Novel Therapeutic Approaches Using Adoptive Regulatory T cell for Alzheimer's disease

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Kyung Hee University

Background

- 기존 치료제의 문제점

02

❖ **근본적인 치료제의 부제**

❖ 병의 진행속도를 늦추고 증상을 완화시키는 약물치료가 주를 이룸

❖ 약물요법의 경우 환자의 증상 개선효과가 신속하고 뚜렷하게 나타나지만 장기복용 시 오심, 구토, 식욕감퇴, 복통 등의 소화기 계통과 저 혈당, 췌장염, 신장기능저하의 부작용이 나타남

- 알츠하이머병에 쓰이는 대표적 약물

구분	제품명/개발사	성분명	주된 부작용	참고
AChE 억제제	아리셉트(Aricept)/에자이	도네페질(donepezil)	오심, 구토, 식욕감퇴, 복통 등의 소화기 계통의 부작용	경증 인지장애의 개선효과는 없는 것으로 밝혀짐
	엑셀론(Exelon)/노바티스	리바스티그민(rivastigimine)		일시적 증상 완화하나 치료효과 부족
	라자딘(Razadyne)/얀센	갈란타민(galanatamine)		
NMDA수용체 길항제	나멘다(Namenda)/앨러간	메만틴(memantine)	두통, 기면, 피로, 불안초조	
	에빅사(Ebixa)/룬드백			
	액수라(Axura)/머츠			
AChE 억제제 + NMDA수용체 길항제	남자릭(Namzaric)/(악티비스)	도네페질 + 메만틴	오심, 설사, 변비, 저혈당, 식욕저하, 췌장염, 신장기능 저하	체중개선제로 사용

Market

- **국내 개발 약물**

구분	주요 경쟁사	보유기술 및 제품	구분	주요 경쟁사	보유기술 및 제품
1	메디포스트	뉴로스템, 줄기세포치료제,1/2a	6	동아쏘시오	DA-9803 천연물(전임상)
2	젬백스앤카엘	Aβ/Tau 축적억제 (펩타이드) GV1001 (임상2상)	7	메디프론	MDR-1339 aggregation/toxicity blocker (임상1상)
3	네이처셀	Aβ 제거 (줄기세포치료제) 아스트로스템(임상1/2상FDA)	8	일동제약	ID1201천염물(임상2상)
4	차바이오텍	Aβ 축적억제(줄기세포치료제) CB-AC-02 (1/2a상)	9	퓨리메드	PM012 천연물(임상3상)
5	대화제약	인지기능 개선 (천연물신약) DHP1401(2b상)	10	SK 케미칼	SK-PC-B70M 천연물 (임상3상)

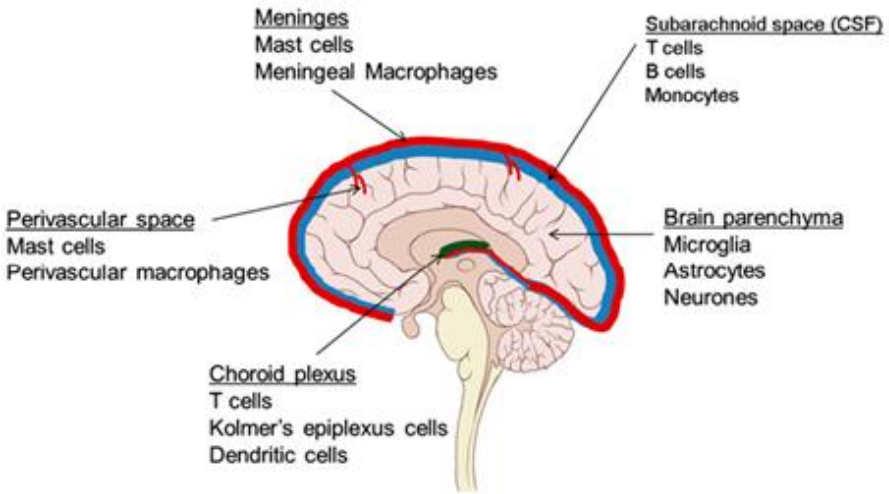
- **국외 개발 약물**

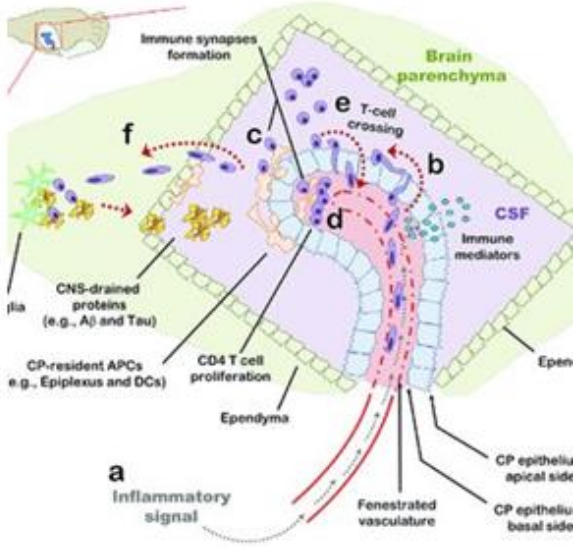
구분	주요 경쟁사	보유기술 및 제품	구분	주요 경쟁사	보유기술 및 제품
1	Merck	MK-7622(**임상2상 중단**)	6	Eli Lilly	Aβ antibody Fab PEG Aβ표적항체(**임상3상 중단**)
2	GlaxoSmithkline	GSK933776 Aβ표적 mAb(**임상2상중단**)	7	AbbVie	ABT-957 Calpain inhibitor(임상1상)
3	Janssen R&D	JNJ-54861911 BACE inhibitor(임상2상)	8	ACImmune	ACI-35 pTau 타겟백신(임상1상)
4	Boehringer Ingelheim	BI409306(임상2상)	9	Alkermes	ALKS7119 CNC modulator(임상1상)
5	AllaChem	AVN-101 5-HT6 수용체(임상2상)	10	QR Pharma	Bisnorcymserine AChEI(임상1상)
11	Biogen	Aβ/Tau 축적억제 (FDA 승인)			

FDA approves Aduhelm, the first new medication for AD in 2021

Background

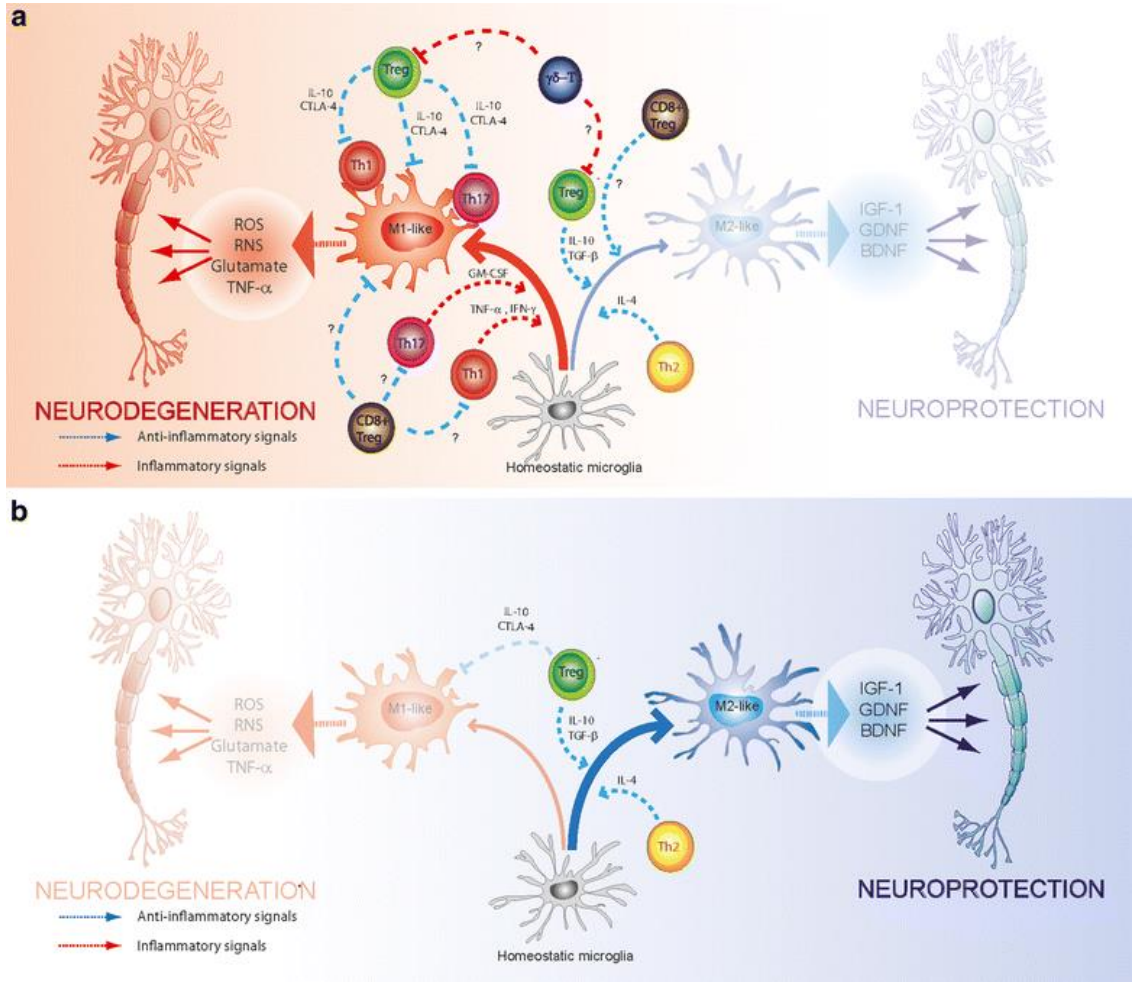
- 최근 기존의 방식에서 벗어나 뇌질환을 바라보는 새로운 시각으로 패러다임의 변화가 생기고 있으며 이는 **면역계와 뇌질환의 상관관계에** 주목하고 있음
- 특히 퇴행성 뇌질환에 의한 면역반응에는 **미세아교세포(microglia)**가 관여하고 있어 일를 중심으로 퇴행성 뇌질환을 이해하려는 연구가 진행 중임
- 또한 인간의 **중추신경계에서도 임파계**가 존재하고 이를 통해 면역세포가 유입됨이 증명되었음 (Structural and functional features of central nervous system lymphatic vessels, *Nature* volume 523, pages 337–341)
- 최근에 말초의 면역세포들도 중추신경계에 침윤할 수 있고 염증을 유발하여 퇴행성 뇌질환을 유발할 수 있다는 사실이 밝혀짐. 면역세포의 뇌로의 유입은 **맥락총(choroid plexus)**을 통과하는 것으로 밝혀짐.
- 알츠하이머 치매환자에서도 $CD4^+$ T임파구의 뇌내 맥락총을 통한 유입이 밝혀졌고 그 조절 핵심 인자가 IFN-γ라는 사실도 확인한 바 있음. 이같은 결과는 면역세포가 염증반응을 통한 면역조절에 있어서 중추신경내로 직접 이동할 수 있다는 것을 증명함으로써 **알츠하이머 치매에 면역조절 치료제의 적용** 가능성을 시사함





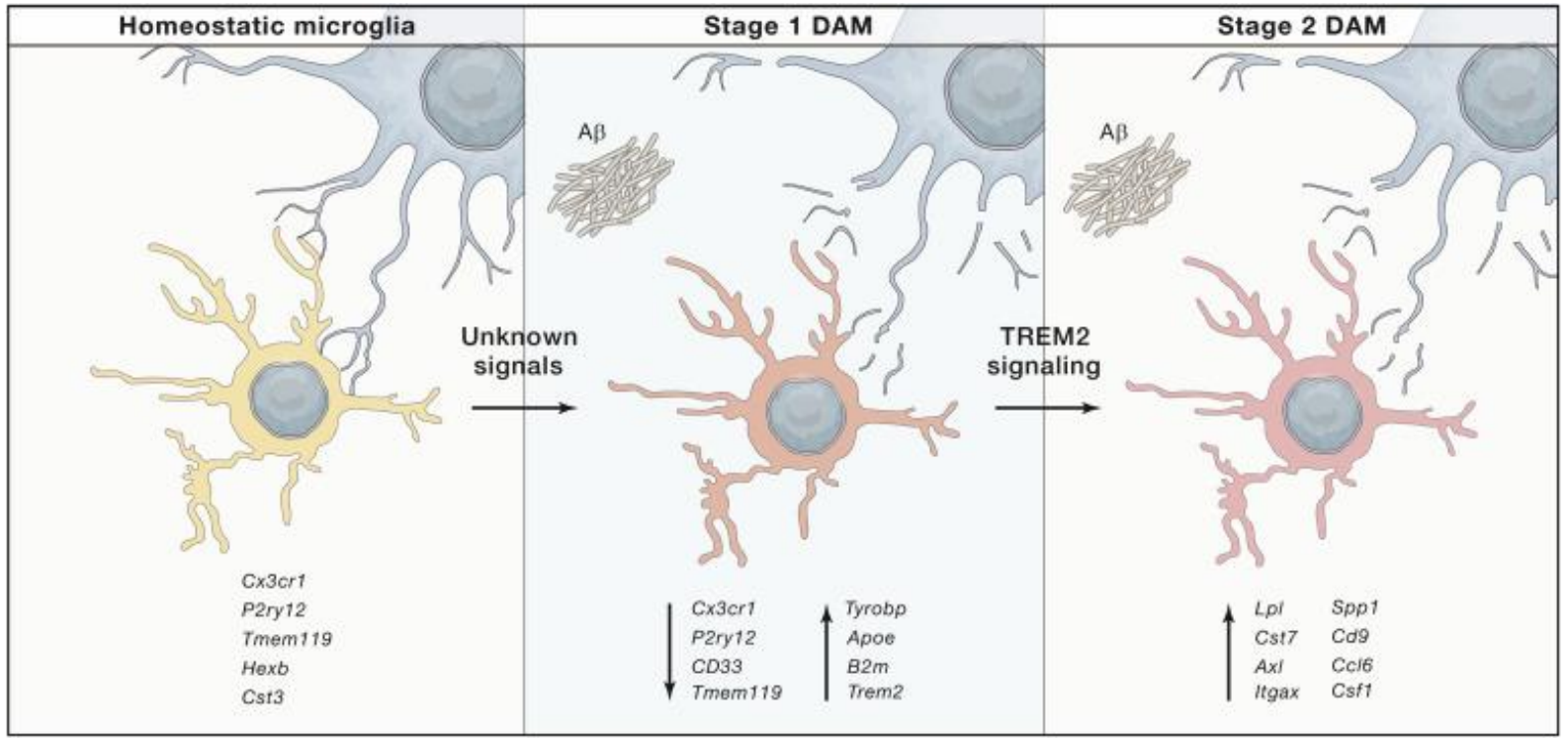
AD target - Treg

- 퇴행성 뇌질환 새로운 치료타겟 Treg (조절 T 임파구)



Disease Associated Microglia (DAM)

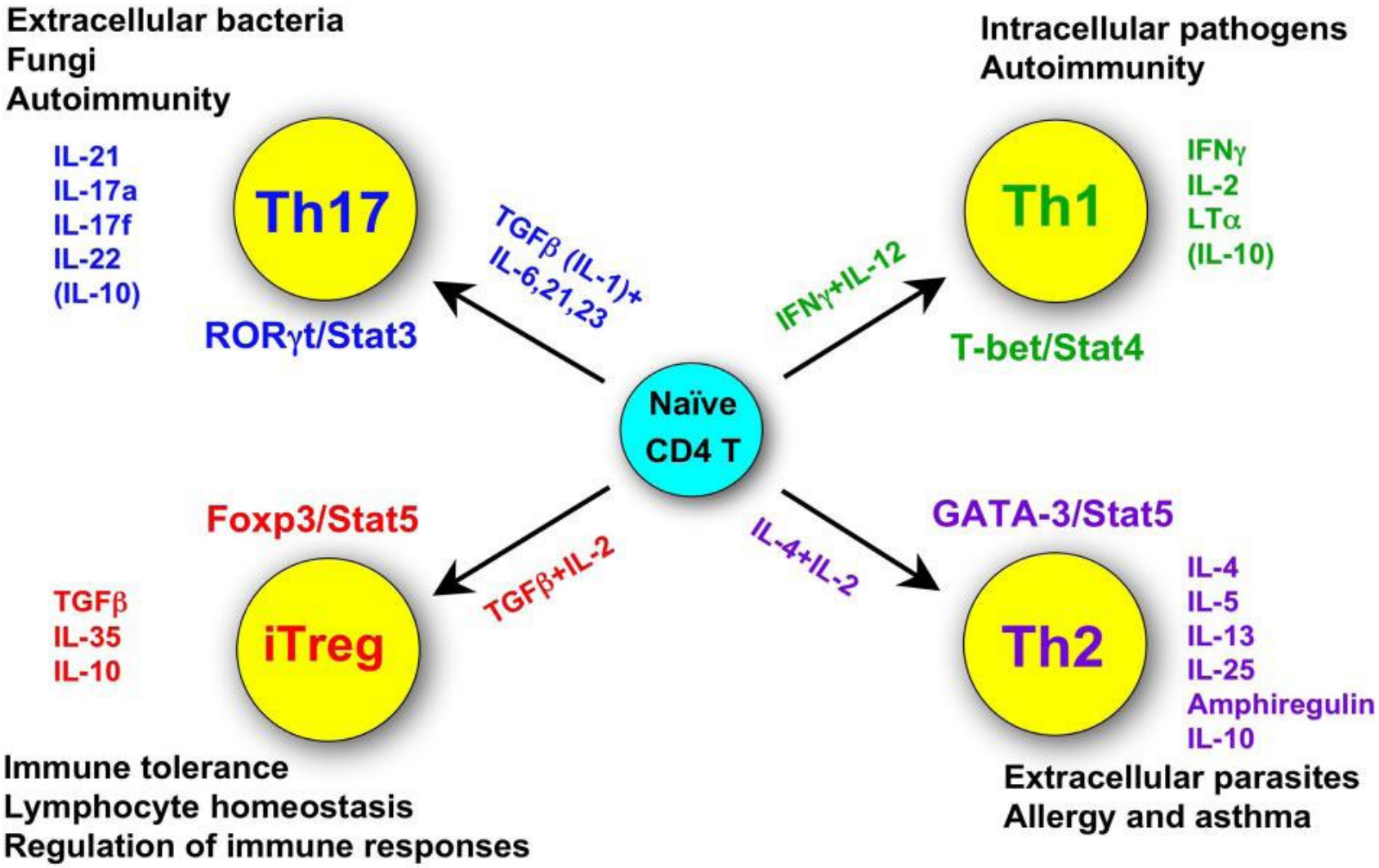
- Alzheimer's disease-associated phagocytic cells conserved in mice and human
- Activated sequentially by Trem2-independent and-dependent pathways



