LSI Medience Corporation, Nonclinical Research Center

Identification of tumor infiltrating lymphocyte subsets using the CT26WT tumor-bearing mouse model

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Objective

Immune checkpoint inhibitors such as anti-PD-1 antibody and anti-CTLA-4 antibody have recently been approved for the treatment of melanoma or non-small cell lung cancer. Further developments of therapeutic methods using their inhibitors have been advanced in other types of cancer. Therefore, it is important to establish an evaluation method for their inhibitors.
In this study, we examined an evaluation method for mouse tumor infiltrating lymphocytes (TILs) subsets (regulatory T cells, $CD8^+$T cells, dendritic cells, bone marrow-derived suppressor cells), which is one of the evaluation items of immune checkpoint inhibitors, using syngeneic mouse model.

Summary in Japanese

免疫チェックポイント阻害薬（抗PD-1抗体，抗CTLA-4抗体など）は，近年悪性黒色腫や非小細胞性肺癌などのがん治療に認可されている．さらに他のがん種においても免疫チェックポイント阻害薬を用いた治療法の開発が進んでおり，これらの薬剤の評価系の確立は重要である．今回我々はシンジェニックマウスモデルを用いて免疫チェックポイント阻害薬の評価項目の1つであるマウス腫瘍浸潤リンパ球（TILs）サブセット（制御性T細胞，$CD8^+$T細胞，樹状細胞，骨髄由来免疫抑制細胞）の解析法について検討した．その結果，腫瘍中のリンパ球サブセットが検出でき，免疫チェックポイント阻害薬による変化が認められたことから，本解析法は免疫チェックポイント阻害薬の評価系として有用と考えられる．

Materials and Methods

【Animal】

Mice, BALB/cAnNCrlCrlj, ♀, 7w (At inoculation of cells)

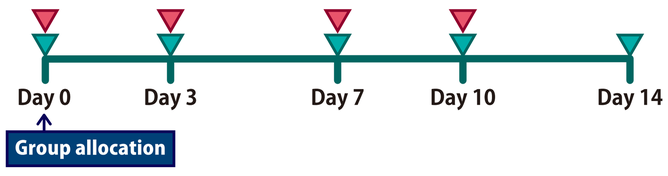
【Cell Line】

CT26WT (ATCC, mouse colon carcinoma)

The cell suspension (1×10^6 cells) was injected subcutaneously into the right abdominal region of each animal.

Experiment Schedule 1 (Anti-tumor study)

▼ : The points of administration (i.p.)
▼ : The points of tumor diameter measurement



Experiment Schedule 2 (TILs measurement)

▼ : The points of administration (i.p.)
▼ : The points of TILs measurement

Antibody

Antibody	Clone	Manufacturer
PD-1	RMP1-14	BioXcell
IgG2a	2A3	BioXcell
CTLA-4	9D9	BioXcell
IgG2b	MPC-11	BioXcell

Group configulation

Anti-tumor study

Group	Dose (μg/body)	Number of animals
Control (IgG2a + IgG2b)	200 + 150	6
PD-1	200	6
CTLA-4	150	6
PD-1 + CTLA-4	200 + 150	6

TILs measurement

Group	Dose (μg/body)	Number of animals
Control (IgG2a + IgG2b)	200 + 150	3
PD-1	200	3
CTLA-4	150	3
PD-1 + CTLA-4	200 + 150	3

【Evaluation】

(1) Measurement of tumor diameter

Tumor diameter was measured using a caliper on Day 0, 3, 7, 10 and 14.
Tumor volume was calculated by the following formula.

Estimated tumor volume (mm^3) =
1/2 × long diameter (mm) × short diameter (mm) × short diameter (mm)

(2) Measurement of tumor infiltrating lymphocytes

At 8 days after administration, tumors were excised from mice and dispersed using gentleMACS Octo Dissociator (Miltenyi Biotec).
Cells were passed through a 70 μm cell strainer, centrifuged at 190×g and supernatant was removed.
Blocking reagent and CD45 microbeads were added to the cell suspension and incubated for appropriate time.
Magnetic cell sorting was performed to isolate CD45 positive cells using QuadroMACS Separator and MACS columns (Miltenyi Biotec).
Cell staining was performed by the antibodies shown in the table below.
Samples were measured by a flow cytometer (FACSLyric, BD Biosciences) and analyzed using BD FlowJo software (BD Biosciences).

