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Hepatic leukemia-associated macrophages exhibit a pro-inflammatory phenotype in Notch1-induced acute T cell leukemia

Xiao Yang[a], Wenli Feng[a], Rong Wang[a], Feifei Yang[a], Lina Wang[a], Shayan Chen[a], Chong Chen[a], Qian Ren[a], Guoguang Zheng[a,b,*]

[a] State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300020, China
[b] Center for Stem Cell Medicine, Chinese Academy of Medical Sciences, Beijing 100730, China

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ABSTRACT

Tumor-associated macrophages (TAMs) are well accepted and the pathological role of macrophages in hematopoietic malignancies have been proposed. Hepatomegaly is frequently observed in T cell acute lymphoblastic leukemia (T-ALL) patients with poor prognosis. However, the role of leukemia-associated macrophages (LAMs) in hepatic microenvironment remains unclear. Here, the characteristics of hepatic LAMs (H-LAMs) were studied in Notch1 induced T-ALL model. Increase in proportion and absolute counts of H-LAMs was detected with infiltration of inflammatory cells. Furthermore, H-LAMs exhibited a more M1-like phenotype distinct from that of TAMs in hepatocellular carcinoma and LAMs from BM or spleen in leukemia. Moreover, H-LAMs expressed increased level of cytokines in charge of recruiting inflammatory cells, which contributed to pro-inflammatory hepatic microenvironment.

1. Introduction

Macrophages are indispensable cellular component in both innate immunity and adaptive immunity. In response to environmental signals, macrophages undergo polarization to diverse activation states. For decades, the M1/M2 classification for activated macrophages, which are stimulated by IFNγ/LPS or IL-4/IL-13 respectively, was established and widely accepted (Martinez et al., 2009; Martinez et al., 2008). A colorwheel hypothesis was also proposed (Mosser and Edwards, 2008). In fact, due to the complexity of different microenvironments, phenotypes of macrophages are continuum and different from classical M1/M2 states. Even in steady state, macrophages in different organs showed different gene expression patterns, which were suggested to be determined by organ specific microenvironments (Yona et al., 2013). Furthermore, local macrophages acquired newly induced identity when transferred to another organ (Lavin et al., 2014). Macrophages in solid tumor microenvironments were suggested as tumor-associated macrophages (TAMs), which promoted the progression and metastasis of tumors in most case by enhancing angiogenesis, stimulating the proliferation, migration, and invasion of tumor cells (Pollard, 2004; Wynn et al., 2013). Typically, TAMs were regarded as M2 or M2-like macrophages (Tiainen et al., 2015).

Macrophages are also important components of hematopoietic niche (Winkler et al., 2010). Previous works demonstrated that macrophages mainly participated in the regulation of hematopoietic stem/progenitor cells (HSC/HPCs) and erythroid differentiation (Ehninger and Trumpp 2011; Chow et al., 2011; Hanspal and Hanspal, 1994). The roles of macrophages in hematopoietic malignancies were also studied. $CD68^+$ macrophages infiltrated in lymphoma sections were suggested as a new biomarker for risk stratification (Steidl et al., 2010). Anti-CD47 antibody alone or in combination with rituximab had desired therapeutic effect on human Non-Hodgkin lymphoma (NHL) and acute myeloid leukemia (AML) by enhancing macrophage phagocytosis (Chao et al., 2010; Majeti et al., 2009). Leukemia is hematopoietic malignancy characterized by early infiltration of leukemia cells to main organs during progression (Colmone et al., 2008). Abnormal activation of Notch signal pathway resulted in leukemia, typically T cell acute lymphoblastic leukemia (T-ALL). Hepatosplenomegaly, which was related to poor prognosis, were observed in 40%-70% T-ALL patients (Shih and Wang, 2007; Kai et al., 1996; Chiaretti and Foa, 2009). We previously

Abbreviations: T-ALL, T cell acute lymphoblastic leukemia; H-LAMs, hepatic leukemia-associated macrophages; TAMs, tumor-associated macrophages; HCC, hepatocellular carcinoma; BM, bone marrow; SP, spleen; HSC/HPCs, hematopoietic stem cells/hematopoietic progenitor cells

* Corresponding author at: State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300020, China.
E-mail address: zhengggtjchn@aliyun.com (G. Zheng).
URL: http://mailto:zhengggtjchn@aliyun.com (G. Zheng).

