.0000000001136868377216160297393798828125、0.00000000005684341886080801486968994140625、0.000000000028421709430404007434844970703125、0.0000000000142108547152020037174224853515625、0.00000000000710542735760100185871124267578125、0.000000000003552713678800500929355621337890625、0.0000000000017763568394002504646778106689453125、0.00000000000088817841970012523233890533447265625、0.000000000000444089209850062616169452667236328125、0.0000000000002220446049250313080847263336181640625、0.00000000000011102230246251565404236316680908203125、0.000000000000055511151231257827021181583340541015625、0.0000000000000277555756156289135105907916702705578125、0.0000000000000138777878078144567552953958351352890625、0.00000000000000693889390390722837764769791756764453125、0.00000000000000346944695195361418882384895878382265625、0.000000000000001734723475976807094411924479391911328125、0.0000000000000008673617379884035472059622396959556640625、0.0000000000000004336808689942017736029811198479778125、0.00000000000000021684043449710088680149055992398890625、0.00000000000000010842021724855044340074527996199453125、0.000000000000000054210108624275221700372639980997265625、0.0000000000000000271050543121376108501863199904986328125、0.00000000000000001355252715606880542509315999524931640625、0.000000000000000006776263578034402712546579997624658203125、0.0000000000000000033881317890172013562732899988123291015625、0.00000000000000000169406589450860067813664499940616455078125、0.0000000000000000008470329472543003390683224997030822765625、0.00000000000000000042351647362715016953416124985154113828125、0.000000000000000000211758236813575084767080624925770569140625、0.0000000000000000001058791184067875423835403124628852845703125、0.00000000000000000005293955920339377119177015623144264228515625、0.00000000000000000002646977960169688559588507811572132114278125、0.00000000000000000001323488980084844279794253905786066057140625、0.000000000000000000006617444900424221398971269528930330285703125、0.0000000000000000000033087224502121106994856347644651651428515625、0.0000000000000000000016543612251060553497428173822325825714278125、0.0000000000000000000008271806125530276748714086911162912857140625、0.00000000000000000000041359030627651383743570434555814564285703125、0.000000000000000000000206795153138256918717852172779072821428515625、0.000000000000000000000103397576569128459358926086389536410714278125、0.000000000000000000000051698788284564229679463043169768205357140625、0.0000000000000000000000258493941422821148397315215848841026785703125、0.00000000000000000000001292469707114105741986576079244205133928515625、0.00000000000000000000000646234853557052870993288039622102566964278125、0.00000000000000000000000323117426778526435499644019811051283482140625、0.000000000000000000000001615587133892632177498220099055256417410703125、0.0000000000000000000000008077935669463160887491100499276282087053515625、0.000000000000000000000000403896783473158044374555024963814104352678125、0.0000000000000000000000002019483917365790221872775124819070521763390625、0.00000000000000000000000010097419586828951109363875624095352608816953125、0.000000000000000000000000050487097934144755546819378120476763044084765625、0.0000000000000000000000000252435489670723777734096890602383815220423828125、0.00000000000000000000000001262177448353618888672084453011919076102119140625、0.000000000000000000000000006310887241768094443360422265055959555510559703125、0.0000000000000000000000000031554436208840472216802111325297977777527798515625、0.00000000000000000000000000157772181044202361084010556626489888887638992578125、0.000000000000000000000000000788860905221011805420052783132449444438194962890625、0.0000000000000000000000000003944304526105059027100263915662247222190974814453125、0.0000000000000000000000000001972152263052529513550131957831123611095487407140625、0.0000000000000000000000000000986076131526264756777565978915561805547743703515625、0.00000000000000000000000000004930380657631323783887829894577809027738718517578125、0.000000000000000000000000000024651903288156618919439149472889045138693592587890625、0.000000000000000000000000000012325951644078309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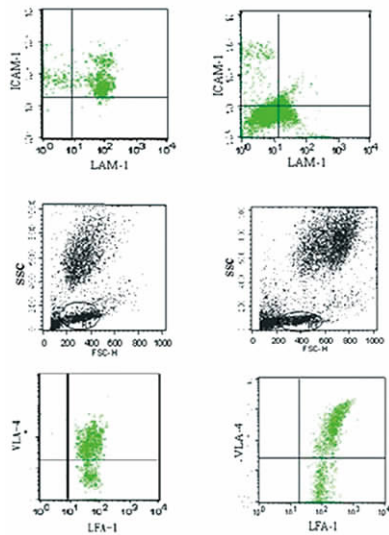


Fig. 2 CD4⁺T lymphocytes surface molecules before and after mobilization by rhG-CSF, R1 is lymphocytes.