Panel 1: Treg

Antigen	Fluorochrome
CD45	PerCP-Cy5.5
CD3	FITC
CD4	PE-Cy7
CD25	BV421
Foxp3	AF647
Live/Dead	FVS510

Panel 2: $CD8^+$T cells

Antigen	Fluorochrome
CD45	APC
CD3	FITC
CD8a	APC-H7
CD69	BB700
PD-1	PE
Live/Dead	FVS510

Panel 3: DC and MDSC

Antigen	Fluorochrome
CD45	PE
CD3	FITC
CD11c	BB700
CD11b	BV421
Gr-1	APC
MHC II	PE-Cy7
Live/Dead	FVS510

Conclusion

(1) In antitumor test, an antitumor effect was observed in the CTLA-4 group and the PD-1+CTLA-4 group.

(2) Change in distribution of TILs was observed as below.

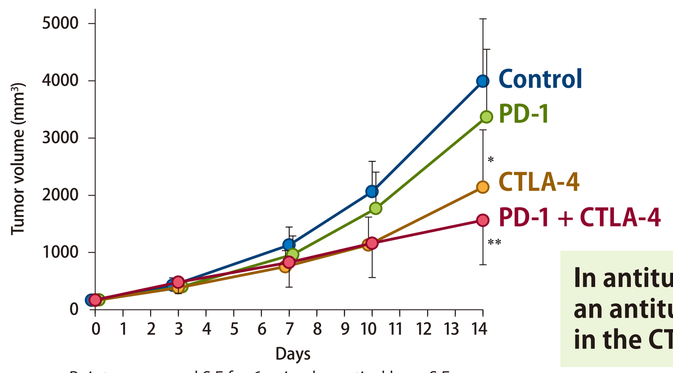
- **The proportion of Treg in tumor decreased with no significant difference in the CTLA-4 group and the PD-1+CTLA-4 group compared to control group.**
- **The proportion of $CD69^+CD8^+$T cells (activated $CD8^+$T cells) in tumor significantly increased in the PD-1+CTLA-4 antibody group compared to control group. On the other hand, the proportion of $PD\text{-}1^+CD8^+$T cells (exhausted $CD8^+$T cells) in tumor significantly decreased in the PD-1 group and the PD-1+CTLA-4 group compared to control group.**
- **No significant difference in the proportion of DC and MDSC in tumor was found in any of the test groups compared to control group.**

In conclusion, TILs subsets in tumor could be detected and the changes in the proportion of TILs subsets by immune checkpoint inhibitors could be observed.

This evaluation method is considered to be useful to evaluate immune checkpoint inhibitors.