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Table 1
Primers used in this study.

Gene	Forward	Reverse
Arg1	5′-CAACCAGCTCTGGGAATCTG-3′	5′-AATCGGCCTTTTCTTCCTTC-3′
Ccl3	5′-CCGAACTGTTACTCTTGGCATCAT-3′	5′-CCTTCTTCTCACTGGGTCTTCTGA-3′
Ccl5	5′-GCCCTCACCATCATCCTCACT-3′	5′-TCTCTGGGTTGGCACACACT-3′
Ccl17	5′-TGCTTCTGGGGACTTTTCTG-3′	5′-TGGCCTTCTTCACATGTTTG-3′
Cd206	5′-CCTGAACAGCAACTTGACCA-3′	5′-GCAATGGCCATAGAAAGGAA-3′
Cd14	5′-CTGCGTGTGCTCGGGATTTC-3′	5′-CGTTGCGGAGGTTCAAGATGTT-3′
Cxcl1	5′-TCGTCTTTCATATTGTATGGTCAAC-3′	5′-CGAGACGAGACCAGGAGAAAC-3′
Cxcl9	5′-AGTGTGGAGTTCGAGGAACC-3′	5′-GAGTCCGGATCTAGGCAGG-3′
Cxcl11	5′-AGCTGCTCAAGGCTTCCTTA-3′	5′-AGTAACAATCACTTCAACTTTGTCG-3′
Gapdh	5′-TGAAGGTCGGTGTGAACGGATT-3′	5′-CTCGCTCCTGGAAGATGGTGAT-3′
Ifngr1	5′-CGAAGCAGCAGAACAGGAAGAAC-3′	5′-TGATAGGCGGTGAGGCTACAAG-3′
IL-1b	5′-TGCCACCTTTTGACAGTGAT-3′	5′-TGTCCTCATCCTGGAAGGTC-3′
IL-6	5′-CCGCTATGAAGTTCCTCTCTGC-3′	5′-ATCCTCTGTGAAGTCTCCTCTCC-3′
IL-10	5′-CCAGAGCCACATGCTCCTA-3′	5′-AGGGGAGAAATCGATGACAG-3′
IL-12b	5′-ATGTGGAATGGCGTCTCTGTCT-3′	5′-TGGGCGGGTCTGGTTTGA-3′
iNOS	5′-CAGCGGAGTGACGGCAAAC-3′	5′-AGACCAGAGGCAGCACATCAA-3′
Mcsf	5′-TCACAACCTCATCCTTCTGCG-3′	5′-GACCCAGTTAGTGCCCAGTGA-3′
Md2	5′-AGACTGAGGGGAACCAATGGATTT-3′	5′-TTACGCTTCGGCAACTCTATGGA-3′
Mmp9	5′-TGAGTCCGGCAGACAATCCT-3′	5′-CCCTGGATCTCAGCAATAGCA-3′
Tgfb	5′-GCTGAACCAAGGAGACGGAAT-3′	5′-GCCTTAGTTTGGACAGGATCTG-3′
TLR4	5′-CCGCTCTGGCATCATCTTCATTG-3′	5′-CTCTGCTGTTTGCTCAGGATTCG-3′
Tnfa	5′-AAGCCTGTAGCCCACGTCGTA-3′	5′- GGCACCACTAGTTGGTTGTCTTTG-3′
TNFR1	5′-CGGTCCTAGTAACTGGCACTTCAT-3′	5′-CATCCACCACAGCATACAGAATCG-3′
Vegfa	5′-TCCTCCTATCTCCACCACCTATCC-3′	5′-CTCACCGCCTTGGCTTGTC-3′

showed that splenic microenvironment enhanced malignant phenotype of leukemia cells and splenectomy effectively prolonged survival of T-ALL mice (Ma et al., 2014). Furthermore, we suggested macrophages in leukemic microenvironment as leukemia-associated macrophages (LAMs) and demonstrated that LAMs from BM and spleen of T-ALL mice were distinct from TAMs reported in the literature (Chen et al., 2015). BM and spleen are important hematopoietic sites in adult, whereas liver is also hematopoietic site in embryonic and fetal mice (Kaushansky et al., 2010; Dzierzak and Medvinsky, 1995). Furthermore hepaomegaly was frequently observed in leukemia patients. However, the dynamic distribution, phenotype and pathological roles of macrophages in leukemic liver have not been established.

