

Institute of Experimental Immunology Neuroinflammation / Prof. Dr. Jan Lünemann		
Date 12.04.2016 C. Keller	Standard Operating Procedure PolySIA Treatment in EAE	Pages 1/1

EAE, Active Immunization

Reagents:

- complete Freund's adjuvant (CFA)
- Pertussis toxin, concentration 2ug/ml (PT); PT stock from Sigma is 0.2mg/ml à 10ul aliquots stored at -20°C. Before use add 1ml of PBS.
- MOG₃₅₋₅₅-peptide (MEVGWYRSPFSRVVHLYRNGK), concentration 5mg/ml

1. Dilute MOG (stock: 5mg/ml) in PBS; emulsify with equal volume of CFA. The final MOG concentration should be 100µg/100µl.
2. DAY 0: Immunize subcutaneously (see SOP: Immunizations and Injections) with 100µl of emulsion into both flanks à total of 200 µl.
3. DAY 0: Inject 100 µl (200ng) of PT i.p.
4. DAY 2: Inject 100 µl (200ng) of PT i.p.
5. For scoring follow 'SOP - Scoring of EAE'

EAE, Adoptive Transfer

1. DAY 0: Immunize donor mice (either WT or 2D2) subcutaneously (see SOP: Immunizations and Injections) with 100µl of emulsion into both flanks à total of 200 µl.
2. DAY 0: Inject 100 µl (200ng) of PT i.p into the lateral abdomen
3. DAY 7: sacrifice mice and remove spleens, inguinal and axillary lymph nodes
4. Transfer organs into petri dish filled with ice-cold PBS or HANKS
5. Follow SOP for lymphocyte isolation from the spleen:
 - homogenate tissue with two glass slides and filter through a 70 µm cell strainer into a 50 ml Falcon tube
 - centrifuge for 5 min at 1500 rpm (400-500g) , 4°C
 - discard supernatant, resuspend pellet in 1 ml of RBC lysing solution per spleen
 - incubate 5min on ice, invert tube a few times
 - fill up with PBS and filter through a 40 µm mesh
 - centrifuge for 5 min at 1500 rpm (400-500g) , 4°C
 - discard supernatant, resuspend in medium
6. count cells, resuspend at 8-10x10⁶ cells/ml in culture medium: RPMI complete (10% FCS, PenStrep, β-Mercaptoethanol) containing 20 ug/ml MOG and 10 ng/ml recombinant IL-23
7. Culture cells for 2 days at 37°C and 5% CO₂. Blasts should be visible under the microscope after two days.
8. One day before transfer of the cells irradiate the recipient mice with 550 rads.
9. DAY 9: Wash cells with PBS twice and adjust volume to desired concentration (5x10⁶ cells/mouse if 2D2 donors were used, 10-15 x10⁶ cells/mouse if WT donors were used)
10. Inject recipient mice i.p. with 200 µl of cells.
11. For scoring follow 'SOP - Scoring of EAE'.

Intraperitoneal injection of polysialic acid after disease onset (DAY 9-13)

1. restrain mice in reclined position with neck skin between thumb and index finger with legs apart towards the researcher and restrain one leg with pinky finger
2. Inject up to 200 µl of liquid containing 10 µg/g body weight for 4 consecutive days into the lateral abdomen using a 25G5/8 needle