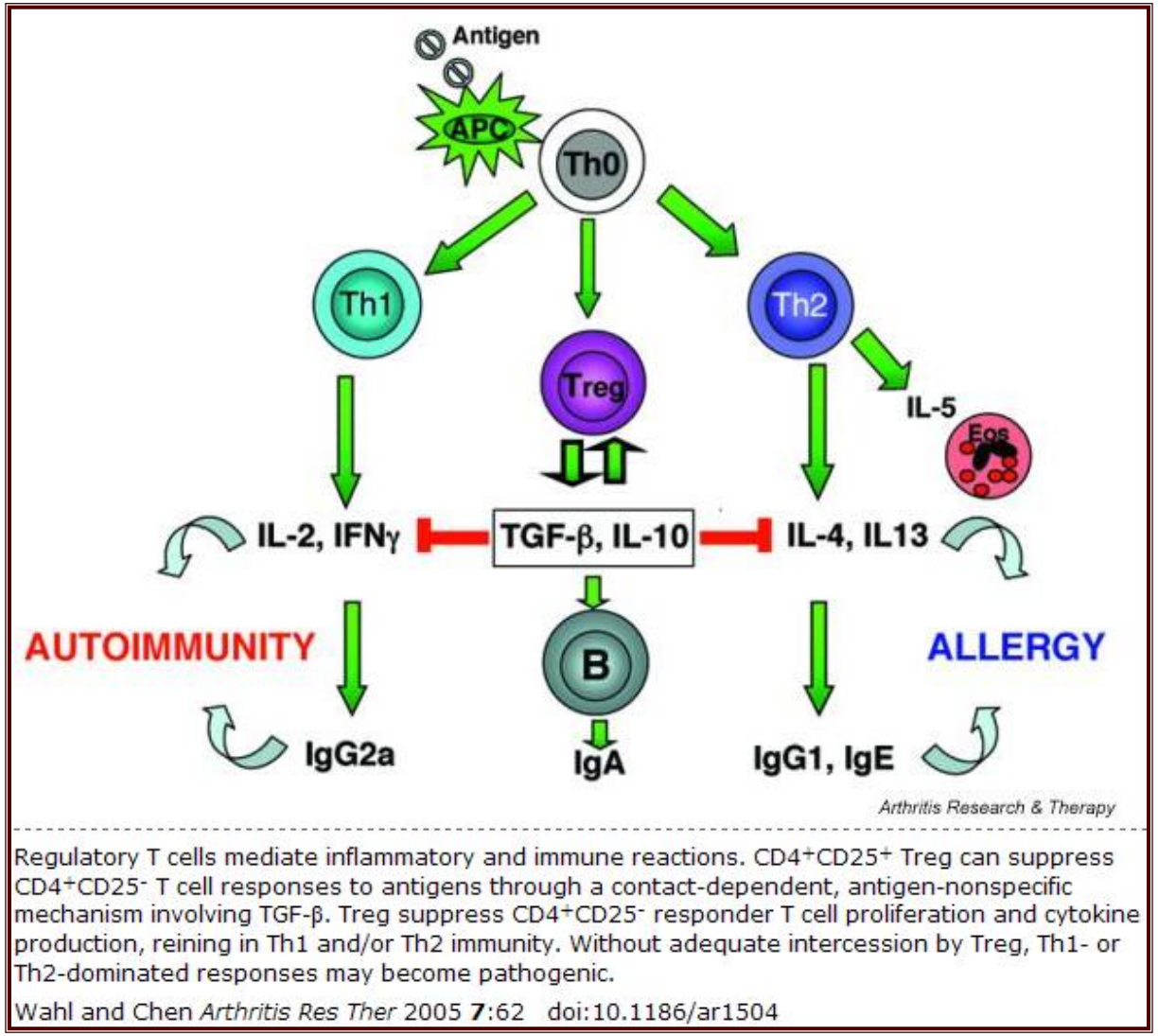
Regulatory T cells (Tregs)

- Treg cells suppress the activation, proliferation, differentiation, and effector functions of many cell types including CD4 and CD8 T cells, B cells, natural killer cells, and dendritic cells, thus **maintaining peripheral tolerance** (preventing autoimmunity) and limiting chronic inflammatory diseases **(immune homeostasis)**

CD4⁺ cell development



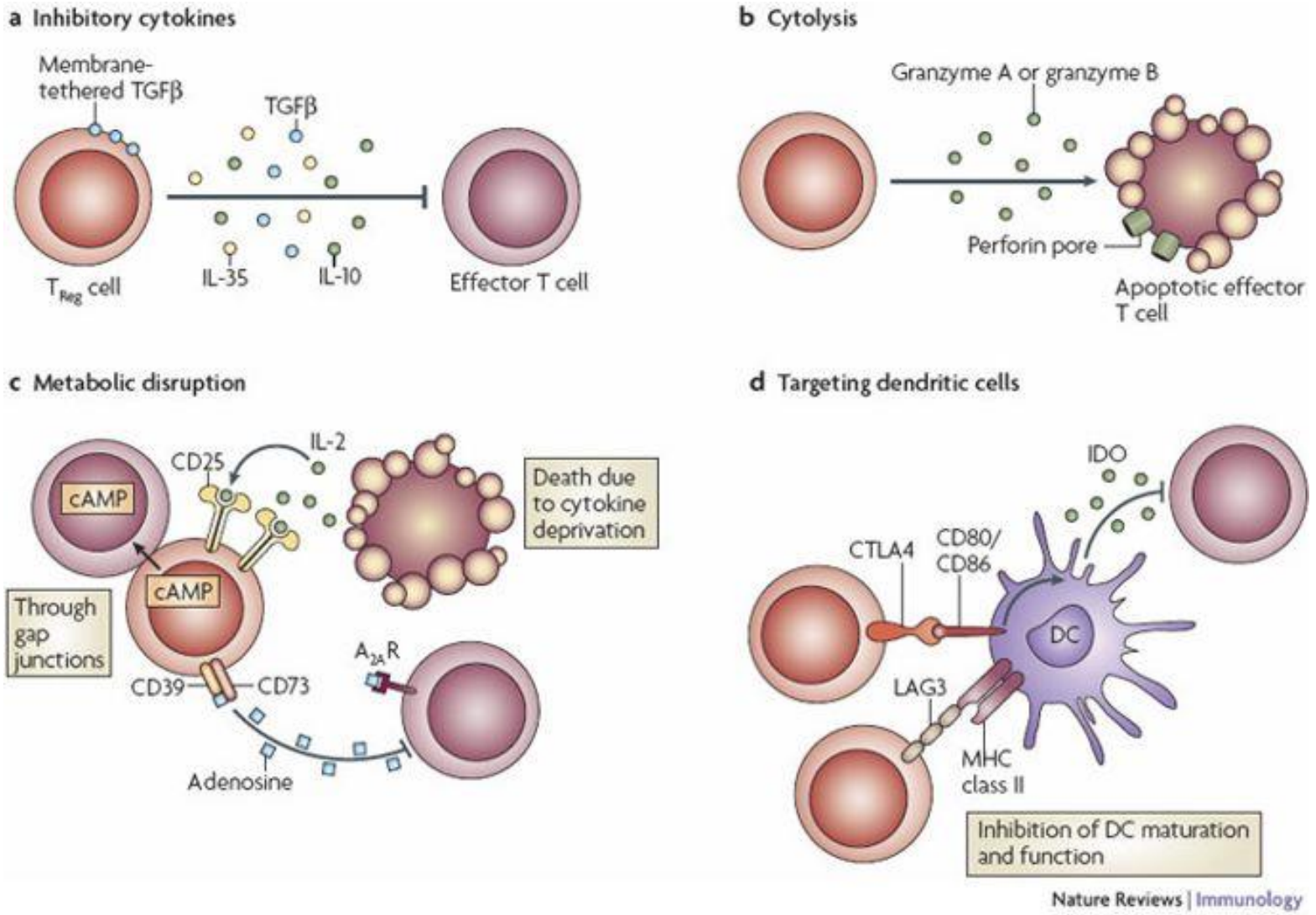
Treg



Regulatory T cells mediate inflammatory and immune reactions. $CD4^+CD25^+$ Treg can suppress $CD4^+CD25^-$ T cell responses to antigens through a contact-dependent, antigen-nonspecific mechanism involving TGF-β. Treg suppress $CD4^+CD25^-$ responder T cell proliferation and cytokine production, reining in Th1 and/or Th2 immunity. Without adequate intercession by Treg, Th1- or Th2-dominated responses may become pathogenic.

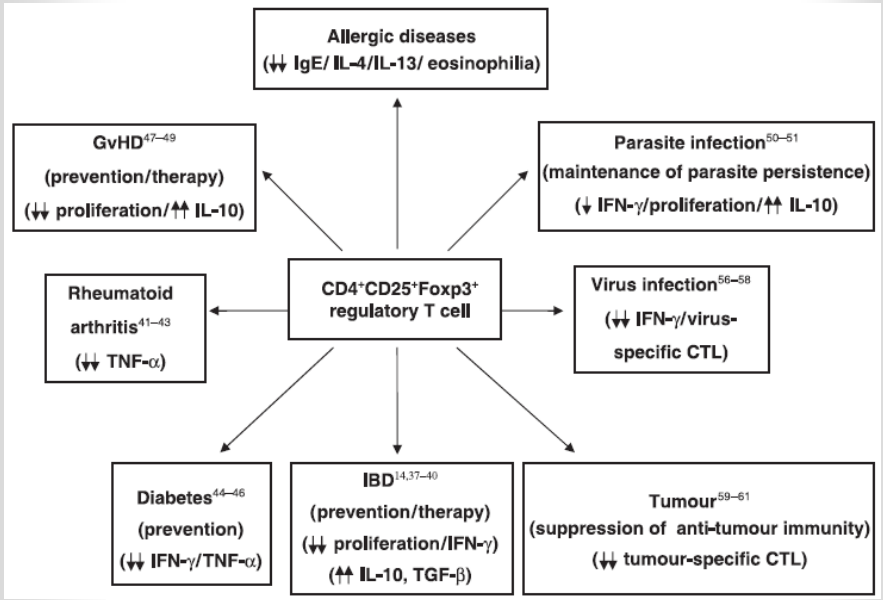
Wahl and Chen *Arthritis Res Ther* 2005 **7**:62 doi:10.1186/ar1504

Proposed Treg suppressive mechanisms



Treg as a drug

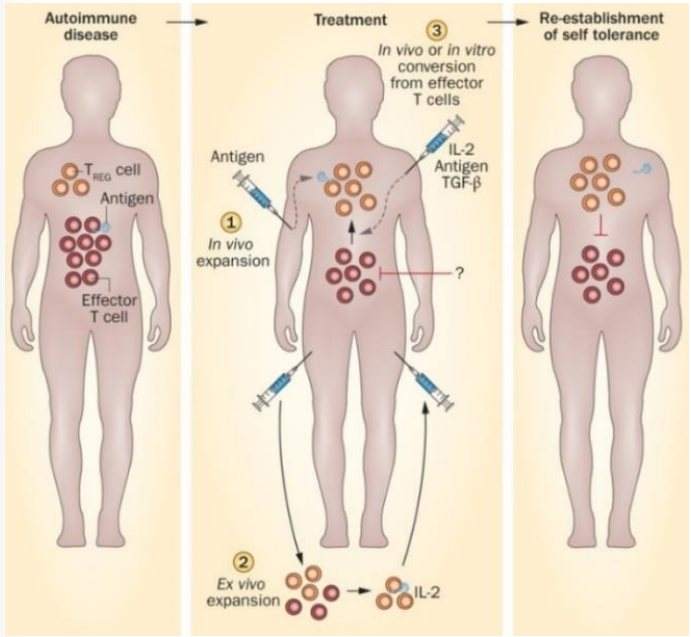
다수 면역질환에서 Treg 세포 효과



[Clinical and Experimental Pharmacology and Physiology, 2006; 33, 519–524]

Treg 세포는 자가면역질환, 알레르기성 질환, 장기이식거부반응 등의 과도한 면역반응을 억제

Treg 세포 치료법



[Nature Review Rheumatology, 2014; 10,543-51]

Treg 세포 증식을 위한 다양한 시도가 진행 중

Treg Clinical trials

ClinicalTrials.gov Search Results 07/19/2019

	Title	Status	Study Results	Conditions	Interventions	Locations
1	Multiple Donor Treg DLI for Severe Refractory Chronic GVHD	Recruiting	No Results Available	•Chronic Graft Versus Host Disease	•Biological: T reg DLI	•University Hospital St. Orsola-Malpighi Polyclinic, Bologna, BO, Italy
2	Safety and Efficacy Study of Regulatory T Cells in Treating Autoimmune Hepatitis	Unknown status	No Results Available	•Autoimmune Diseases	•Biological: Regulatory T cells	
3	Treg Immunotherapy in Crohn's Disease	Not yet recruiting	No Results Available	•Crohn Disease	•Drug: TR004 (Treg immunotherapy) •Other: Placebo	
4	A Phase 1/2 Trial of Donor Regulatory T-cells for Steroid-Refractory Chronic Graft-versus-Host-Disease	Recruiting	No Results Available	•Graft vs Host Disease	•Biological: Donor regulatory T cell adoptive immunotherapy in chronic graft versus host disease	•Instituto Portugues de Oncologia, Lisboa, Portugal •Hospital de Santa Maria, Faculdade de Medicina da Universidade de Lisboa, Instituto de Medicina Molecular, Lisboa, Portugal •Instituto Portugues de Oncologia, Porto, Portugal
5	Amplifying Graft-Versus-Tumor Effect by Donor Regulatory T-Cell Depletion Before Donor Lymphocytes Infusion	Completed	No Results Available	•Hematologic Neoplasms •Relapse	•Procedure: donor lymphocyte infusion	•Hopital Henri Mondor, Creteil, France
6	In-vivo Regulatory T Cell Enhancement With Cyclophosphamide and Sirolimus With or Without Vidaza (Azacitidine) for Steroid-refractory Acute Graft-versus-host Disease	Completed	No Results Available	•Graft Versus Host Disease	•Drug: Cyclophospahmide and Sirolimus •Drug: Low dose IL-2, Cyclophosphamide and Sirolimus •Drug: Low dose IL-2, low dose Vidaza, cyclophosphamide & Sirolimus	•John Theurer Cancer Center at Hackensack University Medical Center, Hackensack, New Jersey, United States
7	Donor Regulatory T-cells for Steroid-Refractory Chronic Graft-versus-host-Disease	Recruiting	No Results Available	•Chronic Graft vs Host Disease	•Biological: Regulatory T-cell enriched infusion	•Hospital Universitario Virgen del Rocío, Sevilla, Seville, Spain
8	Treg Adoptive Therapy for Subclinical Inflammation in Kidney Transplantation	Unknown status	No Results Available	•Late Complication From Kidney Transplant	•Biological: Treg infusion	•University of California, San Francisco, San Francisco, California, United States
9	Donor Alloantigen Reactive Tregs (darTregs) for Calcineurin Inhibitor (CNI) Reduction	Recruiting	No Results Available	•Liver Transplant Recipient •Living Donor (of the Respective Liver Transplant Recipient)	•Biological: darTregs •Drug: Acetaminophen •Drug: Diphenhydramine •Drug: Immunosuppression (IS) Withdrawal •Procedure: Study Mandated Procedures	•University of California at San Francisco, San Francisco, California, United States •Northwestern University Comprehensive Transplant Ctr, Chicago, Illinois, United States •Mayo Clinic in Rochester, Rochester, Minnesota, United States
10	Adoptive TReg Cell for Suppression of aGVHD After UCB HSCT for Heme Malignancies	Suspended	No Results Available	•Acute Lymphoblastic Leukemia •Burkitt Lymphoma •Natural Killer Cell Malignancies •Chronic Myelogenous Leukemia •Myelodysplastic Syndromes •Large-cell Lymphoma •Chronic Lymphocytic Leukemia •Small Lymphocytic Lymphoma •Marginal Zone B-Cell Lymphoma •Follicular Lymphoma •and 8 more	•Biological: Infusion of Treg	•Masonic Cancer Center at University of Minnesota, Minneapolis, Minnesota, United States

470 additional studies not shown

U.S. National Library of Medicine | U.S. National Institutes of Health | U.S. Department of Health & Human Services

2021년 10월 현재 interventional trial 등록 건수 244건
Alzheimer's disease관련 Trial은 1건(VT301) Phase 1

U.S. National Library of Medicine
ClinicalTrials.gov

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Trial record 3 of 309 for: Treg | Interventional Studies | Phase 1

Previous Study | Return to List | Next Study

T1DM Immunotherapy Using CD4+CD127lo/-CD25+ Polyclonal Tregs (Treg)

The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT01210664

Recruitment Status : Completed
First Posted : September 28, 2010
Results First Posted : July 11, 2018
Last Update Posted : July 11, 2018

Sponsor:
University of California, San Francisco

Collaborators:
Juvenile Diabetes Research Foundation
National Institute of Allergy and Infectious Diseases (NIAID)

Information provided by (Responsible Party):
University of California, San Francisco

Study Details | Tabular View | Study Results | Disclaimer | How to Read a Study Record

Study Type	**Interventional**
Study Design	Intervention Model: Single Group Assignment; Masking: None (Open Label); Primary Purpose: Treatment
Condition	Type 1 Diabetes Mellitus
Intervention	Biological: Ex vivo Expanded Human Autologous Polyclonal **Regulatory T Cells**
Enrollment	16

