285~291.造血干细胞移植后淋巴细胞增殖症患者单个核细胞来源DC负载EBV抗原肽制备DC-CIK[J].郭晓玲,魏 雅,朱 平,牛志云,蔡圣鑫,张改玲,达万明,潘 峻.中国肿瘤生物治疗杂志,2010,17(3)

造血干细胞移植后淋巴细胞增殖症患者单个核细胞来源DC负载EBV抗原肽制备DC-CIK 点此下载全文

郭晓玲 魏 雅 朱 平 牛志云 蔡圣鑫 张改玲 达万明 潘 峻

河北医科大学 第二医院 血液内科，河北 石家庄 050051;解放军总医院 血液内科，北京 100036;河北医科大学 第二医院 血液内科，河北 石家庄 050051;北京大学 第一医院 血液内科， 北京 100034;河北医科大学 第二医院 血液内科，河北 石家庄 050051;河北医科大学 第二医院 血液内科，河北 石家庄 050051;河北医科大学 第二医院 血液内科，河北 石家庄 050051;解放军总医院 血液内科，北京 100036;河北医科大学 第二医院 血液内科，河北 石家庄 050051

基金项目：河北省自然科学基金资助项目（No. C2008001097）；国家高等学校博士学科点专项科研基金资助项目（No. 200800890011）；河北省卫生厅重点跟踪项目资助（No. GL200508）

DOI：10.3872/j.issn.1007-385X.2010.3.008

摘要：

目的：以造血干细胞移植后并发淋巴细胞增殖症（post transplant lympholiferative disorder, PTLD)患者外周血单核细胞培养诱导DC，负载抗原肽后制备DC CIK（cytokine induced killer cell），为探索新的PTLD治疗方法奠定基础。方法: 分离造血干细胞移植后EBV（Epstein Barr virus)感染致PLTD患者外周血单个核细胞，贴壁细胞培养诱导DC，悬浮细胞诱导CIK；负载EBV抗原肽LMP2后建立DC CIK共培养体系。流式细胞仪分析共培养前后细胞的免疫表型，ELISA检测共培养前后细胞上清IFN γ的分泌水平，基因扫描仪分析T细胞受体(T cell receptor, TCR)β家族基因谱。结果: 成功制备负载EBV抗原肽的DC CIK，HLA DR +CD86 +DC细胞从诱导前的12.5%增加到91.17%；DC CIK共培养14 d后，两例患者的CIK数量分别增加了5.3和6.8倍；CD3 +、CD8 +、CD3 +CD8 +以及CD3 +CD56 + 细胞比例在DC CIK共培养后均明显升高(均 P <0 05)。抗原肽负载的DC CIK共培养体系中IFN γ的分泌水平明显高于未经抗原肽负载的DC组\[（1 332.6±92.38）pg/ml vs (693.42±62.41) pg/ml, P <0.01）\]。DC CIK培养后细胞的 TCRβ 家族基因在5.2家族出现单克隆表达峰。结论：EBV抗原肽负载后DC可诱导DC CIK共培养体系中CD3 +CD8 +以及CD3 +CD56 + 细胞扩增，并分泌高水平IFN γ，为临床应用DC CIK对移植后EBV感染致PTLD患者进行过继性细胞免疫治疗提供实验基础。

关键词：树突状细胞 CIK细胞 EB病毒 移植后淋巴细胞增殖症 过继性细胞免疫治疗

Preparation of DC CIK using EBV peptide pulsed DC from peripheral blood mononuclear cells of post transplant lymphoproliferative disorder patients after hematopoietic stem cell transplantation Download Fulltext

GUO Xiao-ling WEI Ya ZHU Ping NIU Zhi-yun CAI Sheng-xin ZHANG Gai-ling DA Wan-ming PAN Ling

Department of Hematology, Second Hospital of Hebei Medical University, Shijiazhuang 050051, Hebei, China;Department of Hematology, General Hospital of LPA, Bejing 100036, China;Department of Hematology, Second Hospital of Hebei Medical University, Shijiazhuang 050051, Hebei, China;Department of Hematology, First Hospital of Peking University, Bejing 100034, China;Department of Hematology, Second Hospital of Hebei Medical University, Shijiazhuang 050051, Hebei, China;Department of Hematology, Second Hospital of Hebei Medical University, Shijiazhuang 050051, Hebei, China;Department of Hematology, Second Hospital of Hebei Medical University, Shijiazhuang 050051, Hebei, China;Department of Hematology, General Hospital of LPA, Bejing 100036, China;Department of Hematology, Second Hospital of Hebei Medical University, Shijiazhuang 050051, Hebei, China

Fund Project:Project supported by Natural Science Foundation of Hebei Province (No.C2008001097), and the Research Foundation for the Doctoral Program of Higher Institution of China (No. 200800890011), and the Key Follow up Program of Health Bureau of Hebei Province (N

Abstract:

Objective:To construct a DC CIK (cytokine induced killer cell) co culture system using peripheral blood mononuclear cells (PBMCs) derived DC from hematopoietic stem cell transplantation (HSCT) patients with post transplant lympholiferative disorder (PTLD) after pulsed with EBV special peptides, so as to lay a foundation for new adoptive immunotherapy of patients with PLTD after HSCT. Methods: PBMCs were obtained from patients with PTLD after HSCT; DC was induced from adherent cells; and CIK was induced from suspension cells. DC was further pulsed with EBV special peptides and co cultured with CIK to establish the DC CIK co culture system; the immunophenotype of cells in DC CIK system before and after co culture were determined by FACS, IFN γ secretion was assayed by ELISA, and TCRβ genealogy was examined by genetic analyzer. Results: The ratio of HLA DR +CD86 +DC increased from 12 5% to 91.17% after cytokine stimulation. After co culture with DC for 14 d, the numbers of CIK in two patients with PTLD increased to 5.3 and 6.8 times,respectively. The ratios of CD3 +, CD8 +, CD3 +CD8 +, and CD3 +CD56 + cells were significantly increased after DC CIK co culture. IFN γ level in peptide pulsed DC CIK group was significantly higher than that in peptide unpulsed DC CIK group（\[1 332.6±92.38\] pg/ml vs \[693.42±62.41\] pg/ml, P <0.05\]）; TCRβ genealogy assay found the clone expansion peak of 5.2 TCRβ subfamily in DC CIK co culture system. Conclusion: EBV peptide pulsed DC can induce CD3 +CD8 + and CD3 +CD56 + cell expansion in DC CIK co culture system with high level of IFN γ. DC CIK can be used as a new adoptive immunotherapy to HSCT patients with EBV infection and PTLD.

Keywords:dendritic cell (DC) cytokine induced killer cell (CIK) Epstein Barr virus(EBV) post transplant lymphoproliferative disorder (PTLD) adoptive immunotherapy