流式细胞仪分析 CD4 免疫磁珠试剂分离富集获得的 CD4⁺T 细胞纯度在 94% 以上。SDF-1 α 作用于 CD4⁺T 细胞 4 h 后, 迁移率在 rhG-CSF 动员前后分别为 (28.56 $\pm$ 5.45)% 和 (31.35 $\pm$ 5.33)%, 动员前后 CD4⁺T 淋巴细胞向 SDF-1 α 迁移能力差异无统计学意义 (n=12, P>0.01)。

2.3 动员前后 CD4⁺T 淋巴细胞对 ICAM-1 的黏附能力的影响

CD4⁺T 细胞在 CD3 单克隆抗体作用下对 ICAM-1 的黏附率, rhG-CSF 动员前后分别为 (84.4 $\pm$ 6.45)% 和 (60.69 $\pm$ 10.18)%, 在 CD3 单抗作用下动员前 CD4⁺T 细胞对 ICAM-1 的黏附率显著高于动员后, 差异有统计学意义 (n=12, P<0.01)。

2.4 动员前后外周血清 IFN- γ 和 IL-4 浓度的影响

rhG-CSF 动员前后外周血清 IL-4 浓度分别为 21.15 $\pm$ 7.73 pg/ml 和 12.65 $\pm$ 6.43 pg/ml (n=12, P<0.01)。动员前后外周血清 IFN- γ 浓度分泌分别为 43.03 $\pm$ 18.05 pg/ml 和 20.74 $\pm$ 7.08 pg/ml (n=12, P<0.01) (图 3)。动员后两种细胞因子的浓度均下降, 差异有统计学意义 (P<0.01)。

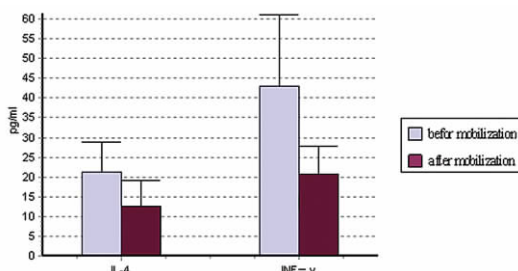


Fig. 3 Concentration of IFN- γ and IL-4 in donors' peripheral blood pre- and post-mobilization by rhG-CSF

3 讨论

rhG-CSF 是异基因外周血造血干细胞移植中最常用的造血干/祖细胞动员剂, 除可以动员造血干/祖细胞进入外周血循环外, 还对人体具有广泛的免疫调节作用。因此, 使用 rhG-CSF 动员的异基因外周血造血干/祖细胞, 可以显著减少

allo-PBSCT 时供者的痛苦, 而且可以加快移植受试者造血及免疫重建的速度。Franzke 等研究证实, 在单细胞水平上 CD4⁺T 细胞能够表达 G-CSF 受体, G-CSF 通过接触 CD4⁺T 细胞上的 G-CSF 受体, 诱导 T 细胞并活化其功能, 以减少 T 细胞的异源性反应能力, 增加 IL-4 分泌^[4,5]。Toh 等研究发现证实, G-CSF 动员后的外周血干细胞供者纯化的 T 细胞, 可以通过 G-CSF 上调 Th2 和 Treg 细胞相关基因, 下调 Th1 细胞和 GVHD 相关的基因诱导免疫耐受^[6]。因此, G-CSF 是有力的 T 细胞免疫调节剂, 通过改变外周血 T 淋巴细胞数量和功能, 进而影响移植后的 GVHD 的发生^[7,8]。故越来越多的研究者在重视 rhG-CSF 对造血干/祖细胞的动员效果的同时, 也更加重视 rhG-CSF 在动员过程中对免疫细胞的影响。