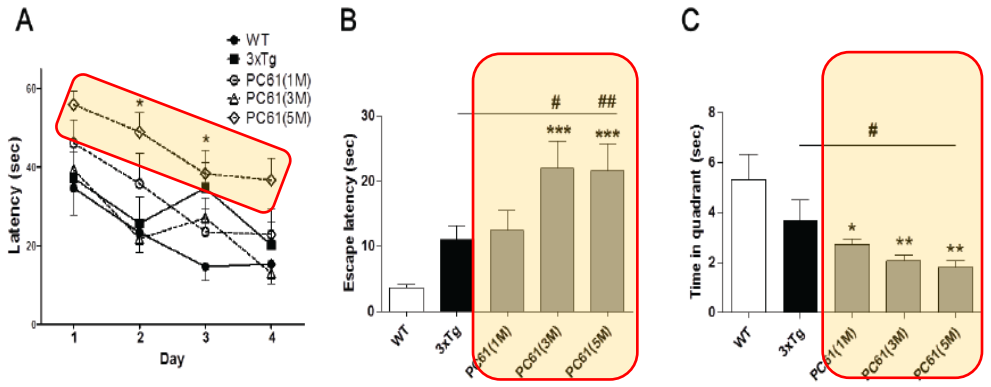
Participant Flow Go to

Recruitment Details				
Pre-assignment Details				
Arm/Group Title	Polyclonal Regulatory T Cells, 0.05 x10^8 Cells	Polyclonal Regulatory T Cells, 0.4 x10^8 Cells	Polyclonal Regulatory T Cells, 3.2 x10^8 Cells	Polyclonal Regulatory T Cells, 26 x10^8 Cells
▼ Arm/Group Description	Cohort 1: Patients with Type 1 Diabetes Mellitus will have their regulatory T cells (Tregs) isolated by researchers and receive 0.05 x10^8 cells of Ex vivo Expanded Human	Cohort 2: Patients with Type 1 Diabetes Mellitus will have their regulatory T cells (Tregs) isolated by researchers and receive 0.4 x10^8 cells of Ex vivo Expanded Human Autologous	Cohort 3: Patients with Type 1 Diabetes Mellitus will have their regulatory T cells (Tregs) isolated by researchers and receive 3.2 x10^8 cells of Ex vivo expanded Human Autologous	Cohort 4: Patients with Type 1 Diabetes Mellitus will have their regulatory T cells (Tregs) isolated by researchers and receive 26 x10^8 cells of Ex vivo expanded Human Autologous

알츠하이머 치매에 미치는 조절 T 세포의 역할

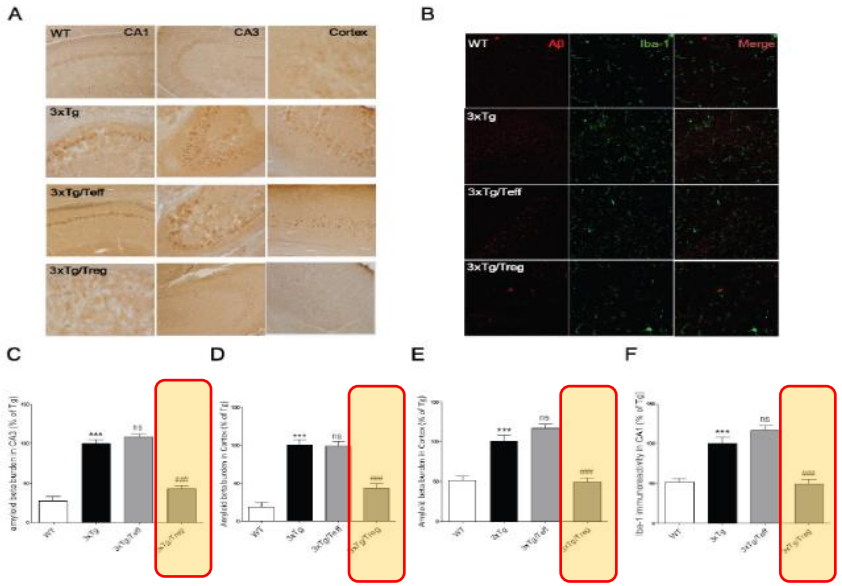
- 조절 T 세포를 **제거**하면 3xTg 마우스에서 알츠하이머 치매 병증이 **악화**됨을 확인
- 조절 T 세포를 **주입**하면 3xTg 마우스에서 알츠하이머 치매 병증이 **완화**됨을 확인

➢**알츠하이머 치매 환자에 조절T세포의 주입으로 치료가 될 수 있음을 시사함**



조절T세포 제거 기간에 따른 인지능력 및 기억력 감소효과

Oncotarget, 2016



해마 부위의 amyloid plaque 및 미세아교세포의 변화

알츠하이머 치매에 미치는 조절 T 세포의 역할

www.impactjournals.com/oncotarget/ Oncotarget, Vol. 7, No. 43

Research Paper: Immunology

Neuroprotective effects of CD4+CD25+Foxp3+ regulatory T cells in a 3xTg-AD Alzheimer's disease model

Hyunjung Baek[1], Minsook Ye[1], Geun-Hyung Kang[1], Chanju Lee[1], Gihyun Lee[1], Da Bin Choi[1], Jaehoon Jung[1], Hyunseong Kim[2], Seonhwa Lee[3], Jin Su Kim[3], Hyun-ju Lee[4], Insop Shim[4], Jun-Ho Lee[5] and Hyunsu Bae[1]

doi:10.1093/brain/aww408 BRAIN 2016: 139; 1237–1251 | 1237

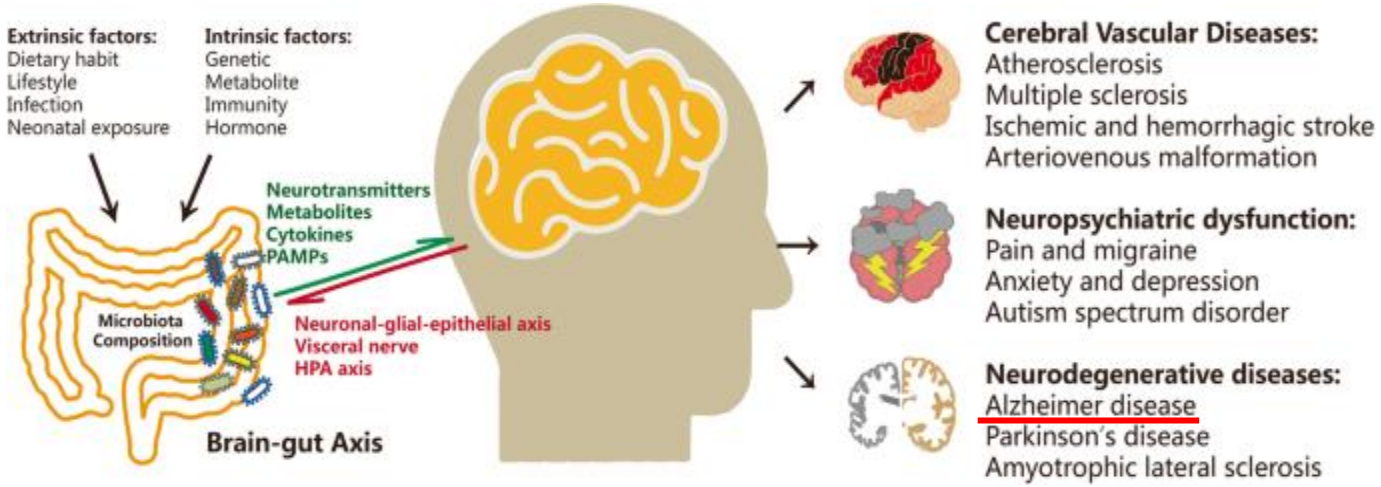
BRAIN
A JOURNAL OF NEUROLOGY

Regulatory T cells delay disease progression in Alzheimer-like pathology

Cira Dansokho,[1,2,*] Dylla Ait Ahmed,[1,2,*] Saba Aid,[2,3,*] Cécile Toly-Ndour,[1,2,*] Thomas Chaigneau,[1,2] Vanessa Calle,[1,2] Nicolas Cagnard,[4] Martin Holzenberger,[2,3] Eliane Piaggio,[5,6] Pierre Aucouturier[1,2] and Guillaume Dorothée[1,2]

알츠하이머 치매와 조절 T 세포 관련 최신 연구결과

- Gut microbiota-activated TH17 cells, characterized by IL-17A, IL-17F, and IL-22 secretion, are responsible for high affinity IgA secretion, memory CD4+ T cell differentiation.
- Bacterial metabolites and products have recently been shown to worsen AD.
- In AD patients, bacteria-derived amyloids (curli, tau, Aβ, α-syn, and prion) can function as initiators to cross-seed and aggregate plaques and tangles



Zhu et al. Journal of Neuroinflammation (2020) 17:25
https://doi.org/10.1186/s12974-020-1705-z

Journal of Neuroinflammation

REVIEW Open Access

The progress of gut microbiome research related to brain disorders

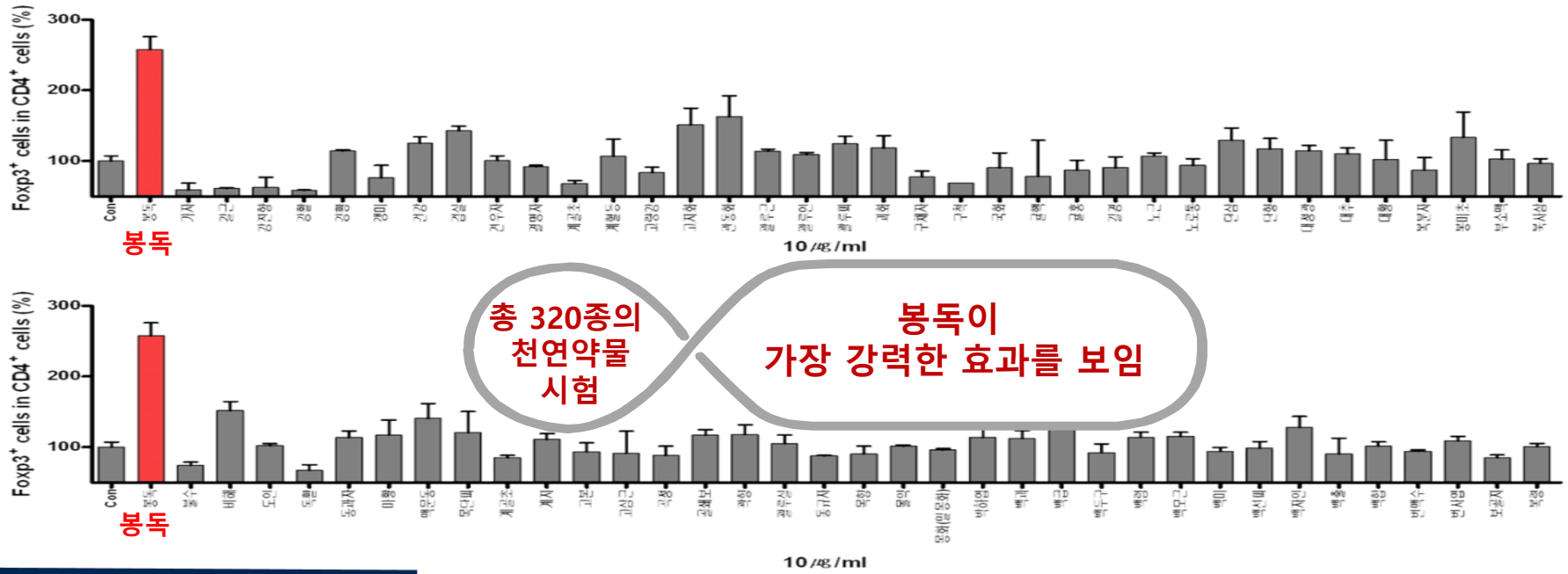
Gut. 2020 Feb;69(2):283-294. doi: 10.1136/gutjnl-2018-317431. Epub 2019 Aug 30.

Transfer of a healthy microbiota reduces amyloid and tau pathology in an Alzheimer's disease animal model

Min-Soo Kim [# 1 2], Yoonhee Kim [# 3], Hyunjung Choi [# 4], Woojin Kim [5], Sumyung Park [5], Dongjoon Lee [3], Dong Kyu Kim [3], Haeng Jun Kim [3], Hayoung Choi [3], Dong-Wook Hyun [1], June-Young Lee [1], Eun Young Choi [5], Dong-Sup Lee [5], Jin-Woo Bae [6], Inhee Mook-Jung [7 4]

The story about bee venom Phospholipase A2

Treg 세포 증식 약물 선별



총 320종의 천연약물 시험

봉독이 가장 강력한 효과를 보임

치료제로서의 봉독 사용

- 히포크라테스(B. C. 460-377): 봉독 "신비한 약(arcanum)"
- 한의학 최초의 침구학 문헌 마황퇴의서(B.C. 168) 봉독 치료 기록
- 1858년 프랑스의 Desjardins가 류마티스성 질환 치료 보고
- 현재 임상에서 경추나 요추의 통증 및 디스크, 퇴행성 관절염, 통풍, 오십견, 섬유근통, 섬유근통, 근막통증 증후군, 산후 관절통, 대상포진, 수근관 증후군, 테니스 엘보, 염좌, 신경염, 다발성 경화증, 발기부전, 우울증, 신경증, 만성피로 증후군, 비만, 탈모, 알레르기 등의 질환에 사용됨
- 본 연구팀은 수년간 봉독의 Treg 세포를 통한 면역조절에 대한 연구를 진행하여 봉독이 다발성 경화증, 천식, 파킨슨병, 치매 등 면역관련 질병에 효과가 있음을 증명하였음

Bee venom on immune diseases

Contents lists available at SciVerse ScienceDirect

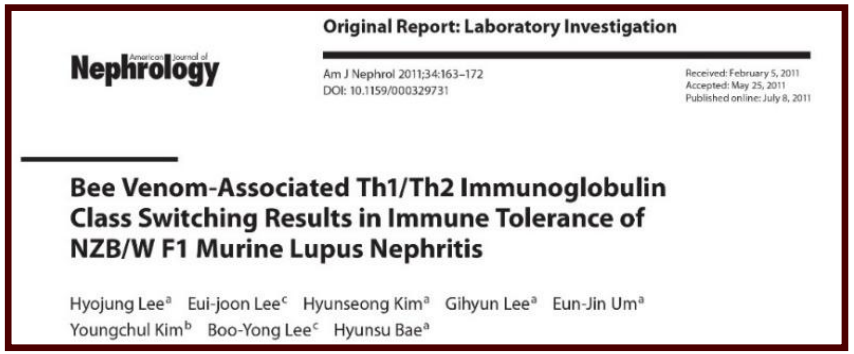
Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Neuro-protective effects of bee venom by suppression of neuroinflammatory responses in a mouse model of Parkinson's disease: Role of regulatory T cells

Eun Sook Chung[a], Himchan Kim[a], Gihyun Lee[a,b], Soojin Park[a,b], Hyunseong Kim[a,b], Hyunsu Bae[a,b,*]

Parkinson's disease



Nephrology

Original Report: Laboratory Investigation

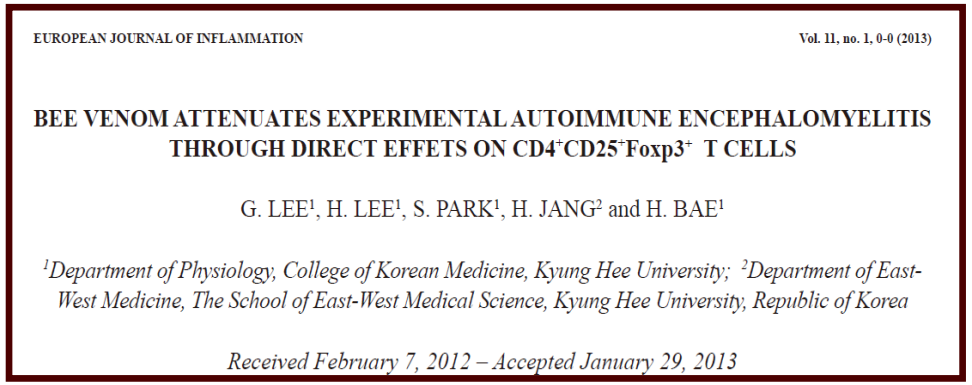
Am J Nephrol 2011;34:163–172
DOI: 10.1159/000329731

Received: February 5, 2011
Accepted: May 25, 2011
Published online: July 8, 2011

Bee Venom-Associated Th1/Th2 Immunoglobulin Class Switching Results in Immune Tolerance of NZB/W F1 Murine Lupus Nephritis

Hyojung Lee[a] Eui-joon Lee[c] Hyunseong Kim[a] Gihyun Lee[a] Eun-Jin Um[a]
Youngchul Kim[b] Boo-Yong Lee[c] Hyunsu Bae[a]

Lupus



EUROPEAN JOURNAL OF INFLAMMATION Vol. 11, no. 1, 0-0 (2013)

BEE VENOM ATTENUATES EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS THROUGH DIRECT EFFETS ON $CD4^+CD25^+Foxp3^+$ T CELLS

G. LEE[1], H. LEE[1], S. PARK[1], H. JANG[2] and H. BAE[1]

[1]Department of Physiology, College of Korean Medicine, Kyung Hee University; [2]Department of East-West Medicine, The School of East-West Medical Science, Kyung Hee University, Republic of Korea

Received February 7, 2012 – Accepted January 29, 2013

Multiple Sclerosis

Contents lists available at SciVerse ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

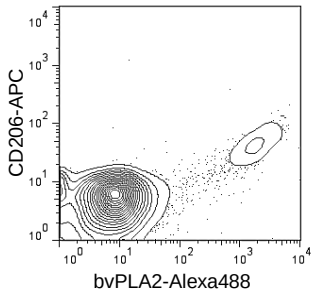
Bee venom ameliorates ovalbumin induced allergic asthma via modulating $CD4^+CD25^+$ regulatory T cells in mice

Myoung Suk Choi[a,1], Soojin Park[b,1], Taewon Choi[b], Gihyun Lee[b], Kyoung-Keun Haam[b], Moo-Chang Hong[b], Byung-Il Min[a], Hyunsu Bae[b,*]

Asthma

bvPLA2-binding on CD206⁺ DCs is critical for Treg differentiation

A



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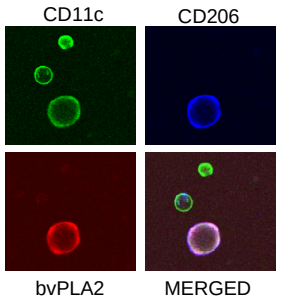

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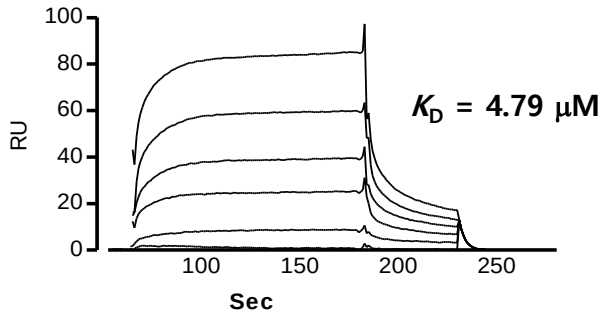