In this study, the distribution and characteristics of hepatic LAMs (H-LAMs) in Notch1-induced mouse T-ALL model were studied. Hepatomegaly was observed with infiltration of inflammatory cells. Furthermore, H-LAMs exhibited unique phenotype distinct from that of TAMs in hepatocellular carcinoma (HCC) and those of LAMs from BM and spleen in leukemia. Moreover, H-LAMs showed a more M1-like phenotype and expressed a panel of cytokines in charge of recruiting inflammatory cells, which contributed to pro-inflammatory microenvironment in liver.

2. Materials and methods

2.1. Mice

C57BL/6J (CD45.2) and $C_{57}B_6$·SJL (CD45.1) mice were provided by the Animal Center of the Institute of Hematology and Blood Diseases Hospital, CAMS & PUMC. 6–8 wks old female mice were used and maintained in the SPF-certified animal facility. The procedures for animal experiments were approved by the Animal Care and Use Committee at the institution.

2.2. Notch1 induced T-ALL mouse model

The establishment of mouse T-ALL model of was described previously (Hu et al., 2009). 2×10^6 leukemia cells, which derived from HSC/HPCs of $C_{57}B_6$·SJL (CD45.1) mice over-expressing intracellular domain of Notch1 (ICN1), were transplanted *i.v.* into the non-irradiated recipient C57BL/6J (CD45.2) mice. All mice suffered leukemia with the survival time of 27–31 days. Mice were sacrificed by cervical dislocation at the indicated time points.

2.3. FACS analysis and cell sorting

LSR II cytometery or Canto II cytometery (BD Biosciences, San Jose, CA) was used for FACS analysis. FACS Aria III (BD Biosciences) was used for cell sorting. Flowjo software (version 7.6.1) was used for data analysis. Standard protocols were followed for all experiments. BM cells were obtained by flushing femurs and tibiae and suspended in PBS buffer with EDTA described previously (Cheng et al., 2015). Spleen or liver cells were also harvested as single-cell suspension in the same buffer. Cells were filtered through graded nylon filters, and red cells were removed using ammonium chloride lysis buffer. Liver cells were resuspended in PBS containing 2% FBS and a combination of Abs (anti-CD45.2, anti-CD11b, anti-F4/80, BioLegend, San Diego, CA), washed, and subjected to FACS for analysis or cell sorting. The hepatic macrophages were gated as the $CD45.2^+GFP^-F4/80^+CD11b^+$ subpopulation. Intracellular staining of iNOS and CD206 were performed using IntraSure Kit (BD Biosciences) following the manufacturers' protocols.

2.4. Quantitative RT–PCR (qRT-PCR)

Total RNA was extracted from hepatic macrophages using RNeasy Mini Kit (Qiagen, Valencia, CA). qRT-PCR assays were performed on the 7900 real-time PCR system (Applied Biosystems). The expression level of target genes was analyzed by the RQ value calculated through ΔΔCt method. The sequences of all primer were listed in Table 1. For each gene, the RQ value of hepatic macrophages from control mice on day 0 was designated 1.000, respectively.

2.5. Statistical analysis

Results are expressed as the means ± SD. Comparisons between two groups were analyzed by unpaired Student's *t* test, whereas comparisons among more than two groups were analyzed by one-way ANOVA with multiple comparison tests. Analysis is done using the SPSS16.0 software package (SPSS, Chicago, IL) or GraphPad Prism 5.0 (GraphPad Software Inc, La Jolla, CA). Statistical significance is accepted when the P values are less than 0.05.