EXPERIMENTAL CELL RESEARCH 327 (2014) 192–208

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/yexcr

Research Article

Downregulation of endogenous STAT3 augments tumoricidal activity of interleukin 15 activated dendritic cell against lymphoma and leukemia via TRAIL

Sumit Kumar Hira[a], *Indrani Mondal*[b], *Debasis Bhattacharya*[b], *Partha Pratim Manna*[a,*]

[a]*Immunobiology Laboratory, Banaras Hindu University, Varanasi 221005, India*
[b]*Department of Pathology, Burdwan Medical College, West Bengal 713104, India*

ARTICLE INFORMATION

Article Chronology:
Received 30 January 2014
Received in revised form
4 August 2014
Accepted 8 August 2014
Available online 17 August 2014

Keywords:
Lymphoma
Dendritic cell
TRAIL
IL-15
STAT3

ABSTRACT

Effector functions in tumor resistance by dendritic cells (DCs) are less well characterized. In this study, we describe that the murine DCs upon stimulation with recombinant IL-15 in vitro or in vivo, expresses TNF superfamily member TRAIL which mediates cytotoxicity and growth inhibition against a murine lymphoma called Dalton lymphoma (DL) via apoptosis. Presence of tumor lysate or intact tumor cells significantly reduces the DC mediated tumoricidal effect, possibly via masking and down-regulating TRAIL in DCs. The antitumor effect of DC derived TRAIL was further augmented by deactivation of STAT3 in tumor cells by cucurbitacin I, which makes it more susceptible to DC derived TRAIL Treatment of tumor cells with cucurbitacin I upregulates TRAIL receptor expression in addition to activation of caspases. Compared to naïve DCs, DCs from tumor bearing mice are significantly impaired in TRAIL expression and consequent antitumor functions against DL which was partially restored by activation with IL-15 or LPS. Priming with recombinant IL-15 prolongs the survival of tumor bearing mice treated with cucurbitacin I. Naïve peripheral blood DCs derived from chronic myeloid leukemia (CML) patients have significant impairment in expression of TRAIL and consequent tumoricidal properties against TRAIL sensitive lymphoma cell lines and primary tumor cells compared to normal control.

Introduction

Dendritic cells (DCs) are increasingly considered to be an important immune cells, critical to bridge innate and adaptive immunity [1]. The roles played by DCs in the initiation of immune responses make them an attractive addition to cancer vaccine strategies [2–4]. Dendritic cells bridge the innate and adaptive immune systems by sampling the cellular environment, signaling to immune effector cells via receptor/ligand interactions or cytokines, and presenting antigen to T cells [5]. Rat splenic DCs may exhibit tumoricidal activity mediated in part by NK cell receptor protein 1 (NKRP1) [6]. Mouse and human DCs may also

Abbreviations: DL, Dalton lymphoma; DCs, Dendritic cells; IL-15, Interleukin 15; Cub, Cucurbitacin I; NDC, DCs from normal; E:T, Effector target ratio

*Corresponding author.
E-mail address: pp_manna@yahoo.com (P.P. Manna).

http://dx.doi.org/10.1016/j.yexcr.2014.08.012
0014-4827/

become cytotoxic against tumor cells, either spontaneously or after activation with IFN-γ or IL-15 [7–9]. Cytotoxic DCs identified in rat spleen exhibits a $CD4^-$ $CD103^+$ MHC class II^{low} phenotype [10], whereas splenic and bone marrow-derived cytotoxic DCs (BMDCs) identified in mice either express $CD8\alpha^+$ or B220 and NK1.1 [11].

IL-15 is an inflammatory and anti-apoptotic T-cell growth factor which shares similar properties with IL-2, and plays an important role in autoimmune disorders and transplant rejections [12,13]. IL-15 facilitates the survival of $CD8\alpha^+$ memory T cells, including self-directed memory cells [14,15]. Earlier, we reported the antitumor potential of IL-15 activated human peripheral blood DCs against various breast cancer cell lines in vitro [8]. Later, IL-15 was established as the major cytokine for the development of NK like killer DC (NKDC) and interferon producing killer DC (IKDC) [16,17]. IL-15 acts as an immunotherapeutic agent for cancer treatment because of its critical role for the proliferation and activation of natural killer (NK) and $CD8\alpha^+$T cells [18,19]. The importance of IL-15 has been evaluated in many tumor models in combination with anti-cancer drugs or anti CD40 [19–22]. Tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL), a TNF super family member binds to the death promoting receptors TRAIL R1 or TRAIL R2 causing receptor oligomerization followed by apoptosis via caspase 3 activations [23,24].