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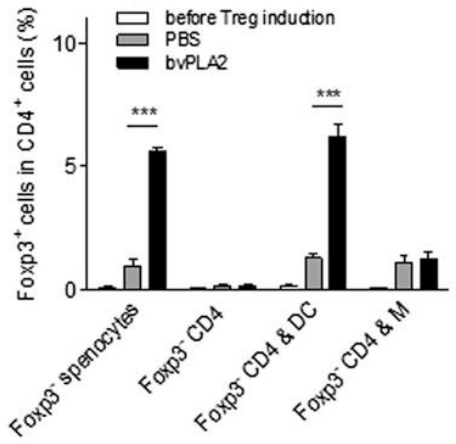

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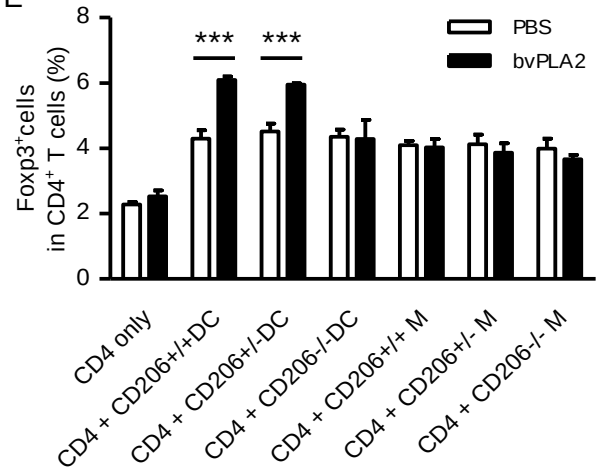
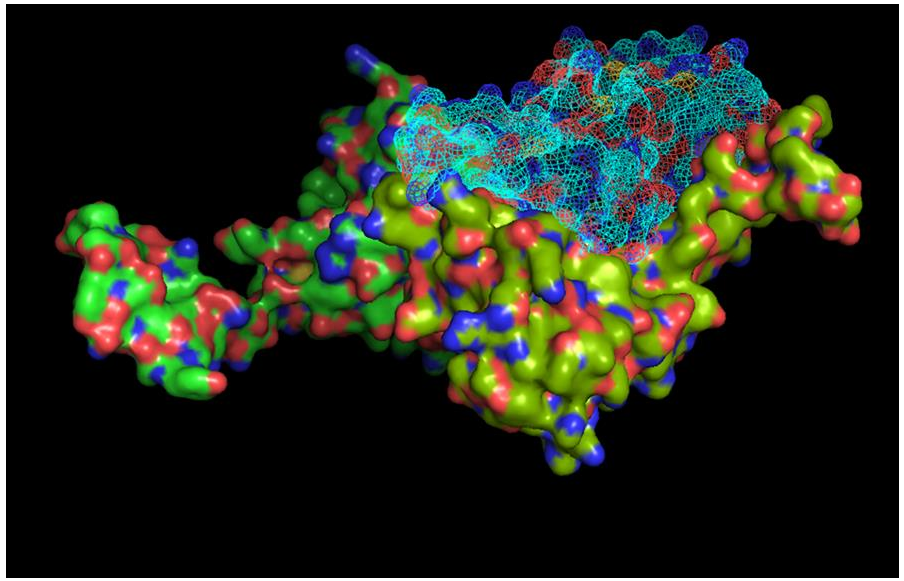
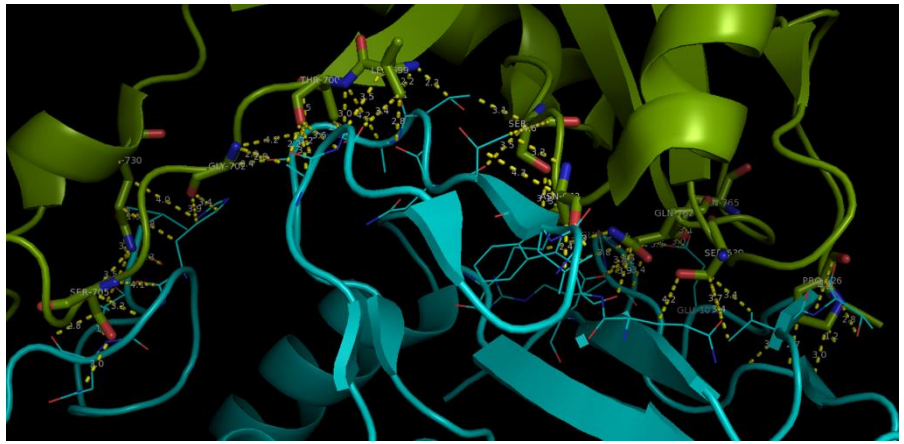




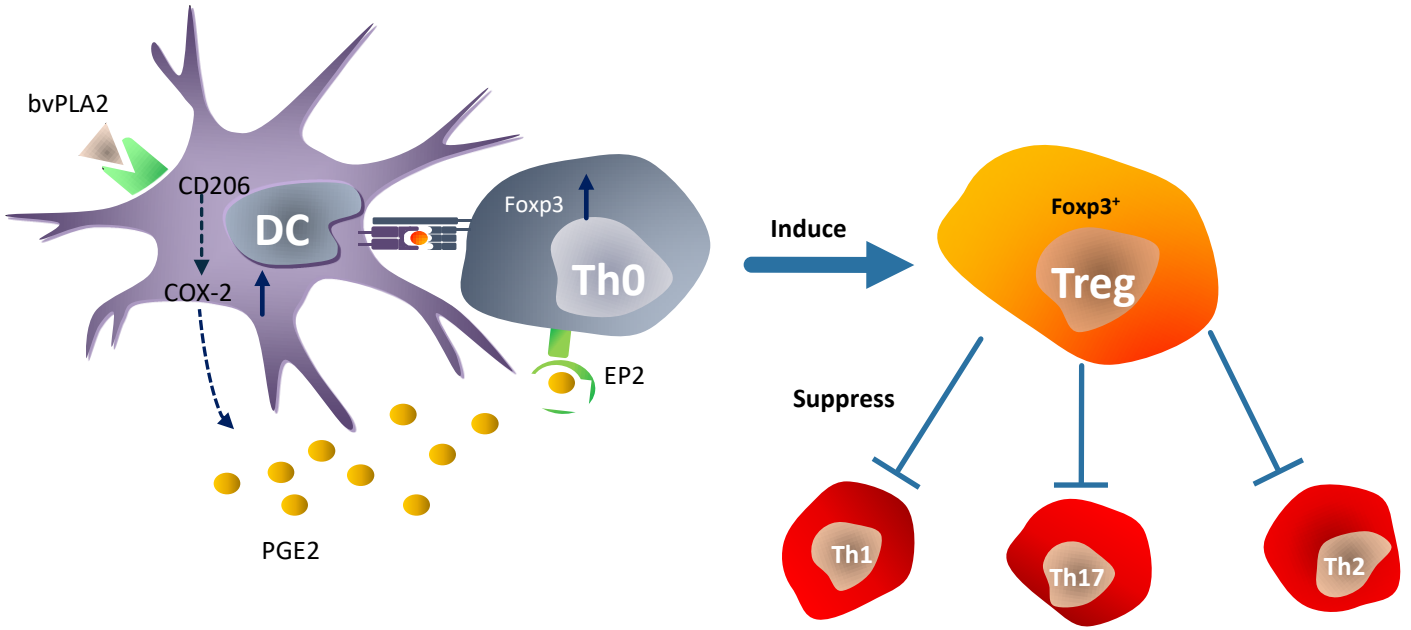


Mode of Action of bvPLA2

PLA2의 Treg 세포 분화 증가 효과를 세계 최초로 규명

[Journal of Immunology, 2015; doi:10.4049/jimmunol.1500386]

봉독 PLA2가 Treg 세포 분화를 유도함과 그 기전을 명확히 규명



치매, 파킨슨씨병/ Multiple sclerosis/Athritis/Asthma

- **bvPLA2 binding on CD206 of Dendritic cells results in PGE2 secretion**
- EP2 receptor signal in naïve T cells induces Treg which inhibit inflammatory diseases

동물모델: bvPLA2에 의한 알츠하이머성 치매 치료효과

* 봉독 유효성분 투여에 의한 인지기능 및 기억력 향상 효과
* 뇌의 해마 부위에 축적된 아밀로이드플라크의 감소효과

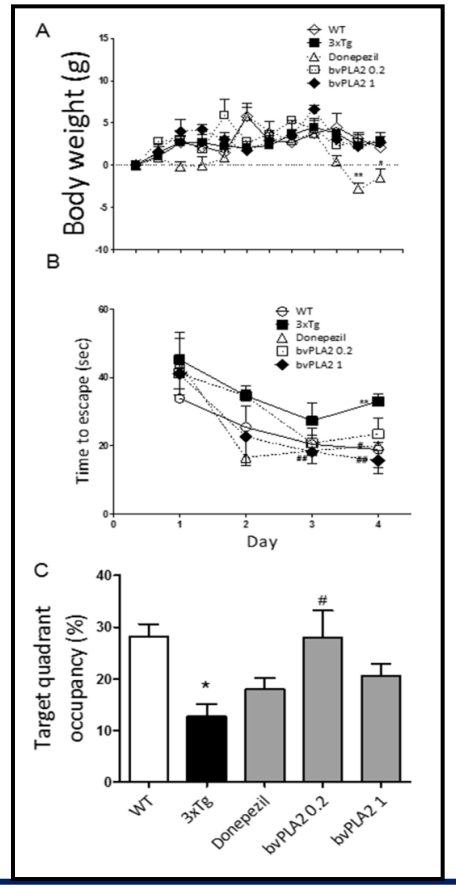
RESEARCH Open Access

Neuroprotective effects of bee venom phospholipase A2 in the 3xTg AD mouse model of Alzheimer's disease

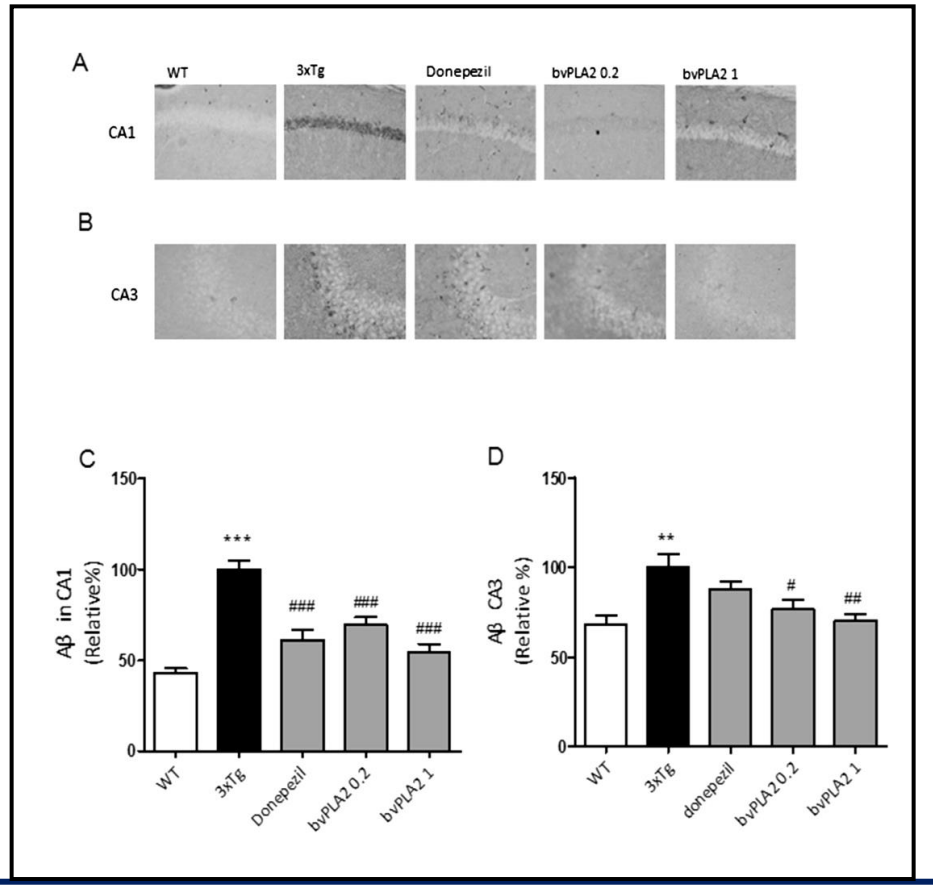
Minsook Ye[1], Hwan-Suck Chung[2], Chanju Lee[1], Moon Sik Yoon[1], A. Ram Yu[3], Jin Su Kim[3], Deok-Sang Hwang[4], Insop Shim[5] and Hyunsu Bae[1*]

Journal of Neuroinflammation (2016) 13:10

Effect on behavior



Effect on Ab plaque formaiton



bvPLA2에 의한 치료효능 논문

The Journal of Immunology
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Bee Venom Phospholipase A_2, a Novel Foxp3$^+$ Regulatory T Cell Inducer, Protects Dopaminergic Neurons by Modulating Neuroinflammatory Responses in a Mouse Model of Parkinson's Disease

Eun Sook Chung*,1, Gihyun Lee*,1, Chanju Lee*, Minsook Ye*, Hwan-suck Chung*, Hyunseong Kim*, Sung-joo S. Bae†, Deok-Sang Hwang‡ and Hyunsu Bae*

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1 E.S.C. and G.L. contributed equally to this work.

OPEN

Experimental & Molecular Medicine (2016) 48, e244; doi:10.1038/emm.2016.49

www.nature.com/emm

ORIGINAL ARTICLE

Bee venom phospholipase A2 ameliorates motor dysfunction and modulates microglia activation in Parkinson's disease alpha-synuclein transgenic mice

Minsook Ye[1], Hwan-Suck Chung[2], Chanju Lee[1], Joo Hyun Song[1], Insop Shim[3], Youn-Sub Kim[4] and Hyunsu Bae[1]

α-Synuclein (α-Syn) has a critical role in microglia-mediated neuroinflammation, which leads to the development of Parkinson's disease (PD). Recent studies have shown that bee venom (BV) has beneficial effects on PD symptoms in human patients or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxin-induced PD mice. This study investigated whether treatment with BV-derived phospholipase A2 (bvPLA2) would improve the motor dysfunction and pathological features of PD in human A53T α-Syn mutant transgenic (A53T Tg) mice. The motor dysfunction of A53T Tg mice was assessed using the pole test. The levels of α-Syn, microglia and the M1/M2 phenotype in the spinal cord were evaluated by immunofluorescence. bvPLA2 treatment significantly ameliorated motor dysfunction in A53T Tg mice. In addition, bvPLA2 significantly reduced the expression of α-Syn, the activation and numbers of microglia, and the ratio of M1/M2 in A53T Tg mice. These results suggest that bvPLA2 could be a promising treatment option for PD.

Experimental & Molecular Medicine (2016) **48**, e244; doi:10.1038/emm.2016.49; published online 8 July 2016

Immunity, Inflammation and Disease
Open Access
ORIGINAL RESEARCH

Bee venom phospholipase A2 suppresses allergic airway inflammation in an ovalbumin-induced asthma model through the induction of regulatory T cells

Soojin Park†, Hyunjung Baek†, Kyung-Hwa Jung, Gihyun Lee, Hyeonhoon Lee, Geun-Hyung Kang, Gyeseok Lee, & Hyunsu Bae

Department of Physiology, College of Korean Medicine, Kyung Hee University, #1 Hoeki-Dong, Dongdaemoon-Gu, Seoul 130-701, Republic of Korea

basic research
http://www.kidney-international.org

Phospholipase A2 inhibits cisplatin-induced acute kidney injury by modulating regulatory T cells by the CD206 mannose receptor

Hyunseong Kim[1], Hyojung Lee[1], Gihyun Lee[1], Hyunil Jang[1], Sung-Su Kim[2], Heera Yoon[1], Geun-Hyung Kang[1], Deok-Sang Hwang[3], Sun Kwang Kim[1], Hwan-Suck Chung[4] and Hyunsu Bae[1]

[1]Department of Physiology, College of Korean Medicine, Kyung Hee University, Dongdaemoon-gu, Seoul, Republic of Korea; [2]Soram Korean Medicine Hospital, Gangnam-gu, Seoul, Republic of Korea; [3]Department of Obstetrics and Gynecology, College of Korean Medicine, Kyung Hee University, Dongdaemoon-gu, Seoul, Republic of Korea and [4]Cancer Preventive Material Development Research Center, Kyung Hee University, Dongdaemoon-gu, Seoul, Republic of Korea

toxins
MDPI
Article

Regulatory T Cells Contribute to the Inhibition of Radiation-Induced Acute Lung Inflammation via Bee Venom Phospholipase A_2 in Mice

Dasom Shin [1,†], Gihyun Lee [1,†], Sung-Hwa Sohn [2], Soojin Park [1], Kyung-Hwa Jung [1], Ji Min Lee [2], Jieun Yang [1], Jaeho Cho [2,*] and Hyunsu Bae [1,*]

1 Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul 130-701, South Korea; ssd060@naver.com (D.S.); glee@khu.ac.kr (G.L.); ih_sj@naver.com (S.P.); jhkh242@naver.com (K.-H.J.); yje2020@hanmail.net (J.Y.)
2 Department of Radiation Oncology, Yonsei University College of Medicine, Seoul 120-752, South Korea; 19980769@hanmail.net (S.-H.S.); carol114@yuhs.ac (J.M.L.)
* Correspondence: jjhmd@yuhs.ac (J.C.); hbae@khu.ac.kr (H.B.); Tel.: +82-2-2228-8095 (J.C.); +82-2-961-9316 (H.B.)
† These authors contributed equally to this work.

