Identification of Therapeutic Targets of Multiple Sclerosis through MALDI- Imaging Mass Spectrometry of Experimental Autoimmune Encephalomyelitis (EAE) mouse model

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Introduction
Experimental autoimmune encephalomyelitis (EAE) is the most commonly used animal model for the studies of multiple sclerosis (MS). Here we applied matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) in search of tissue biomarker from EAE mouse brain / spinal cord at proteome level.

We introduce a novel therapeutic strategy of identifying candidate protein markers, blocking their production in test animal, and making substantial MALDI imaging measurements to evaluate.

Methods
Animals: EAE was induced in female SJL/J mice by immunization with 0.1 ml/head myelin proteolipid protein (PLP) peptide-complete Freund's adjuvant (CFA) emulsion with pertussis toxin (PT).

Compounds: Anti target protein compound was administered orally every day and on every other day with 5 mg/kg from the immunized day 0 as prevention and from day 12 as therapy, respectively.

Sample preparation: Brains and spinal cords were removed and snap-frozen in liquid nitrogen. Sagittal sections (10 μm) of the brains were obtained from cryostat.

MALDI Imaging: The MALDI measurement and image analysis was carried out on UltraflexTwin (Bruker Daltonik GmbH) and rapifleX issuetyper (Bruker Daltonik GmbH). Digital images of IHC were obtained on the LSM710 (Carl Zeiss) and processed using ZEN 2.1 software (Carl Zeiss).

Data analysis: MALDI-IMS data was analyzed using the FlexControl 3.0, FlexControl 5.0 FlexImaging 3.0, FlexImaging 5.0, SCLS Lab 2019(SCLS GmbH) and the ClinProTools 2.2 software (Bruker Daltonik GmbH). Digital images of IHC were obtained on the LSM710 (Carl Zeiss) and processed using ZEN 2.1 software (Carl Zeiss).

Results

Selected ion images of intact proteins from lesions of EAE brain

Fig. 2A: MALDI-IMS of the hippocampus area of acute EAE brain. 2B: Localization of S100A9 protein in the hippocampus from eEAE. MALDI-IMS data (upper panel) and IHC with anti-S100A9 antibody (lower panel). a: corpus callosum, b: lateral ventricle, c: infiltrated immune cells.

A novel therapeutic strategy of EAE targeting S100A9 proteins

Fig. 3 EAE clinical score with or without therapeutic administration of anti-S100A9 compounds (T and L). Each graphs represent EAE clinical score , (A) to (F) represent each experimental group described in the figure. P.O. = Per os, administered orally.

Conclusions

• MALDI-IMS at intact proteome level was applied for murine EAE brains.
• We have developed a novel therapeutic strategy of multiple sclerosis by targeting proteins promoted through MALDI-IMS on EAE brains.
• The current strategy leads a successive result for both prevention and treatment of EAE.
• MALDI-IMS can be applied for elucidating pharmacological effects of a novel compound on murine EAE brains.

Histopathological observation of the brains from spontaneous and treated EAE

Fig. 4 Histopathology of the brains from acute EAE and control. Hematoxylin and Eosin staining. Arrows indicate infiltrated immune cells in the corpus callosum and hippocampus. (c, c') administrated compound-T from day0, (d, d') administrated compound-L from day0.

MALDI-IMS of EAE brains treated with a novel strategy

Fig. 5 Distribution of intact protein at m/z 7545 in acute EAE mouse brains. a, b, c, and d: Optical image. a', b', c', and d': MALDI-IMS data. Compounds were preventively administrated from day 0.

Fig. 6 Segmentation map obtained from multivariate analysis with MALDI-IMS. (a") control. (b'') acute phase of spontaneous EAE. (c'') acute phase of EAE mice preventively administrated with compound-T. (d'') acute phase of EAE mice preventively administrated with compound-L.

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<th>Table 1: Clinical Score of EAE</th>
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<td>a. Clinical normal</td>
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<td>b. Decreased tail tone or weak hind limb</td>
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<td>c. Both decreased tail tone and weak hind limb</td>
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<tr>
<td>d. Partial paralysis of hind limb</td>
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<td>e. Completely paralyzed of hind limb</td>
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<td>f. Dying (unable to move)</td>
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Fig. 1 It shows the two independent EAE experiments. The 1st as blue and 2nd as red. Each spot represents the average score of more than 8 mice. The clinical findings were followed (Table 1) (Sakai T. et al HUPO 2013)

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