IL-9 Contributes to Immunosuppression mediated by Regulatory T cells and Mast Cells in B-cell NHL

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Introduction

➢Regulatory T (Treg) cells have the potent ability to suppress host immune responses, thus preventing anti-tumor immune responses. Mast cells (MCs) promote tumor angiogenesis and tumor growth because of their properties as inflammatory cells. IL-9 is a key cytokine which can be produced by activated Treg cells and it is one of the most important MC growth factors. Additionally, MCs have been found to attract Treg cells infiltration indirectly in tumor microenvironment and induce IL-9 production by Treg cells.
➢In this study, we aim to elucidate the underlying interactions among Treg cells, MCs and IL-9 in immunosuppression of B-cell NHL.

Patients and Methods

➢Blood samples and tumor tissues were collected from 32 patients who were diagnosed with B-cell NHL for the first time. Reactive lymphadenitis tissues were set as control group.
➢Treg cells and MCs-related protein expressions in tumors were examined by IHC and Western-blot. IL-9 level in serum was measured by ELISA.
➢A murine model of lymphoma was established A20 mouse lymphoma cells. Tumor-bearing mice received 100 μg of anti-mIL-9 Ab or isotype control Ab treatment. Mice were sacrificed at day 18 and blood samples and tumor draining lymph nodes were harvested at the same time for further study.
➢The expressions of Treg cells and MCs-related genes were examined by quantitative PCR. The impacts of IL-9 on Treg cells function and apoptosis were determined by H^3-TdR incorporation assay and FACS. The effects of IL-9 on induction of bone-marrow-derived mast cells (BMMCs) and MC-related genes expression were examined by FACS and quantitative PCR.

Results

➢. **Expressions of Foxp3, CD117 and IL-9 protein in B-cell NHL patients**.
●More Foxp3+ Treg cells and CD117+ MCs were found in NHL tumor tissues. A significant up-regulation of Foxp3 and CD117 in protein level can also be observed in tumor tissues of NHL subjects (Figure 1A).
●Sera from NHL patients contained increased levels of IL-9 in a higher frequency than the sera from controls. (Figure 1B).
➢ **IL-9 is required for protumor effect in tumor microenvironment.**
●Higher levels of IL-9 were detected in sera from lymphoma models compared to the sera from normal BALB/c mice (744.18±76.88v. 388.94±54.74pg/ml, n=6, P<0.01).
●Tumor growth was significantly retarded in anti-mIL-9 Ab-treated mice, compared to mice receiving control Ab (1.16±0.31 v. 3.98±0.36g, n=6, P<0.01).
➢**IL-9 remodels expressions of Treg cell and MC related genes in mice tumor draining lymph nodes.**
Treg cells and MCs related genes (Foxp3, CD117, Fcer1a, Mcpt1 and Mcpt5) were up-regulated in tumor group compared with normal lymph nodes, while significantly down-regulated in anti-mIL-9 blocking antibody treated group.
➢ **IL-9 produced by activated Treg cells modulates Treg cells function and apoptosis.**
Activated Treg cells produced high levels of IL-9. IL-9 could enhance functions of Treg cells and protect Treg cells against apoptosis (Figure 1C and Figure 1D).
➢**Effects of IL-9 on IL-3 and SCF-dependent BMMC development and on MC-related genes expressions.**
IL-9 increased expression of MC-related genes (CD117, Fcer1a, Mcpt1 and Mcpt5) and apparently enhanced BMMCs induction (Table 1).

Conclusion

➢. Treg cells and MCs played important roles in immune-system which are associated with risks of B-cell NHL progress
➢IL-9 is an essential mediator in Treg cells and MCs mediated immune tolerance in B-cell NHL.
➢IL-9 promotes tumor growth not only by affecting natural Treg cells functions and apoptosis, but also by promoting MCs functions and inductions in vivo.
➢There may be a close loop among Treg cells, MCs and IL-9 in tumor immunosuppression. Pharmacological or targeted inhibition of IL-9 activity may find utility as an adjunctive in NHL therapy.

Figure and Table

Fig.1

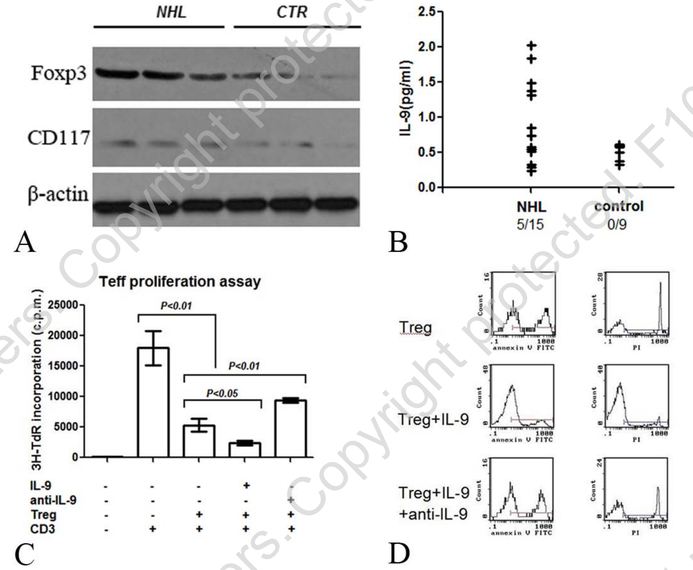

Table1

Group	BMMC purity (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Bone marrow cells+rIL-3 +rSCF	1.39	30.3	54.7	77.6	87.2
Bone marrow cells+rIL-3 +rSCF+rIL-9	1.46	54.7	87.3	98.8	99.8

(Note: There is no conflict of interest.)