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Received: 10 March 2016; Accepted: 26 April 2016; Published: 30 April 2016

PLOS ONE
RESEARCH ARTICLE

Bee Venom Phospholipase A2 Protects against Acetaminophen-Induced Acute Liver Injury by Modulating Regulatory T Cells and IL-10 in Mice

Hyunseong Kim[1], Dong June Keum[1], Jung won Kwak[1], Hwan-Suck Chung[1]*, Hyunsu Bae[1,2]*

1. Department of Physiology, College of Korean Medicine, Kyung Hee University, 1 Hoeki-Dong, Dongdaemoon-gu, Seoul 130-701, Republic of Korea, **2.** Institute of Korean Medicine, Kyung Hee University, 1 Hoeki-Dong, Dongdaemoon-gu, Seoul 130-701, Republic of Korea

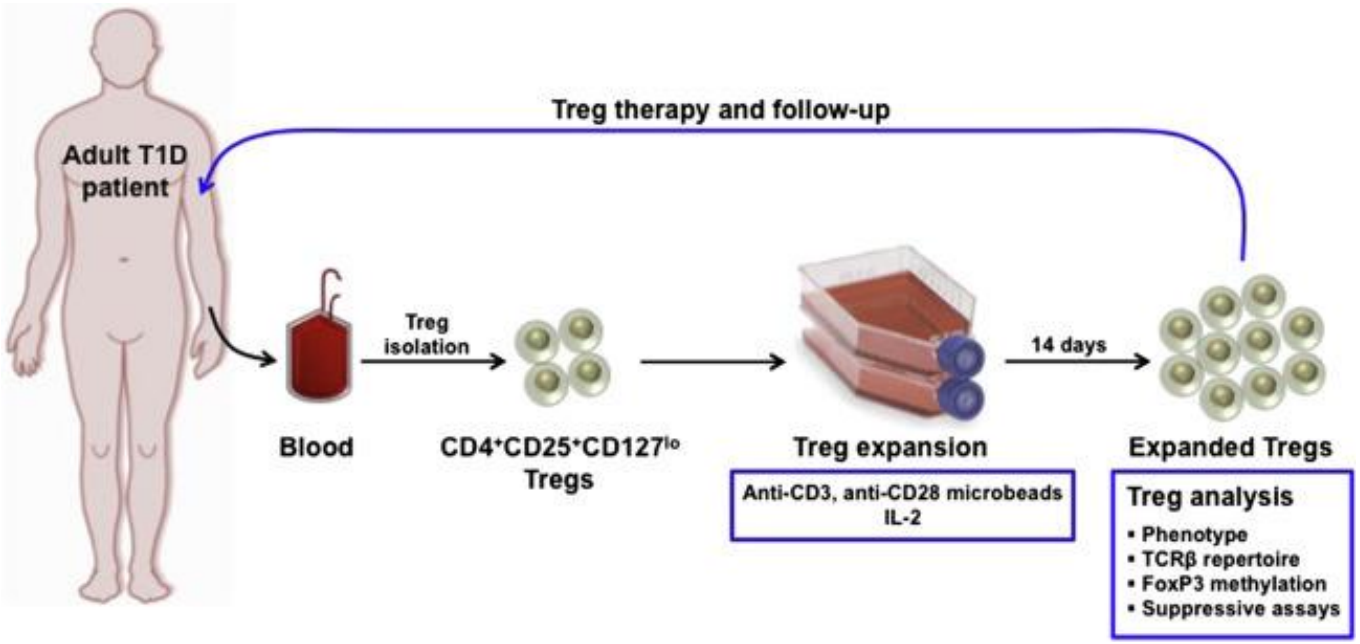
*hbae@khu.ac.kr (HB); sock21@hanmail.net (HSC)

Conclusion I

- Bee venom PLA2 is a novel Treg inducer
- bvPLA2 can be used for *ex vivo* Treg expansion

Adoptive Treg cell therapy

- Treg therapy is a promising therapeutic strategy for the treatment of patients with severe chronic inflammatory and autoimmune diseases
- Treg cells are isolated from a patient, enriched, expanded ex vivo, and reinfused
- Trials using polyclonal Tregs to induce tolerance after solid organ transplantation are currently ongoing for liver and kidney transplantation



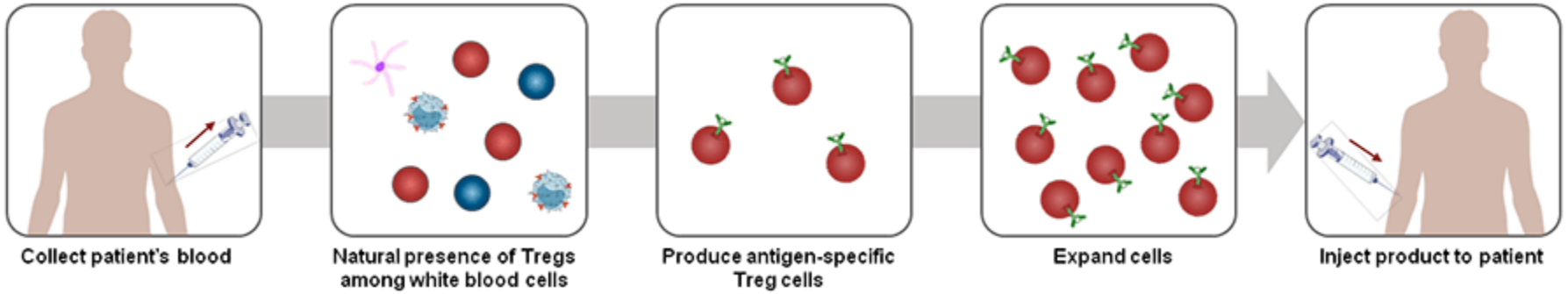
nTreg therapy: polyclonal or specific

- **Clinical trials using human polyclonal nTregs;** graft versus host disease (GVHD) following kidney transplantation, T1D, lupus, and liver disease

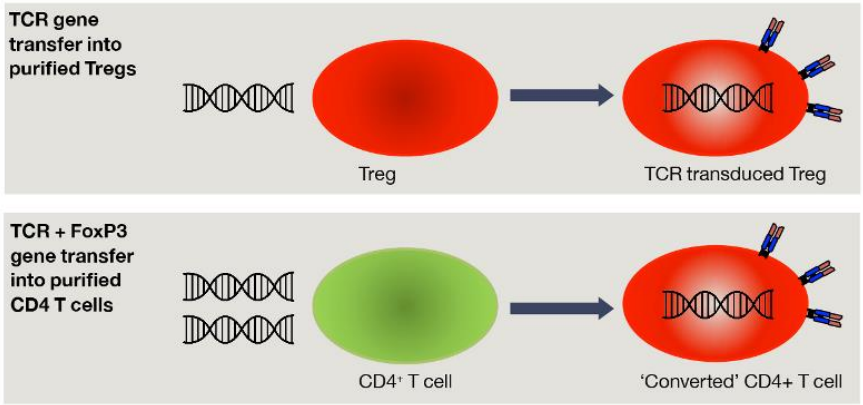
- **Limitations**
  - tumor occurrence/recurrence; correlation between Tregs and tumor survival
  - possibility of expanded Tregs reverting to Tconv cells
  - not homogenous population; may introduce unwanted variability and a lack of efficacy to their therapeutic potential

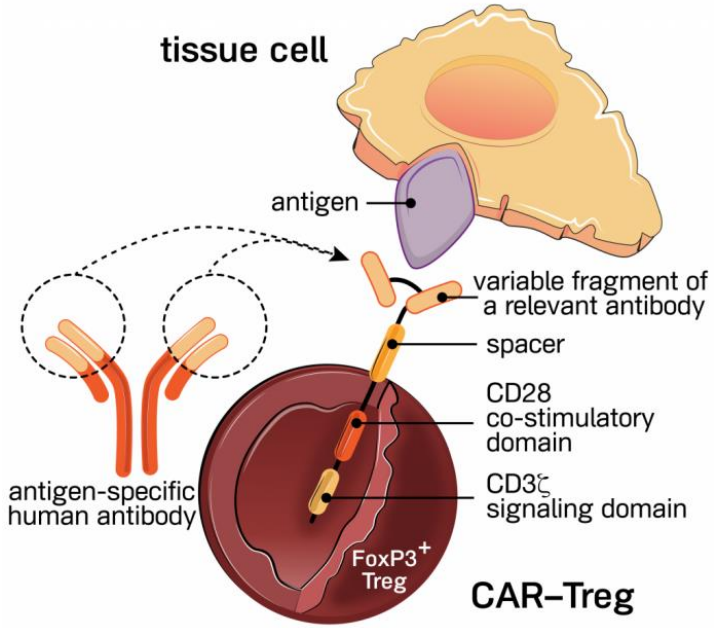
→**antigen-specific Tregs of a defined homogenous population**

Antigen-specific Treg therapy



Gene engineering to produce antigen-specific Treg





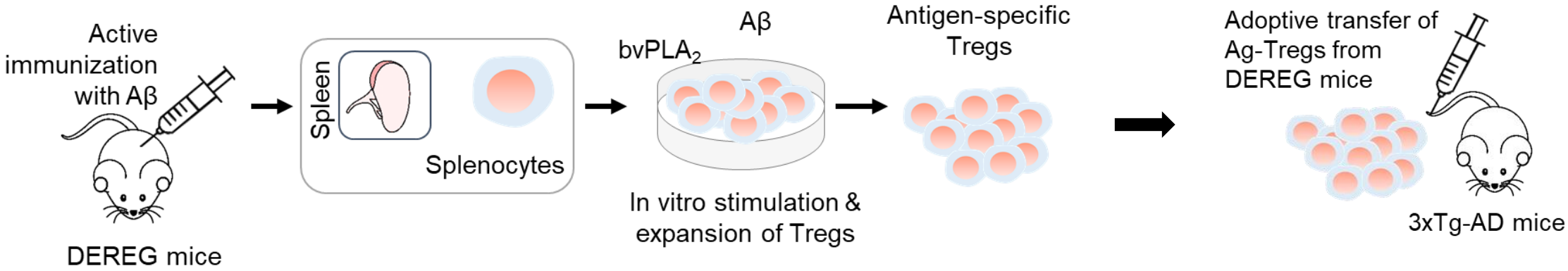
Therapeutic Treg for Alzheimer's disease

- **Restoring or expanding amyloid beta-specific Treg cells by bvPLA2 treatment might be beneficial for the treatment of Alzheimer's disease**

  ; investigate whether adoptive transfer of the Aβ-specific Treg populations would improve the cognitive function and Aβ pathology in 3xTg AD mice

  ; investigate the effects of bvPLA2, novel Treg inducer, for the Aβ-specific Treg expansion

Experimental schedule



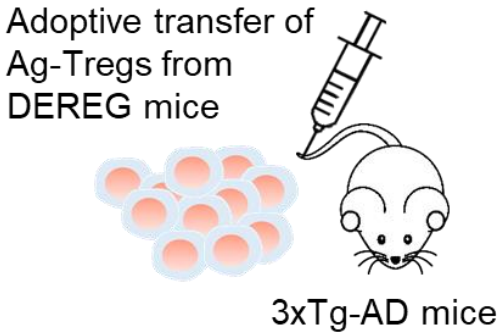
Treg;

- ✓ PBS (**poly-Treg**)
- ✓ Aβ
- ✓ bvPLA2 (PLA2)
- ✓ Aβ+bvPLA2 (**Ag-Treg**)

DEREG mice: depletion of regulatory T cell C57BL6-Tg(Foxp3-DTR/EGFP) mice

Distinction of Manufactured Ag-Treg

A



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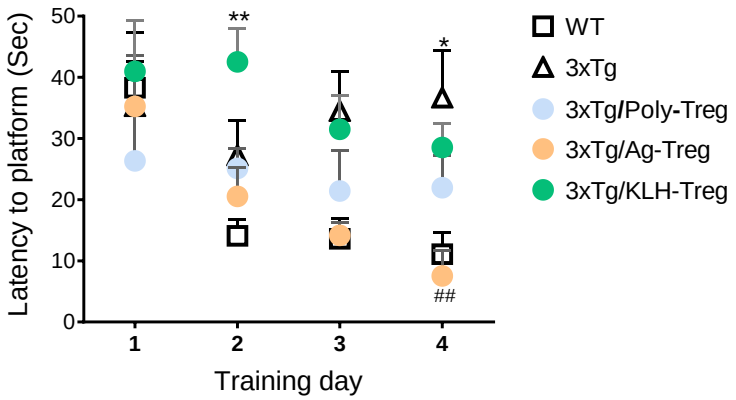

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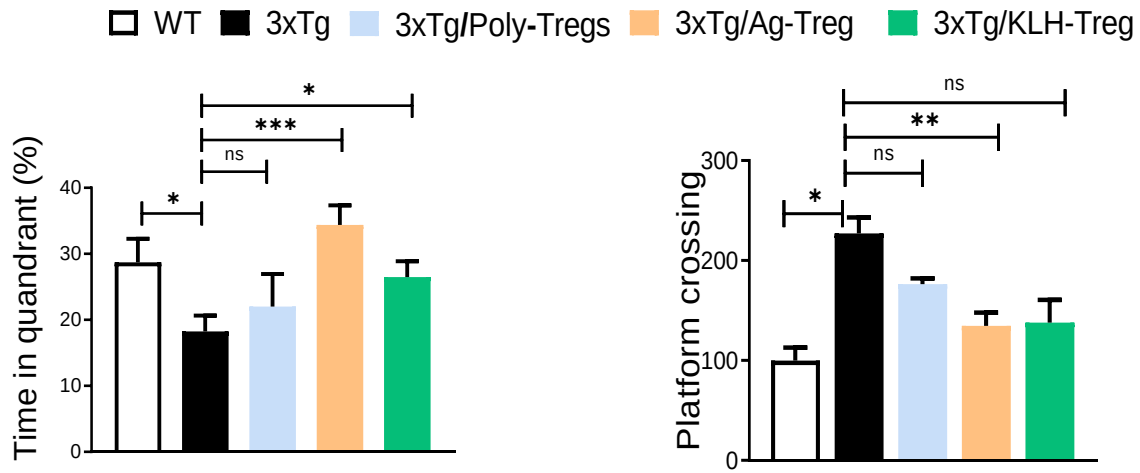
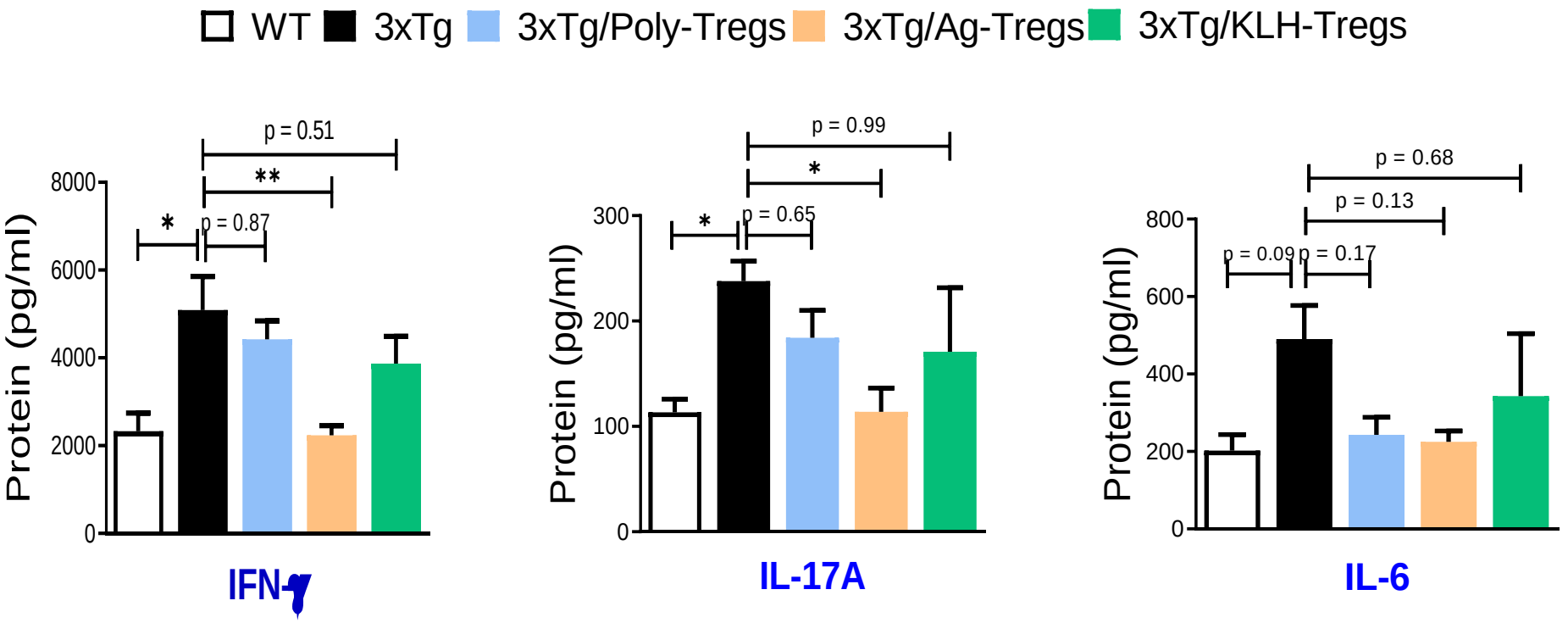
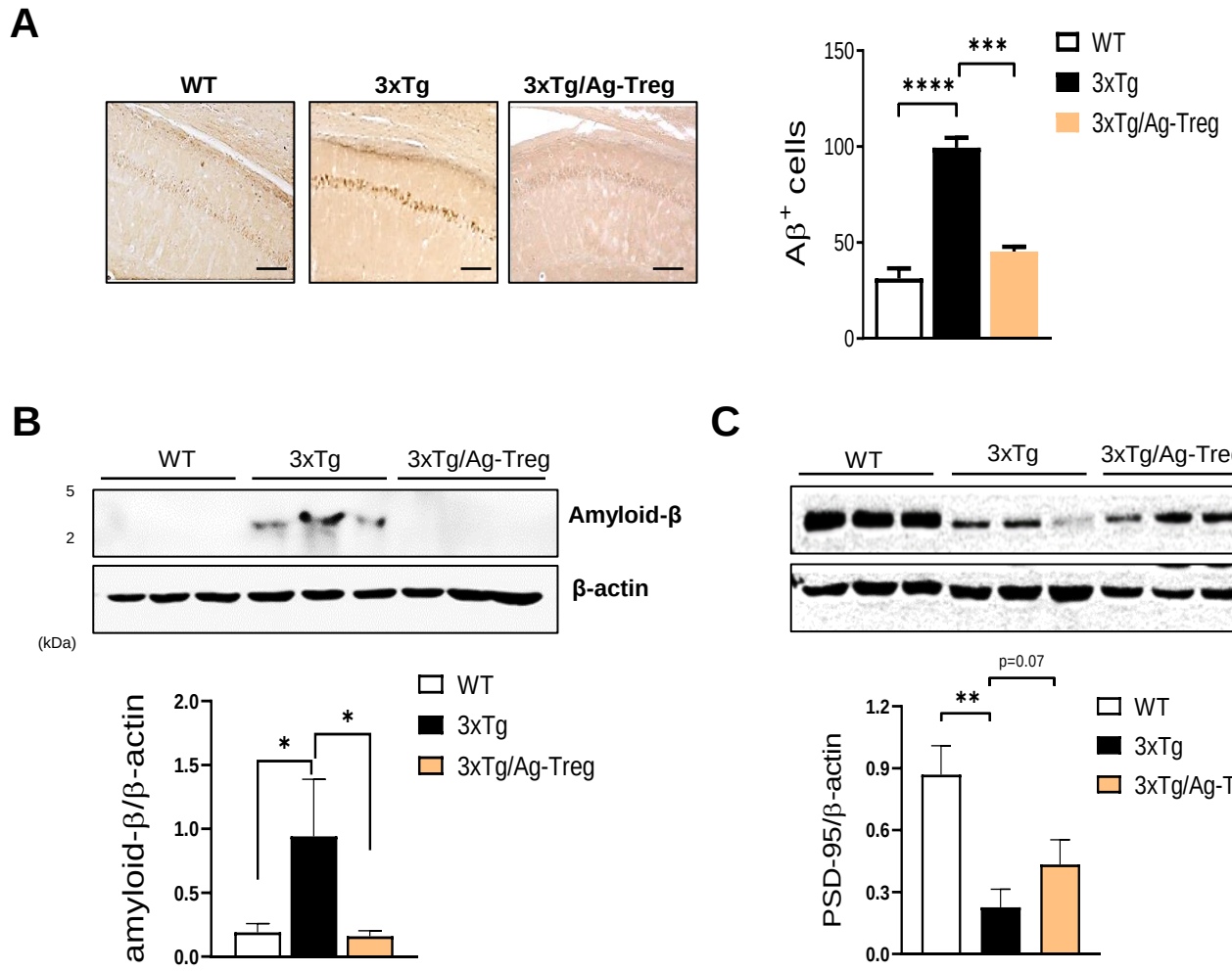


