

Experimental Scheme in the Institute of Innate Immunology, University of Bonn

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2015/1/12-2015/2/13

Date	Contents
1/12 (Mon)	Visit the lab and greet to everyone. Get to know the campus and facilities in the institute.
	Talk with Professor Latz about the research progress and plan, and get instructions on the experiments to do.
1/13 (Tue)	Learn to use the Confocal Laser Scanning Microscope with the functions of autofocus and position-sensing for screening a large number of samples or observation in a long time-course.
1/14 (Wed)	Learn to use the Confocal Laser Scanning Microscope for live cell imagine.
1/15 (Tue)	Group meeting of Latz Lab.
1/16 (Fri)	ASC-knocked in HEK cell culture and seeding.
1/19 (Mon)	Culture of HEK cells.
	Seeding of human primary macrophages for inflammasome activation by liposomes.
	Experiment of inflammasome activation by liposomes in murine immortalized macrophages.
1/20 (Tue)	Confocal Microscopic observation of ASC specks in murine immortalized macrophages by liposome stimulation for 24 hr.
	HEK293 cell seeding for the toxicity experiment.
1/21 (Wed)	Add liposomes to HEK293 cells for 24 hr incubation.
	Collect the supernatant from human primary macrophages for ELISA and toxicity tests.
1/22 (Thu)	Lab meeting.
	LDH release assay to evaluate the cytotoxicity of cationic liposomes in caspase-1 knocked-in HEK cells.
1/23 (Fri)	Confocal Microscopic observation of ASC specks in murine immortalized macrophages by liposome stimulation for 24 hr.
1/26 (Mon)	Cell culture of HEK cells.
	Seeding of primary human macrophages in 96-well cell culture plate.
	ELISA plate coating for detection of human IL-1beta and TNF-alfa.
1/27 (Tue)	Liposome stimulation of primary human macrophages and caspase-1 KO murine macrophages.
	Cytotoxicity assay of liposomes to HEK cells: liposome addition
	ELISA test of human IL-1beta and TNF-alpha of the samples stored from last week.
1/28 (Wed)	Morphological observation of primary human macrophages stimulated by liposomes for 1d.
	Collection of the supernatant of HEK cells incubated with high concentration of liposomes for 18hr.
1/29 (Tue)	Lab meeting.
	Microscopy of liposome-stimulated murine macrophages (lower concentration; longer time)
	Collection of the supernatant of liposome-stimulated primary human macrophages for ELISA and LDH release assay.
	Collection of the supernatant of HEK cells in a lower concentration liposomes for LDH assay.

	ELISA plate coating of human IL-1beta and TNF-alpha.
	HEK cell culture.
1/30 (Fri)	ELISA assay of human IL-1beta and TNF-alpha of the samples got yesterday.
	Microscopic observation of ASC-specks in murine macrophages by stimulation with high concentration of liposomes for short time (5-6 hr).
2/2 (Mon)	HEK cell culture
	LDH release assay of the sample got on 1/28 and 1/29
2/3 (Tue)	NLRP3 inflammasome activation using liposomes for overnight incubation
	Quantification of lysosome rupture by liposomes using acridine orange: sample preparation for FACS tmr
	ELISA plate coating for detection of human IL-1beta and TNF-alpha tmr.
2/4 (Wed)	ELISA testing of human IL-1beta and TNF-alpha (sample got on 2/3)
	FACS screening of lysosomal rupture by liposomes (100 Mm, O/N)
	LDH release assay (sample got on 2/3)
2/5 (Thu)	Group meeting
	ELISA plate coating and liposome addition to the primary human macrophages: testing the release of IL-1beta without LPS stimulation
	Cell (immortalized casp1-KO murine macrophages) seeding for the flow cytometric quantification of lysosome rupture by liposomes.
2/6 (Fri)	ELISA test of IL-1beta (the sample prepared from 2/5)
	Flow cytometry of the lysosome rupture by liposomes (the sample prepared from 2/5)
	Culture of HEK cells
2/9 (Mon)	Cell culture of murine macrophages for lysosome rupture experiment
2/10 (Tue)	Cell culture of wild-type murine macrophages
2/11 (Wed)	Lysosome rupture assay: sample preparation and FACS detection
2/12 (Tue)	Lab meeting: presentation and discussion about the results
	ELISA assay in murine macrophages: LPS priming and liposome stimulation
	ELISA plate coating
2/13 (Fri)	ELISA tests of the expression of murineIL-1beta and TNF-alpha