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

ASMS 2020

○Nami Tanaka1, Hiroki Yamashita1, Takashi Nirasawa2, Ryo Kajita2, Katsutoshi Taguchi3, Masaki Tanaka3, Takayuki Kondo4, Nobuto Kakuda1, Masaya Ikegawa1

1. Doshisha University, Kyoto, Japan
2. Bruker Japan K.K., Yokohama, Japan
3. Department of Anatomy and Neurobiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto-city, Japan
4. Kansai Medical University Medical Center, Hirakata, Japan

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 subsequent MALDI Imaging measurements to evaluate.

Methods

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

Table 1	Clinical Score of EAE
0	Clinically normal
1	Decreased tail tone or weak hind limb
2	Both decreased tail tone and weak hind limb
3	Partially paralyzed of hind limb
4	Completely paralyzed of hind limb
5	Dying (unable to move)

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)

Compounds; Anti target protein compound was administered orally every day and or 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. The sections were transferred to conductive Indium-TinOxide (ITO) coated glass slides. ImagePrep station (Bruker Daltonik GmbH) and airbrush sprayer were used for a sinapinic acid (SA) as a matrix and trypsin deposition.

MALDI Imaging ; The MALDI measurement and image analysis was carried out on Ultraflextream (Bruker Daltonik GmbH) and rapifleX tissuetyper (Bruker Daltonik GmbH) . MALDI measurements were done in positive ion mode with a mass range of 2,500-25,000 Da. The spatial resolution was set to 50-80 µm.

Data analysis; MALDI-IMS data was analyzed using the FlexControl 3.0, FlexControl 5.0 FlexImaging 3.0 , FlexImaging 5.0 , SCiLS Lab 2019(SCiLS 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

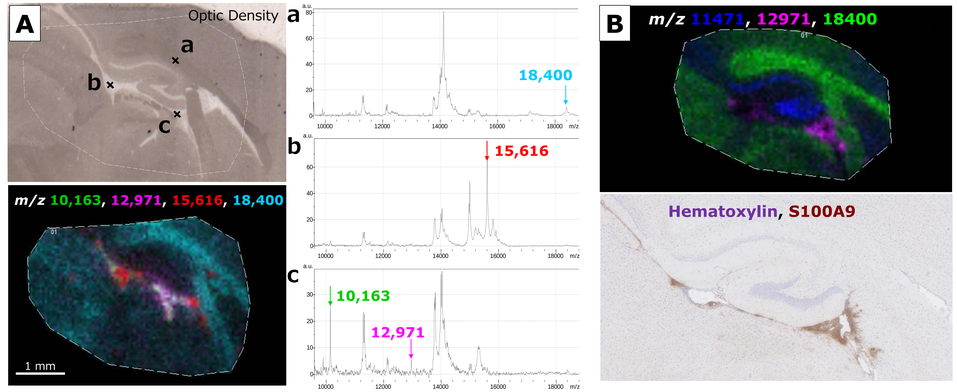

Fig. 2A: MALDI-IMS of the hippocampus area of acute EAE brain. 2B: Localization of S100A9 protein in the hippocampus from pEAE. MALDI-IMS data (upper panel) and IHC with anti-S100A9 antibody (lower panel). a: corpus callosum, b: lateral ventricle, c: infiltrated immune cells.

A novel therapeutic strategy of EAE targeting S100A9 proteins

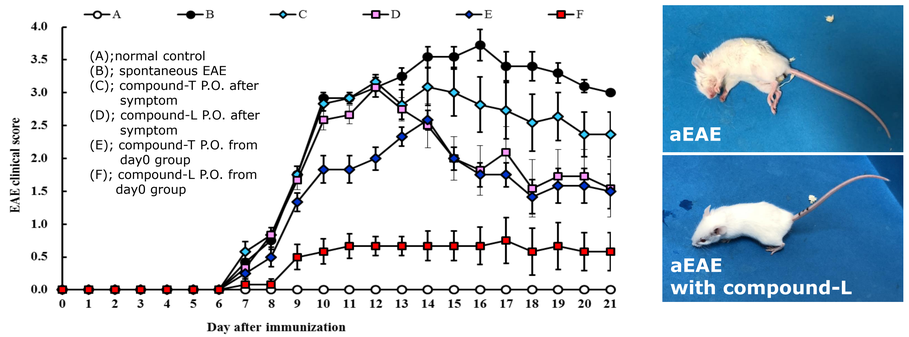

Fig. 3 EAE clinical score with or without therapeutic administration of anti S100A9 compounds (-T and –L). Each graphs represent EAE clinical score , (A) to (F) represent each experimental group described in the figure. P.O. = Per os, administered orally.

Histopathological observation of the brains from spontaneous and treated EAE

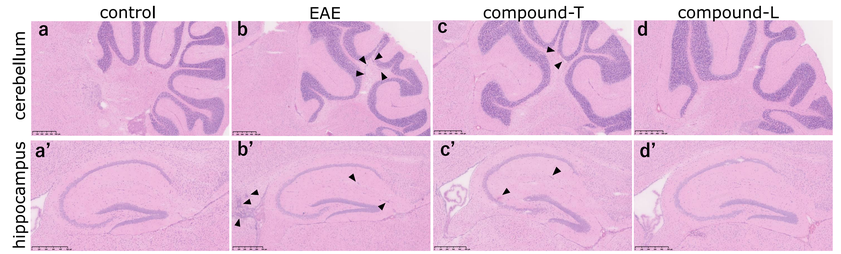

Fig. 4 Histopathology of the brains from acute EAE and control . Hematoxylin and Eosin staining. Arrows indicate infiltrated immune cells in the cerebellum and hippocampus. (c, c') ; administrated compound-T from day0. (d, d') ; administrated compound-L from day0.

MALDI-IMS of EAE brains treated with a novel strategy

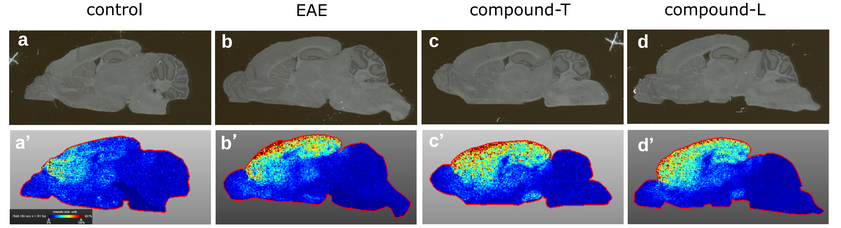

Fig. 5 Distribution of intact protein at m/z 7545 in acute EAE mouse brains. a, b, c, and d : Optical image. a', b', c', and d': MALDI-IMS data. Compounds were preventively administrated from day 0.

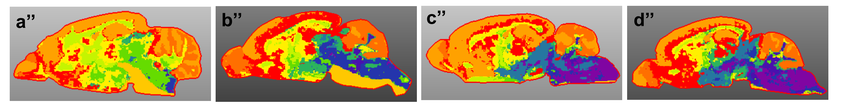

Fig. 6 Segmentation map obtained from multivariate analysis with MALDI IMS. (a'') control. (b'') acute phase of spontaneous EAE. (c'') acute phase of EAE mice preventively administered with compound-T. (d'') acute phase of EAE mice preventively administrated with compound-L.

Conclusions

＊MALDI-IMS at intact proteomic level was applied for murine EAE brains.

＊We have developed a novel therapeutic strategy of multiple sclerosis by targeting proteins nominated 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.