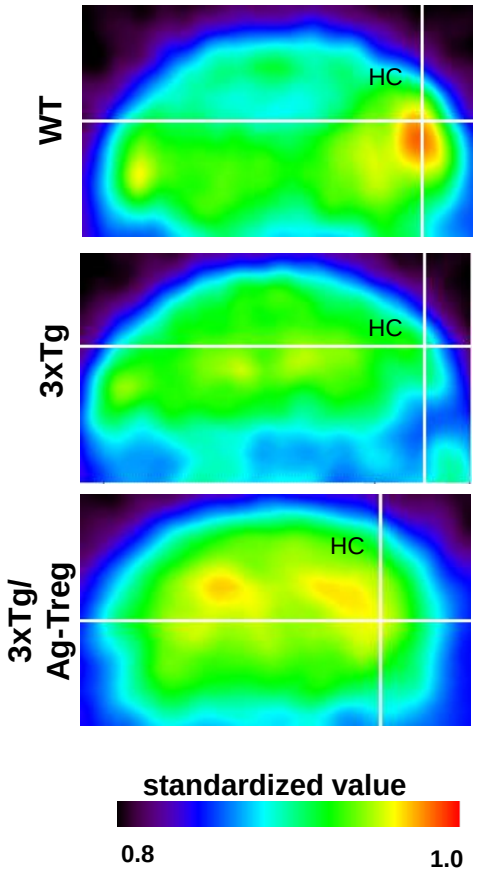

WT
3xTg
3xTg/Poly-Tregs
3xTg/Ag-Tregs
3xTg/KLH-Tregs
Protein (pg/ml)
8000
6000
4000
2000
0
p = 0.51
**
*
p = 0.87
IFN-γ
300
200
100
0
p = 0.99
*
*
p = 0.65
IL-17A
800
600
400
200
0
p = 0.68
p = 0.13
p = 0.09
p = 0.17
IL-6

Amyloid beta deposition



포도당대사 생체영상 micro PET 결과

A



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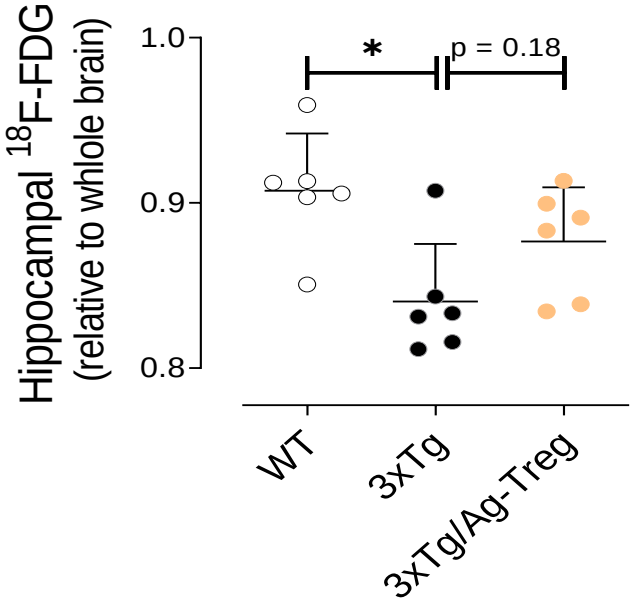
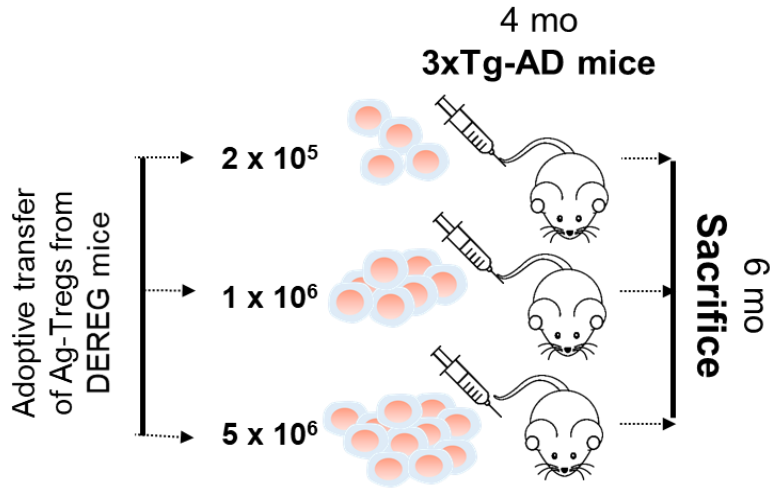


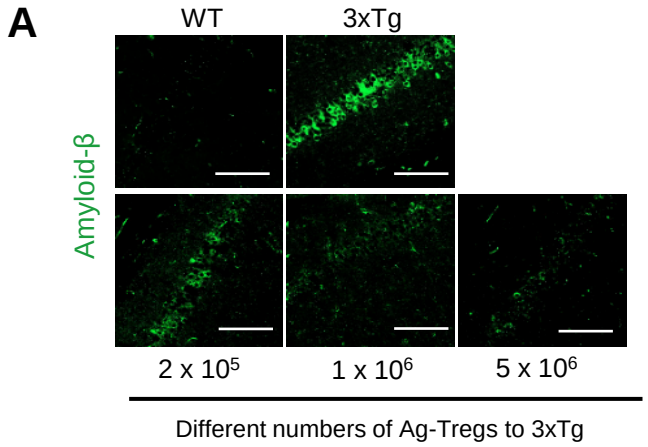
Treg adoptive transfer ;Dose response study

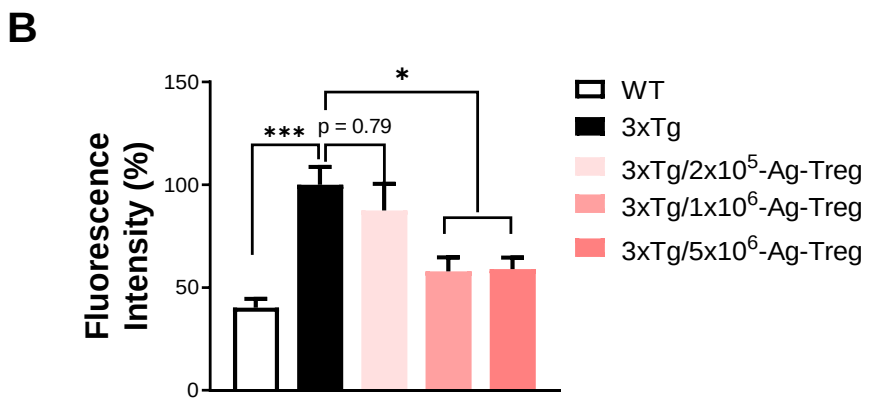
Experimental groups

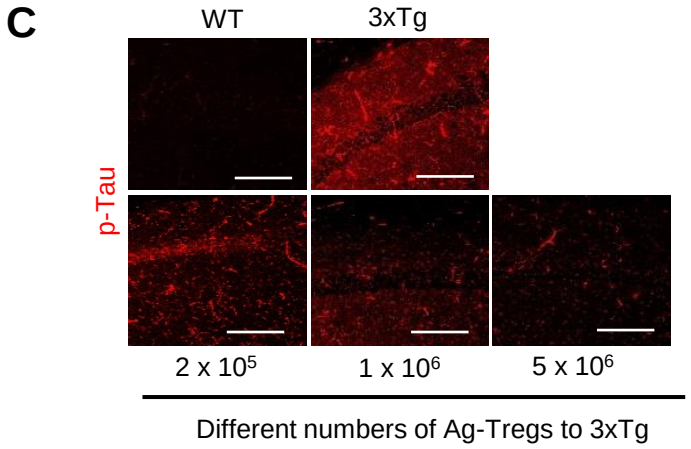
- **WT (B6129SF2/J)**
- **3xTg**
- **3xTg/Ag-Tregs : 2 x 10^5 cells/mice**
- **3xTg/Ag-Tregs : 1 x 10^6 cells/mice**
- **3xTg/Ag-Tregs : 5 x 10^6 cells/mice**

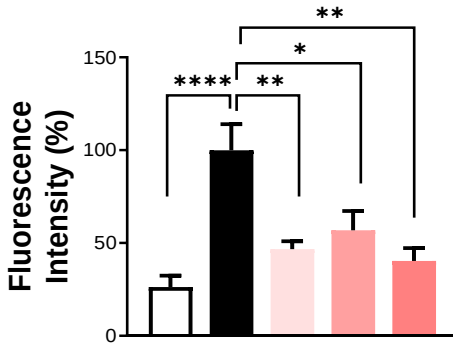


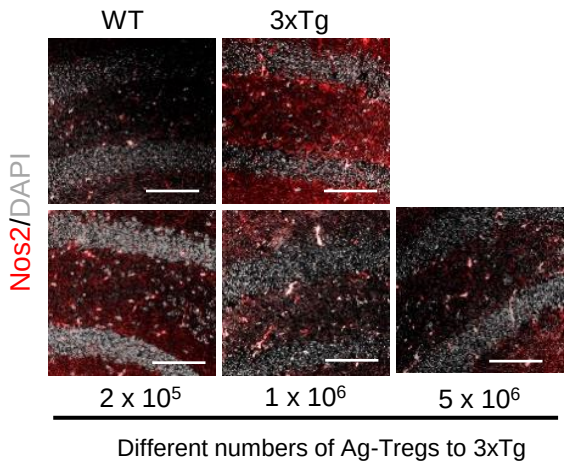
Amyloid beta & p-Tau deposition

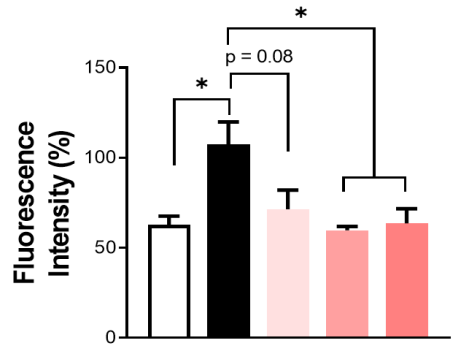








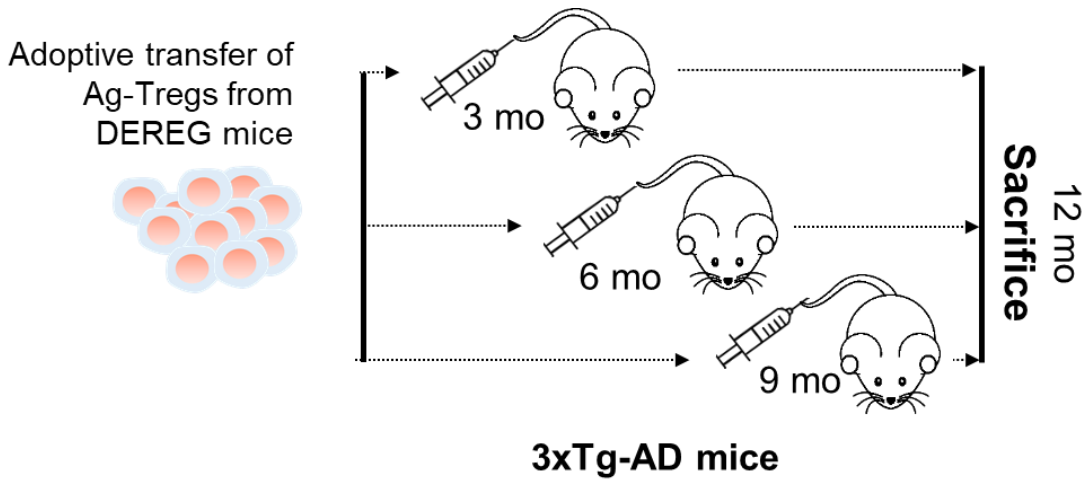




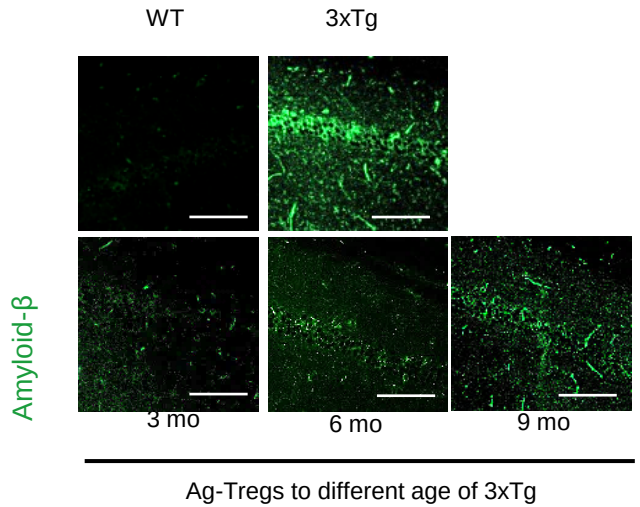
Does disease stage matter?

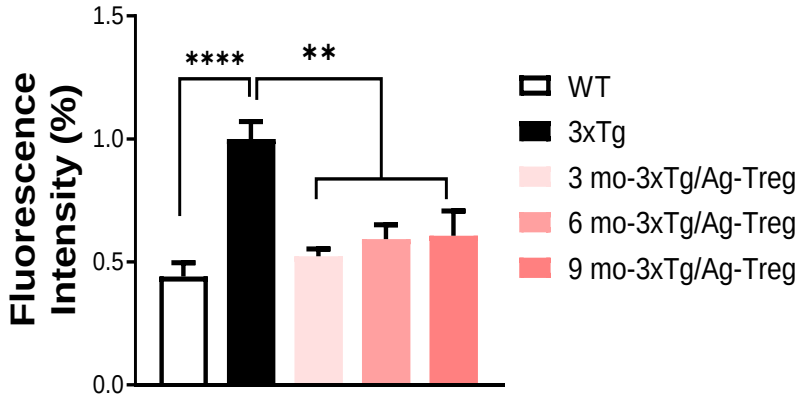
Experimental groups

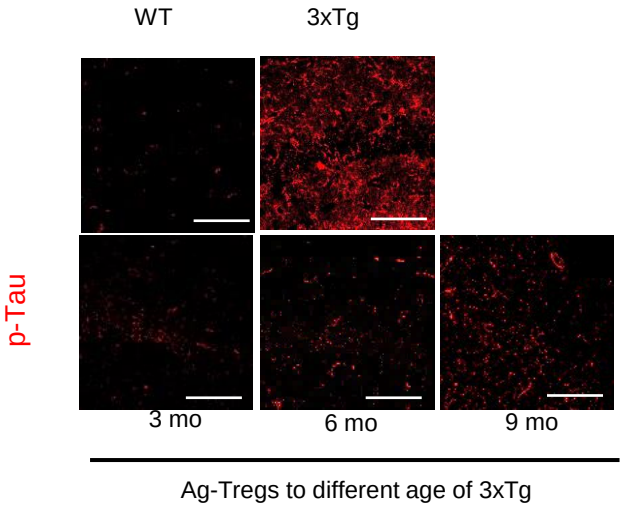
- **WT (B6129SF2/J)**
- **3xTg**
- **3 mo 3xTg/Ag-Treg**
- **6 mo 3xTg/Ag-Treg**
- **9 mo 3xTg/Ag-Treg**

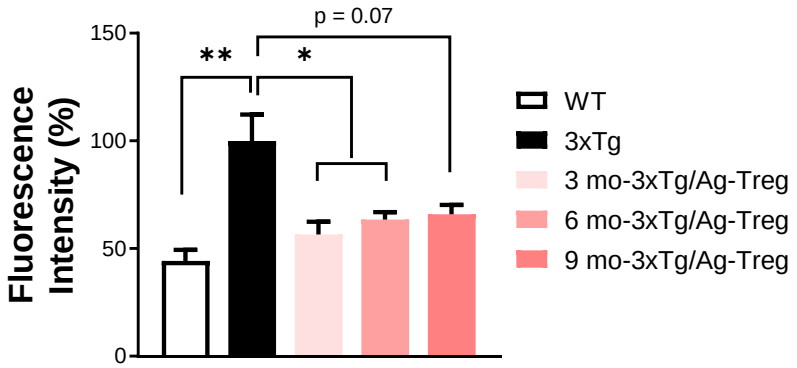


Does disease stage matter?