Results

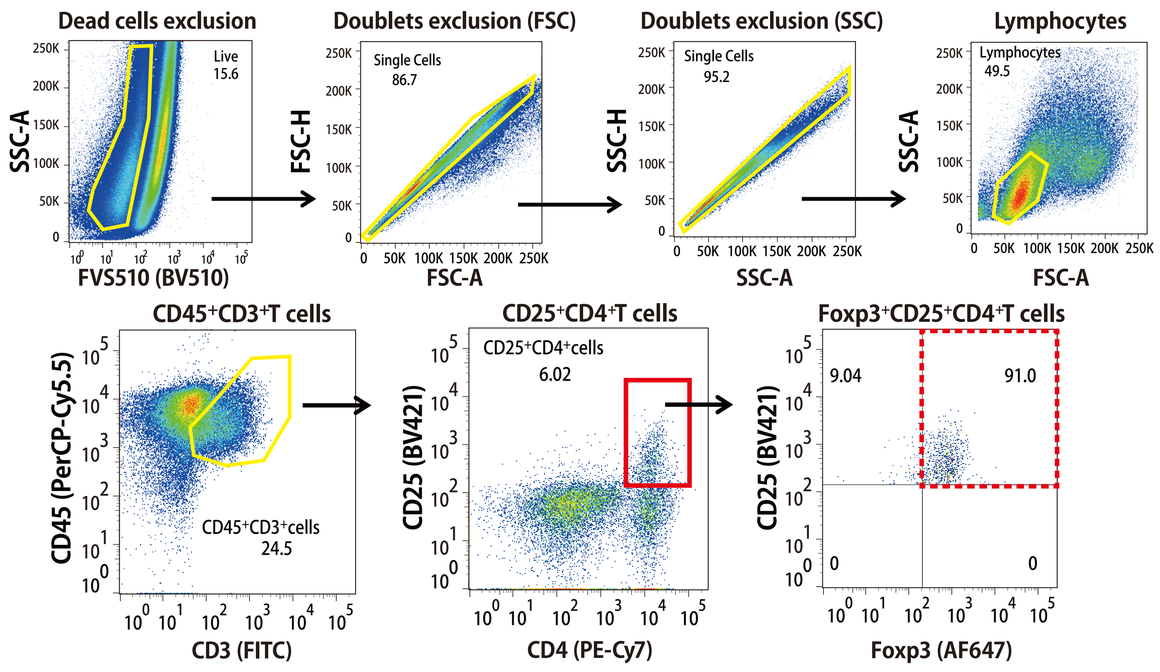
Tumor growth treated with anti-PD-1 antibody and/or anti-CTLA-4 antibody



Points, mean and S.E for 6 animals; vertical bars, S.E.
*: p<0.05, **: p<0.01; Significant difference from control group. (Student's t-test)

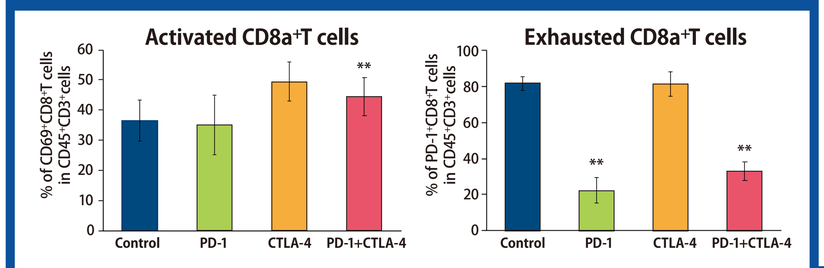
In antitumor test, an antitumor effect was observed in the CTLA-4 group and the PD-1+CTLA-4 group.

Regulatory T cells (Treg : $Foxp3^+CD25^+CD4^+$T cells) in TILs

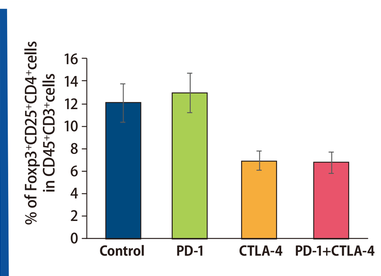


The proportion of Treg in tumor decreased with no significant difference in the CTLA-4 group and the PD-1+CTLA-4 group compared to control group.

Each $CD8^+$T cells subsets in TILs



The columns are the mean and S.E. of 3 animals.
**: p<0.01; Significant difference from control group. (Student's t-test)