越来越多的研究表明, 黏附分子介导的 T 淋巴细胞的迁移和归巢在 GVHD 的发生发展过程中起重要作用。LAM-1 (CD62L) 作为 T 细胞与内皮细胞相互作用的归巢受体, 不仅可以介导淋巴细胞与内皮细胞的黏附以及参与细胞间的信号传导, 还可作为特异性归巢受体介导某些淋巴细胞亚群, 归巢于外周淋巴器特定部位。LFA-1 与 ICAM-1 互为配体, 能够在淋巴细胞、单核细胞以及巨噬细胞等多种免疫细胞表面表达。LFA-1/ICAM-1 是 T 细胞表面的一组黏附分子, 与 T 细胞活化和功能密切相关^[9-11]。VLA-4 (CD49d/CD29) 作为 T 细胞黏附分子的受体, 多表达于淋巴细胞表面, 免疫球蛋白超家族成员血管黏附分子-1 (VCAM-1) 是其相应配体。VLA-4/VCAM-1 主要是在协助内皮细胞与 T 细胞黏附并跨内皮游出时发挥作用。Jilma 等研究发现, 由于 CD62L 在动员后表达减弱, G-CSF 刺激白细胞后, 使其表面的 CD62L 活性呈一过性增强, 然后在 5~10 min 后脱落^[12]。也有报道小鼠移植后给予 CD62L 单抗, 可明显延迟 T 细胞进入淋巴结并使进入淋巴结的 T 细胞数量下降, 从而减低 aGVHD 的发生率并推迟发生时间, 而造血重建不受影响^[13]。Li 等研究证实, 抗 $\alpha 4$ 和 CD62L 抗体与供鼠脾细胞共同孵育, 结果迁移到外周淋巴结的能力大大减弱, 并且可以明显延迟 GVHD 死亡^[14]。Aaron 等的试验也发现, 人 T 细胞 CD62L 表达下降或缺失导致 T 细胞从 T 细胞感染区或聚集区进入到外周血^[15]。Kobayashi 等发现, GVHD 发生后外周血中 CD3⁺T 细胞表面的 ICAM-1 (CD54)、VLA-4 (CD49d) 和 VLA-5 表达上调^[16]。而 Isobe 等用特异性抗体联合阻断 LFA-1 和 ICAM-1, 其预防 GVHD 和植入失败的有效率明显高于比任何一种单一阻断^[17]。在本研究中, 动员后 CD4⁺T 细胞表面的 CD62L 表达率显著下降。由此可以推测, 在 G-CSF 动员后供者 CD4⁺T 细胞表面 CD62L 表达的降低, 可能减弱 CD4⁺T 细胞植入受者后的归巢能力, 阻碍其进入外周淋巴器官或靶器官, 进而影响 CD4⁺T 细胞的免疫效应。而 LFA-1 与 VLA-4 两种分子的表达在 rhG-CSF 动员前后差异无统计学意义, 但 ICAM-1 的表达显著下降, 从而影响了 T 细胞的功能。可见, rhG-CSF 动员后的 CD4⁺T 细胞黏附分子的表达下调可能会改变 T 细胞的迁移、归巢能力, 从而降低 GVHD 的发生率^[18]。

静息状态下的 T 细胞表面 LFA-1 不与其配体 ICAM-1 结合, 当 T 细胞受到刺激后, T 淋巴细胞受体 (TCR) 通路的第一信号引起 LFA-1 磷酸化, 从而使 LFA-1 与 ICAM-1 发生结合^[19]。