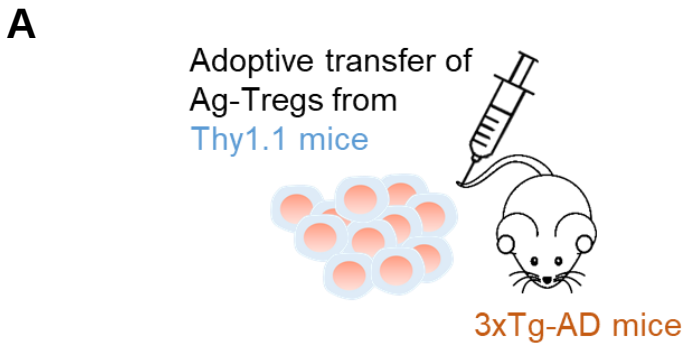


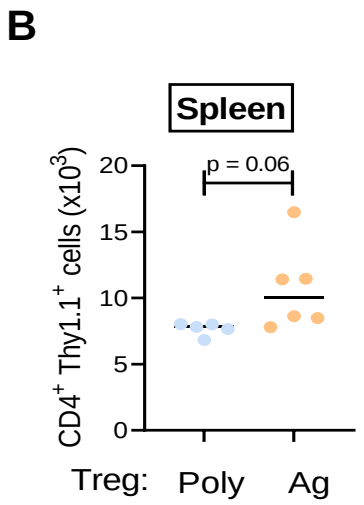


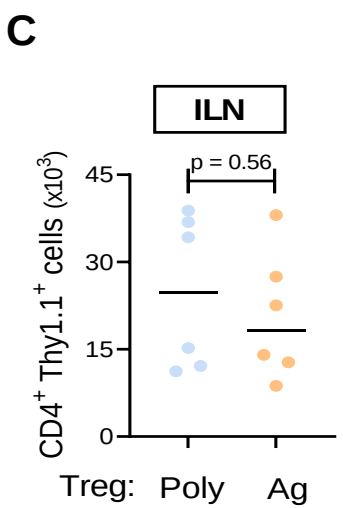


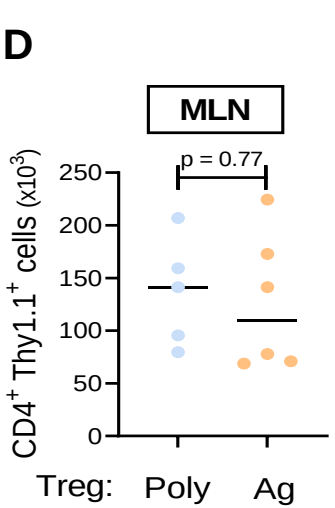


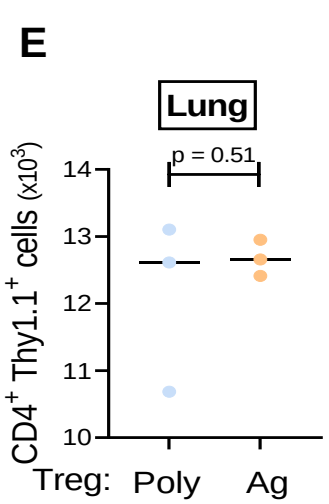
Does Treg migrate to the Brain?

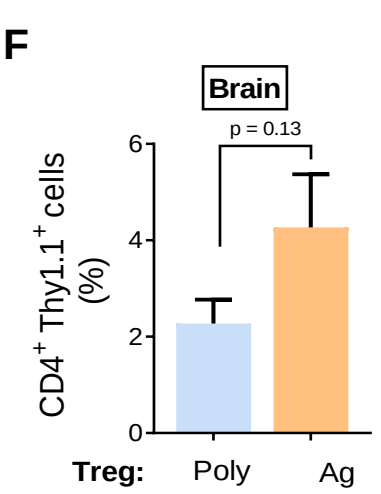


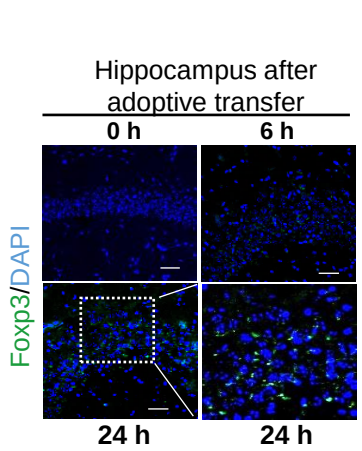


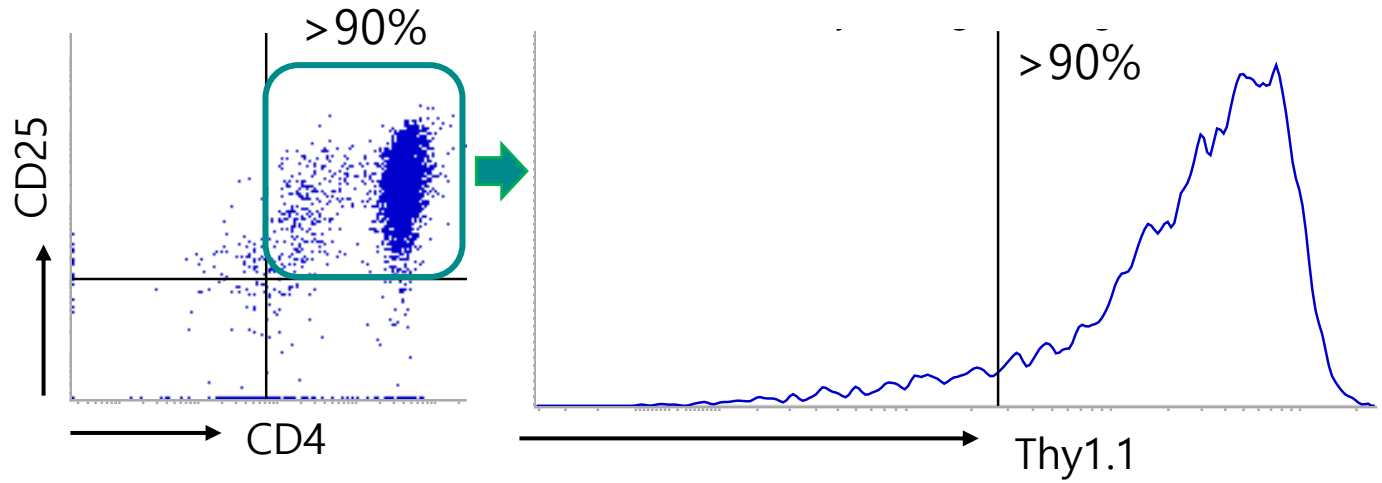












Thy 1.1⁺Treg
Isolation
&
Adoptive Transfer

56 days

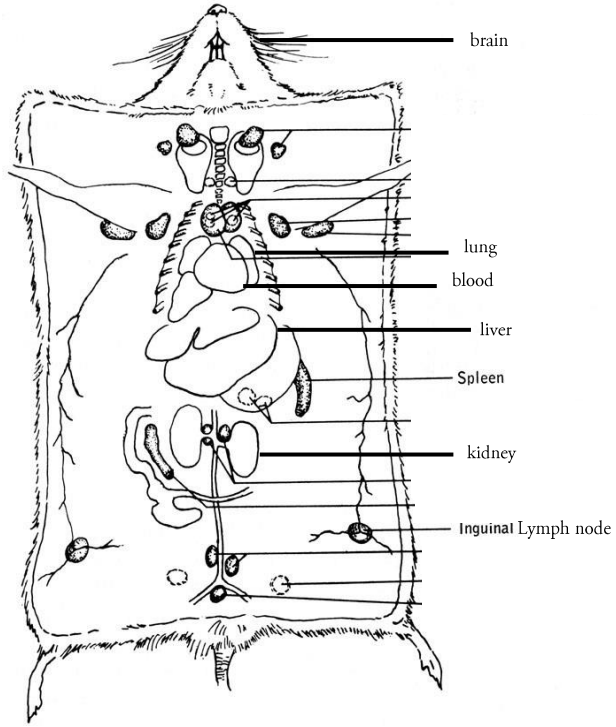
28 days

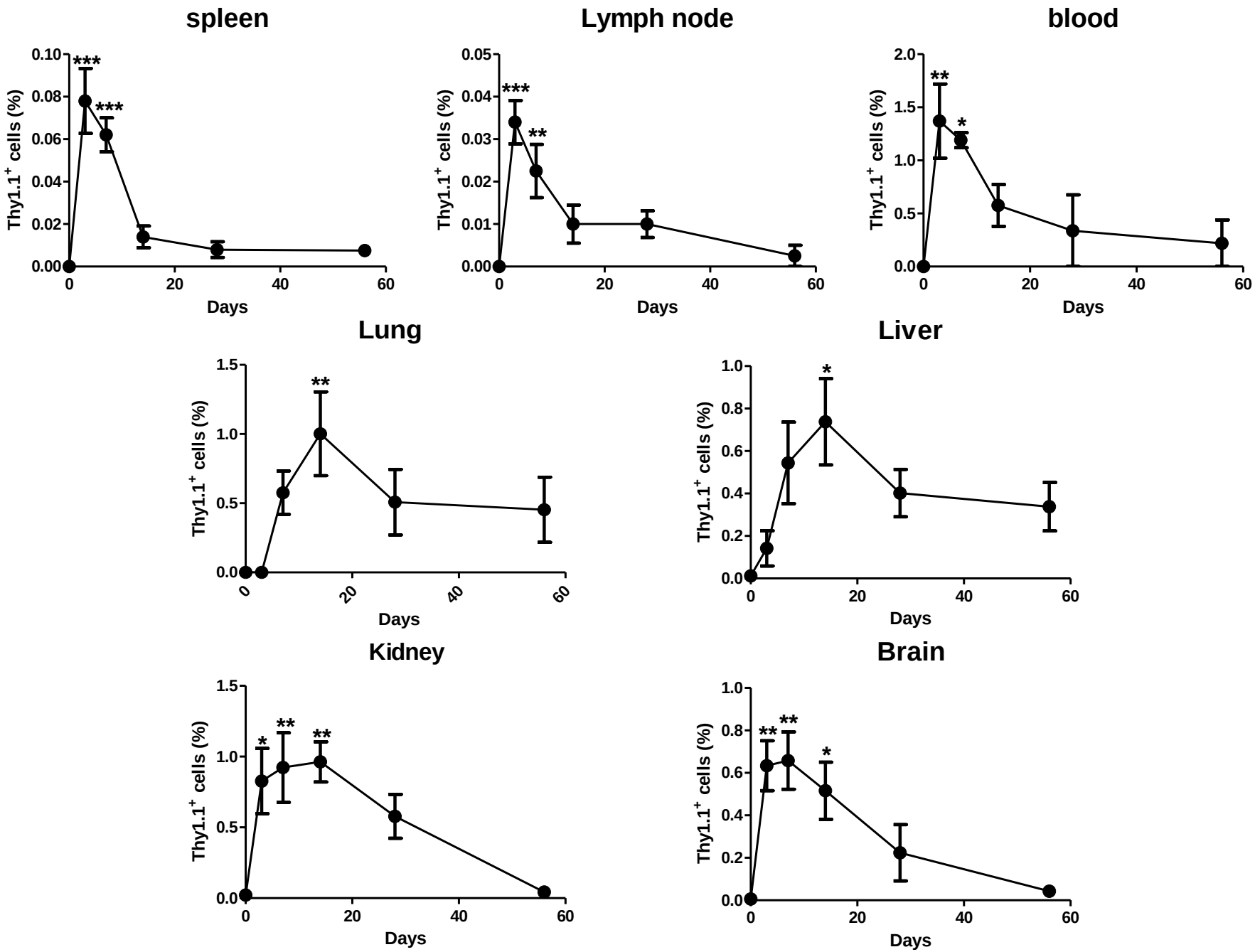
14 days

7 days

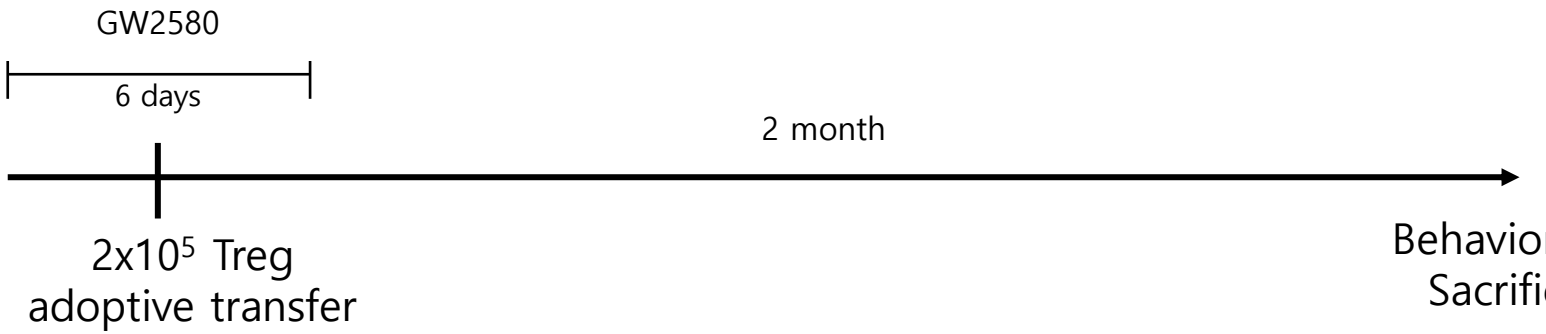
3 days

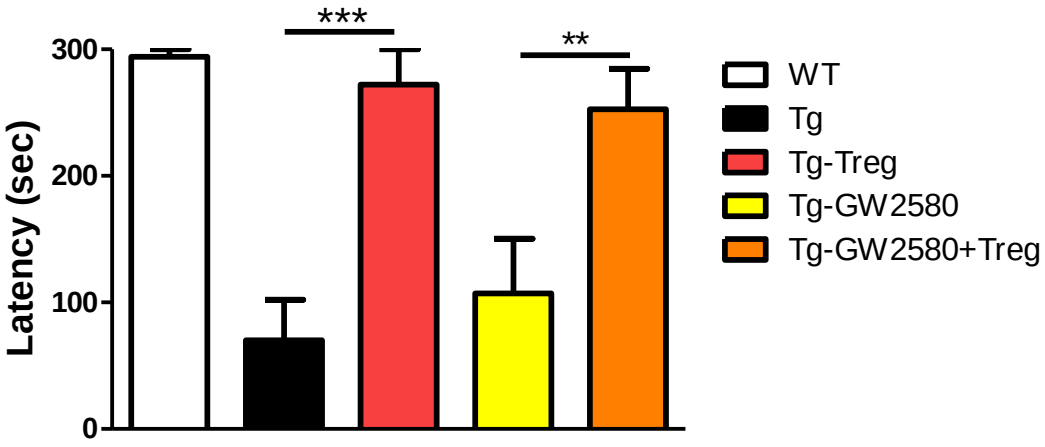
Sacrifice
&
FACS Analysis



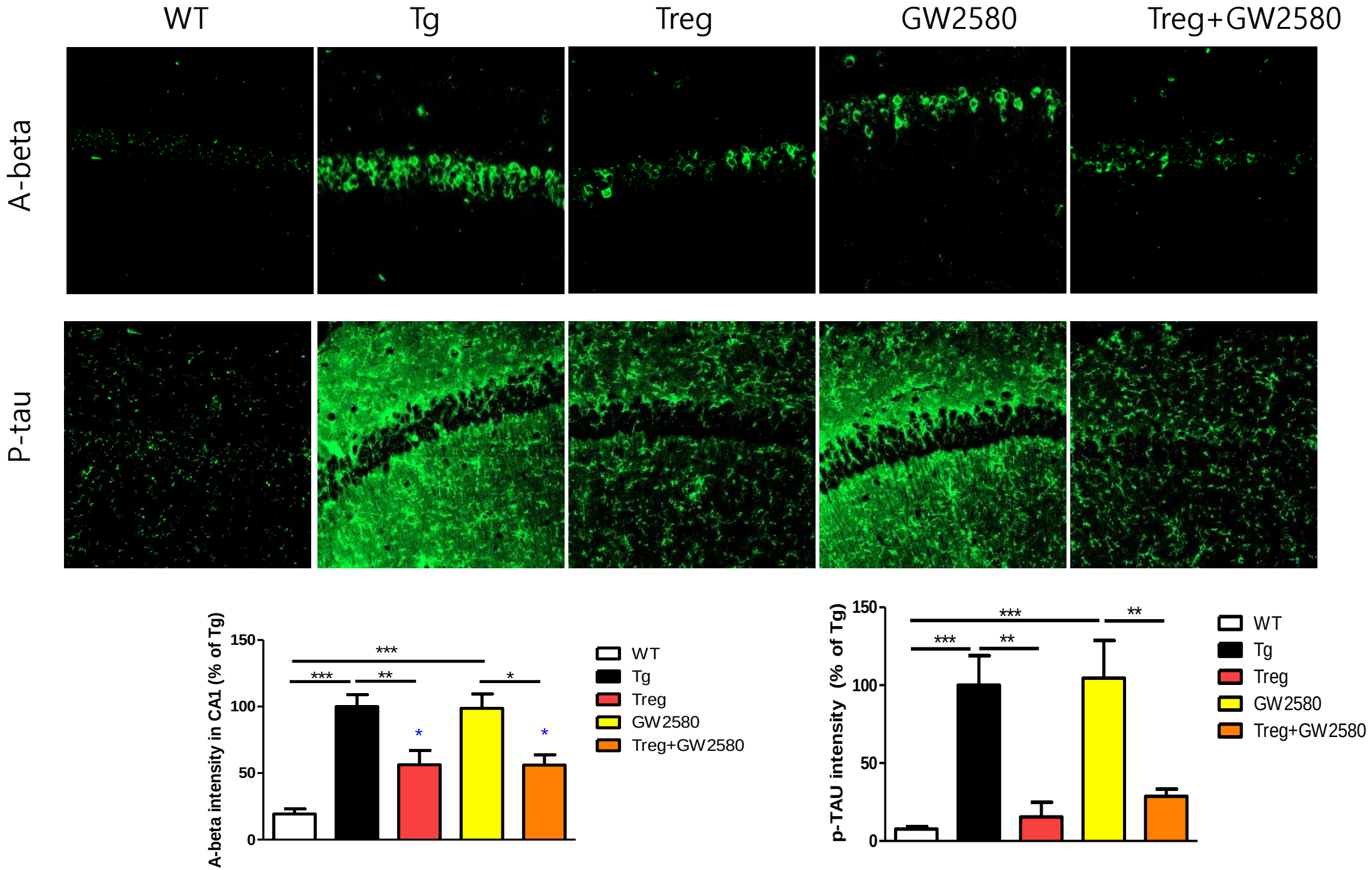

spleen
Lymph node
blood
Lung
Liver
Kidney
Brain
Thy1.1+ cells (%)
Days
0.10
0.08
0.06
0.04
0.02
0.00
0.05
0.04
0.03
0.02
0.01
0.00
2.0
1.5
1.0
0.5
0.0
1.5
1.0
0.5
0.0
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
***
**
*