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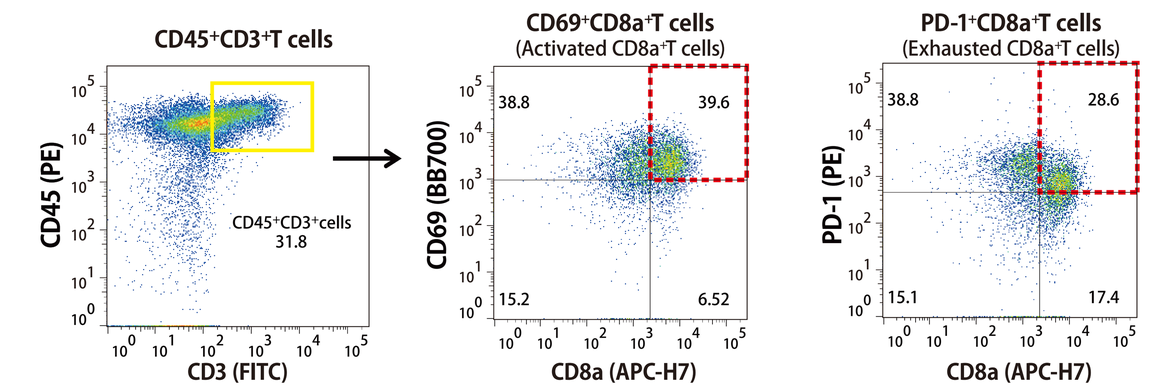
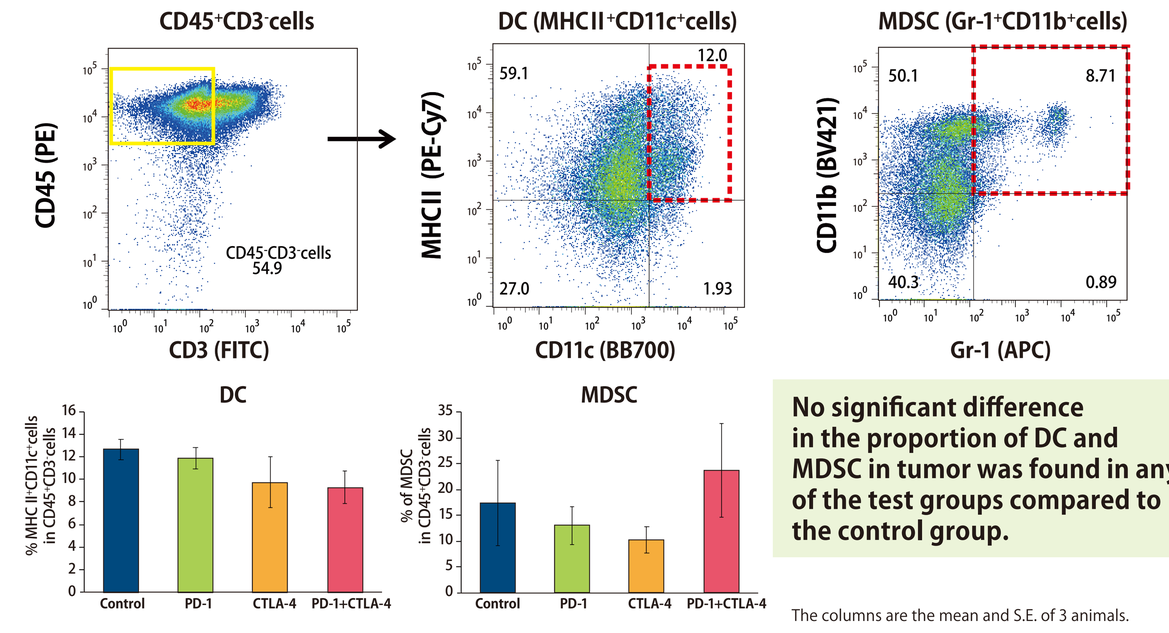


The proportion of $CD69^+CD8^+$T cells (activated $CD8^+$T cells) in tumor significantly increased in the PD-1+CTLA-4 group compared to control group. On the other hand, the proportion of $PD\text{-}1^+CD8^+$T cells (exhausted $CD8^+$T cells) in tumor significantly decreased in the PD-1 group and the PD-1+CTLA-4 group compared to control group.

DC (Dendritic cells) and MDSC (Myeloid-derived suppressor cells) in TILs



No significant difference in the proportion of DC and MDSC in tumor was found in any of the test groups compared to the control group.

The columns are the mean and S.E. of 3 animals.

COI 開示
☑ 発表内容に関連し，過去３年間，開示すべき COI 関係にある企業などはありません
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