In this report, we demonstrated the tumoricidal properties of DC derived TRAIL following stimulation with IL-15 against a highly invasive and vigorously metastatic $CD3^-$ $CD11b^+$ $CD19^+$ murine lymphoma called Dalton Lymphoma (DL), originally described by Goldie and Felix [25–27]. Our findings suggest that murine DCs upregulates the expression of TRAIL in vitro and in vivo following IL-15 stimulation and become cytotoxic against lymphoma and leukemia, via caspase dependent mechanisms. It apparently works downstream to STAT3 since, inhibition of STAT 3 by cucurbitacin I, a selective Janus kinase/STAT3 inhibitor [28,29] accentuate the TRAIL mediated antitumor effects derived from DCs or by recombinant TRAIL. IL-15 priming prolonged the survival of tumor bearing mice in combination with cucurbitacin I, partly by restoring the TRAIL expression in DCs. DCs derived from the tumor bearing mice and CML patients are impaired in TRAIL expression and tumoricidal activities against established lymphoma and leukemia cells. Besides that, CML patient's DCs are also impaired in killing of tumor cells derived from the patients compared to age and sex matched control.

Materials and methods

Reagents and antibodies

Mouse (m) GMCSF, human GMCSF, Flt3 ligand, and IL-4 were purchased from Peprotech, USA. Mouse and human APC conjugated CD11b and CD86, PE conjugated anti-mouse CD4, CD8, H2d, CD11c, CD40, FITC conjugated anti-mouse CD3, CD14, CD19, H2k, anti-human CD1a, CD86, HLA-DR and CD40, purified mouse and human anti-CD3, CD14, CD19 and CD56 and anti-mouse TRAIL (neutralizing antibody) and PE conjugated anti-human CD34 were purchased from BD Biosciences (San Diego, CA). Recombinant human and mouse IL-15, anti-mouse and human IL-15 Rα antibodies were purchased from R&D, MN, USA. Recombinant mTRAIL and pan caspase inhibitor ZVAD-FMK were from Enzo Life sciences, USA. Anti-mouse STAT3, p-STAT3 (Tyr 705) and β-actin were from Cell Signaling, USA. PE conjugated anti-mouse TRAIL, FITC conjugated anti-mouse CD69, PE conjugated anti-human TRAIL, purified anti-human TRAIL (neutralizing antibody), DR4 and DR5, stem cell growth factor, IL-3, IL-6 and G-CSF, were purchased from Bio Legend, USA. LPS was purchased from Sigma Chemical Co. (St. Louis, MO). Cucurbitacin I was from Calbiochem, USA. Neutralizing anti-human DR4 and DR5 antibodies were purchased from R&D, MN, USA. Anti caspase 3 and 8 antibodies were purchased from Cell Signaling Technology, USA. Human IL-15 ELISA MAX™ Deluxe kit was from Biolegend with sensitivity of 4 pg/ml. Mouse IL-15 ELISA Kit (ab100701) with a sensitivity of <0.5 ng/ml was from Abcam. CFSE was from Molecular Probes, Eugene, OR.

Tumor cells

Dalton lymphoma (DL) was maintained in the peritoneum of AKR (H2k) mice by periodic transfer of tumor cells via intraperitoneal injection. YAC-1, THP1, U937, and K-562 were kind gift of Dr. Santu Bandyopadhyay, IICB, Kolkata, India. The cells were maintained in RPMI 1640 (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA) henceforth, called as complete medium. The cell lines used in the study were free from mycoplasma. DL tumor cell lysate was prepared by sonication of tumor cells followed by centrifugation. The supernatant was collected, filtered (0.45 μ) and stored at $-80\,^{\circ}C$ until use.

IL-15 administration and tumor transplant in mice

AKR/J mice were maintained and bred under pathogen-free condition of the central animal house facility of the department. Use of mice was approved by the Institutional Animal Ethics Committee. All animal experiments were performed according to the National Regulatory Guidelines issued by Committee for the Purpose of Supervision of Experiments on Animals, Ministry of Environment and Forest, Government of India. Female 4–6 weeks old normal mice were received daily intraperitoneal (i.p.) injections of recombinant IL-15 (8 μg kg^{-1} body weight in 100 μl PBS) for 7 days. Tumors (DL) in mice were maintained by transplanting fresh tumor cells in PBS (3×10^4 cells/mouse) intraperitoneally. All tumor measurements were performed in a blinded fashion.

Animal model

Mice ($n=12$/group) were transplanted with tumor and after 96 h were treated with cucurbitacin I (0.5 mg kg^{-1} body weight) or IL-15 (8 μg kg^{-1} body weight) either alone or in combination for 12 days. Altogether 5 cucurbitacin doses and 10 IL-15 doses were given in PBS at an interval of 72 h or 24 h respectively. Mice were under observation for 50 days when final data collection was made for Kaplan–Mayer survival analysis.

Patient sample

A total of 11 chronic myeloid leukemia (CML) patients attended the Department of Pathology, Burdwan Medical College with age range between 24 and 55 years, median age being 30 ± 9.80 years