^[20]。因此,本研究中使用 CD3 单克隆抗体活化 TCR/CD3,激活 CD4⁺T 细胞,使 LFA-1 与 ICAM 发生结合。研究结果显示 rhG-CSF 动员后 CD4⁺T 细胞在 CD3 单克隆抗体作用下显著降低对 ICAM-1 的黏附率。提示 rhG-CSF 动员虽然不影响 CD4⁺T 细胞表面 LFA-1 的表达,但可能还可以通过影响与 LFA-1/ICAM-1 相关的信号通路或改变 LFA-1 的分子结构阻碍 CD4⁺T 细胞表面 LFA-1 与 ICAM-1 的黏附,从而阻碍 CD4⁺T 淋巴细胞结合到内皮血管。与此同时,CD4⁺T 淋巴细胞与 ICAM-1 黏附率的下降也阻碍与靶细胞或 APC 的结合,进而影响 CD4⁺T 淋巴细胞的活化和免疫效应的发挥,以上这些都有可能是不增加 GVHD 的原因^[21]。此外,本研究发现 rhG-CSF 动员可引 LFA-1/ICAM-1 信号通路障碍,这可能与 CD4⁺T 细胞分泌大量的 Th2 类细胞因子以及向 Th2 亚群极化有关;并可通过损伤 T 细胞的 CD28/B7 协同刺激信号,抑制 IL-4 等 Th2 类细胞因子生成。这与 Vasconcelos 和黄文荣等人的研究结果一致^[22,23]。由于 Th1、Th2 类细胞因子对 GVHD 的发生起着正负调节作用,具有互相抑制对方细胞分泌细胞因子作用,因此如果 rhG-CSF 动员没有影响 CD28/B7 以外的其它调节细胞因子生成的信号通路,在 IL-4 等 Th2 类细胞因子生成减低时 Th1 类细胞因子生成应该增加。但我们的研究显示, rhG-CSF 动员后 CD4⁺T 细胞分泌 IL-4 和 IFN- γ 均下降。具体机制尚不清楚,还需进一步研究。SDF-1 α 对 T 细胞具有很强的趋化作用,能使 T 细胞滚动和黏附于活化的内皮细胞^[24]。SDF-1 α 通过使淋巴细胞产生强大的剂量依赖性的钙离子流动而增强其迁徙能力,并激活微血管内皮细胞表达 VCAM-1 而通过 VLA-4/VCAM-1 途径使淋巴细胞完成跨内皮移动。本研究的研究发现, rhG-CSF 动员没有影响 CD4⁺T 淋巴细胞 VLA-4 表达率,也没有影响 CD4⁺T 淋巴细胞向 SDF-1 α 迁移,提示 rhG-CSF 动员可能并不影响 CD4⁺T 细胞的迁移功能,这与黄文荣等^[20]的研究结果一致。

rhG-CSF 动员不增高 aGVHD 发生率可能是由多种因素导致的。正如在本研究中所证实的, rhG-CSF 动员会引起 CD4⁺T 淋巴细胞中 LFA-1/ICAM-1 信号通路的改变以及其相关功能受损,这可能是 allo-PBSCT 后不升高 aGVHD 的发生率的一个重要原因。但 rhG-CSF 动员引起的受试者中 CD4⁺T 淋巴细胞功能改变以及移植造血重建过程中 CD4⁺T 淋巴细胞功能的改变是否会影响 GVHD 还需要更加深入的研究。总之, rhG-CSF 动员对 CD4⁺T 淋巴细胞具有广泛的免疫调节作用^[25-27],其下调 CD4⁺T 淋巴细胞上黏附分子的表达和 CD4⁺T 淋巴细胞功能改变为解释 rhG-CSF 的免疫调节作用增添了新的观点。因此,进一步探讨 rhG-CSF 动员对供着 CD4⁺T 细胞上黏附分子的表达和功能改变与临床预后的关系具有重要的临床意义。

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如此,EGFR 是 Grb2 主要的结合分子^[15,16]。除了作为受体主要效应分子,还有其它的机制证明 Grb2 参与到肿瘤形成的进程之中^[18]。此可以推断通过各种方法能阻碍 Grb2 的表达都可能对肿瘤的生长有一定程度的影响。

本研究成功地构建了 plenti-Grb2-SH2 重组慢病毒载体,用 BLAST 与 Grb2-SH2 基因序列进行对比,质粒所携带基因与 Grb2-SH2 序列全部一致,通过慢病毒转染 293T,转染效率比较理想;为建立 Grb2-SH2 基因真核稳定转染细胞系和进一步研究其在肿瘤中的确切机制提供了坚实的实验基础。

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