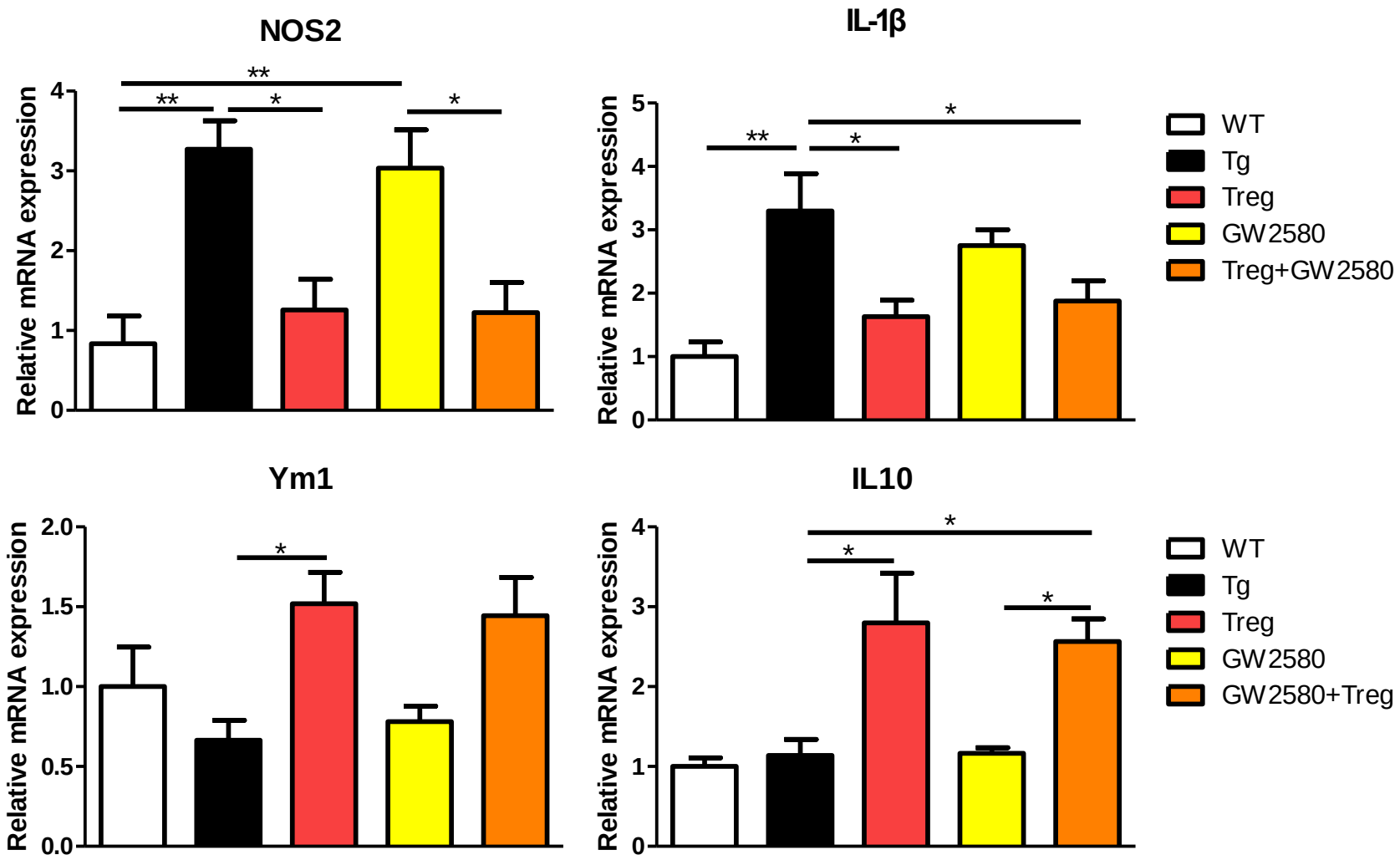
Microglia depletion do not affect the anti-AD effects



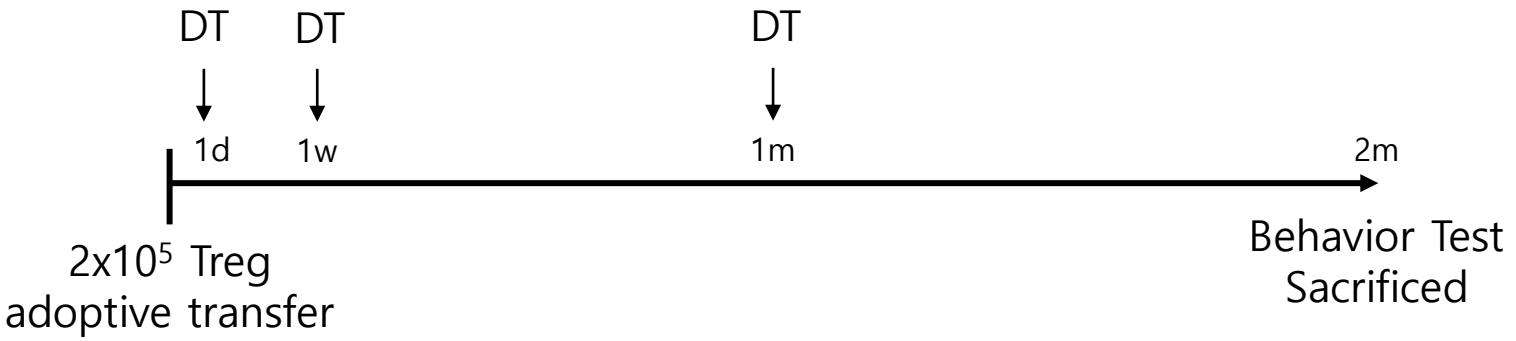


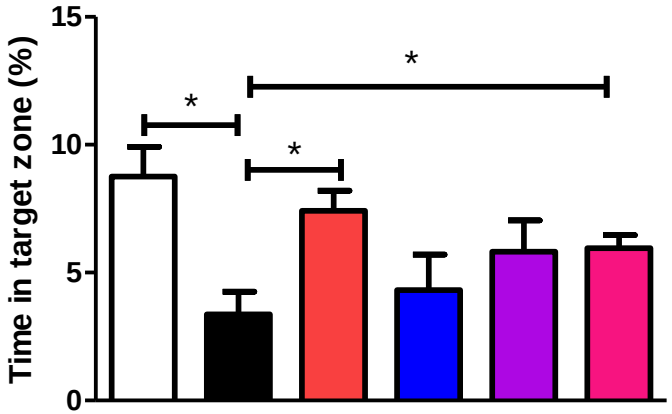
"GW2580"
CSF-1R inhibitor
Depleting microglia

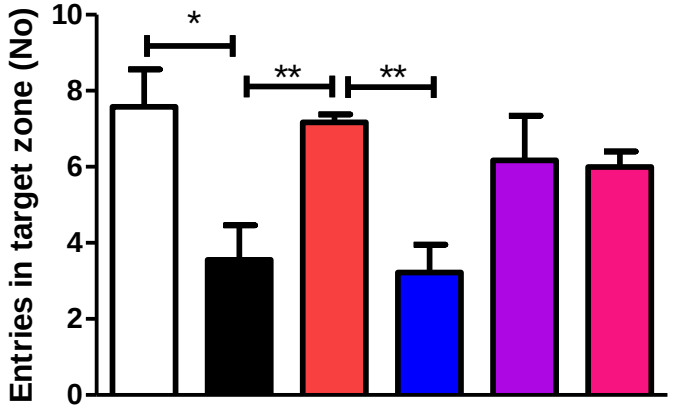

WT
Tg
Treg
GW2580
Treg+GW2580
A-beta
P-tau
A-beta intensity in CA1 (% of Tg)
150
100
50
0
***
***
**
*
*
*
WT
Tg
Treg
GW2580
Treg+GW2580
p-TAU intensity (% of Tg)
150
100
50
0
***
***
**
**
WT
Tg
Treg
GW2580
Treg+GW2580

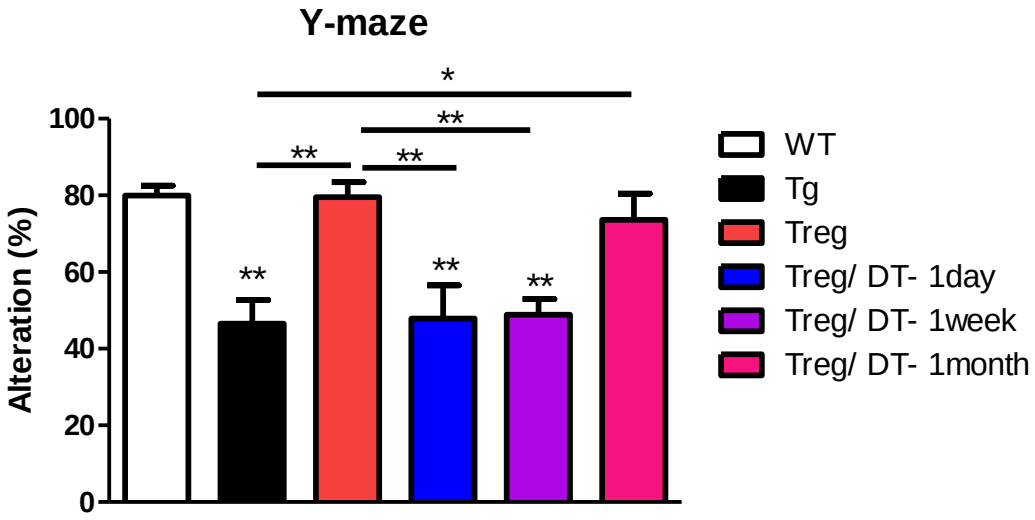

NOS2
Relative mRNA expression
0
1
2
3
4
**
**
*
*
IL-1β
Relative mRNA expression
0
1
2
3
4
5
**
*
*
WT
Tg
Treg
GW2580
Treg+GW2580
Ym1
Relative mRNA expression
0.0
0.5
1.0
1.5
2.0
*
IL10
Relative mRNA expression
0
1
2
3
4
*
*
*
WT
Tg
Treg
GW2580
GW2580+Treg

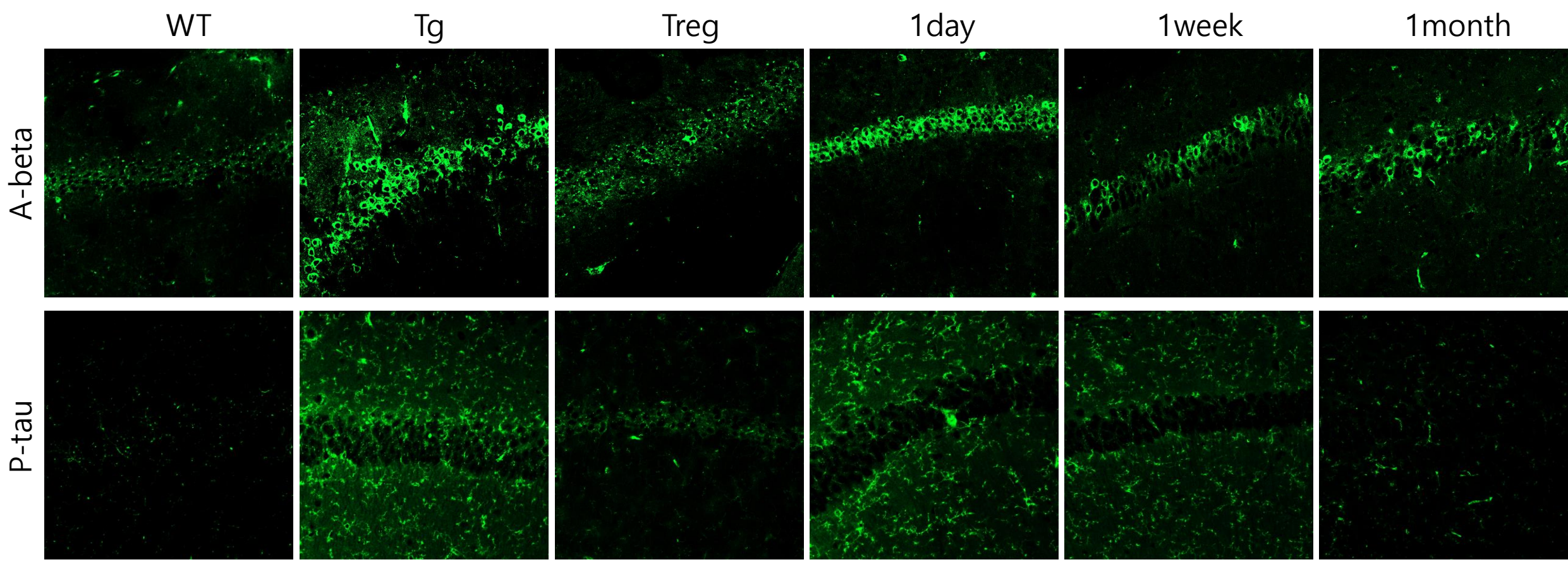
Does depletion of transferred Treg affect anti-AD effects ?

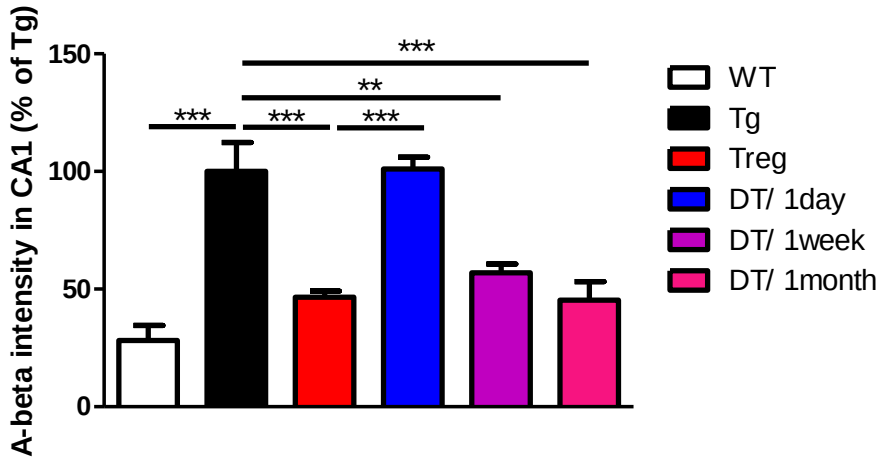


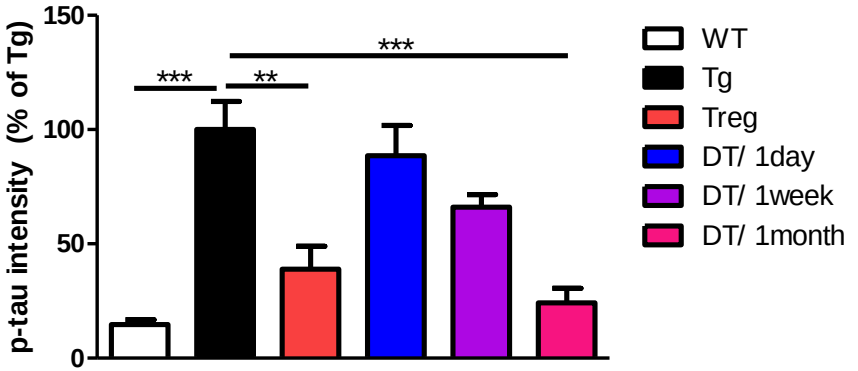


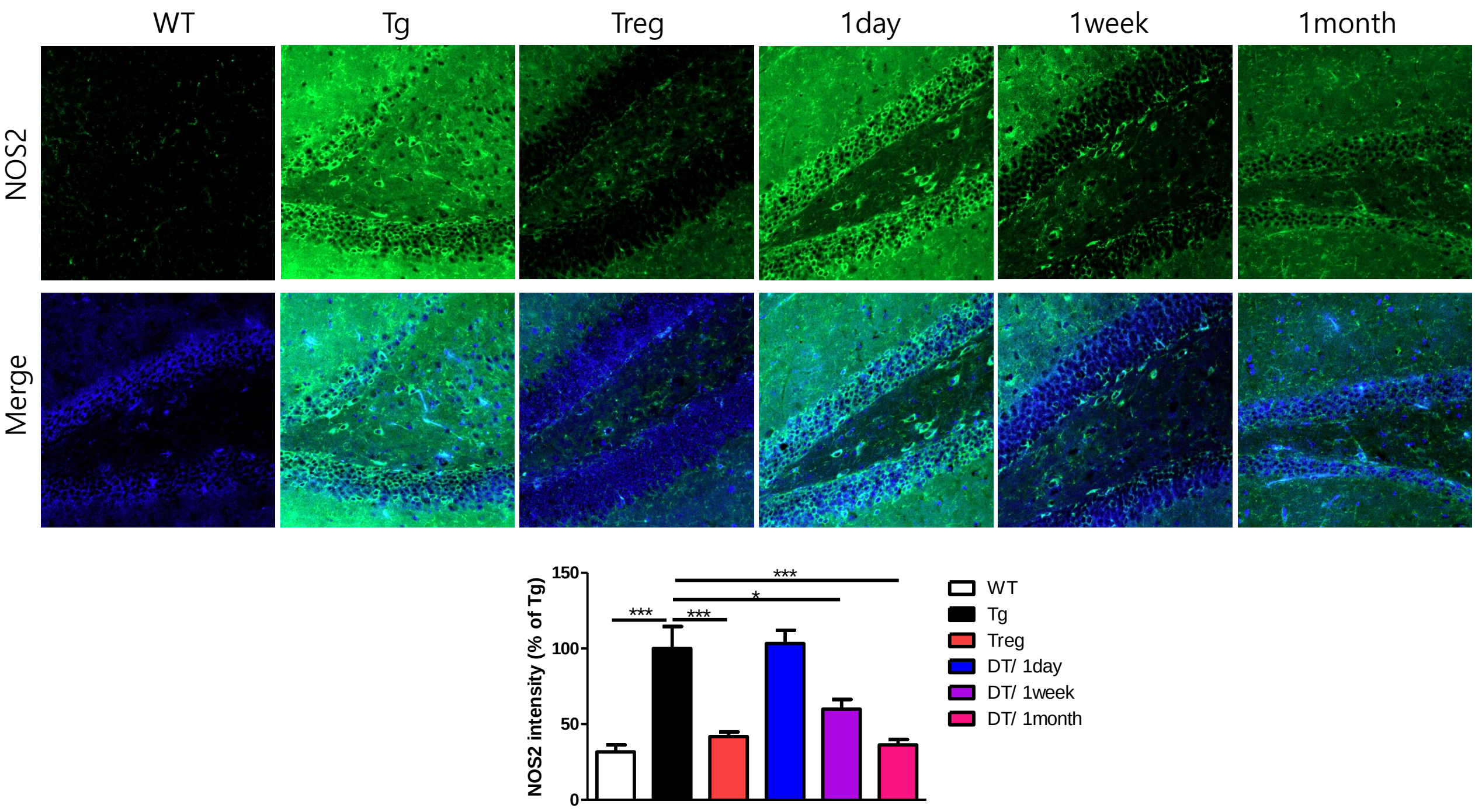





WT
Tg
Treg
1day
1week
1month
A-beta
P-tau

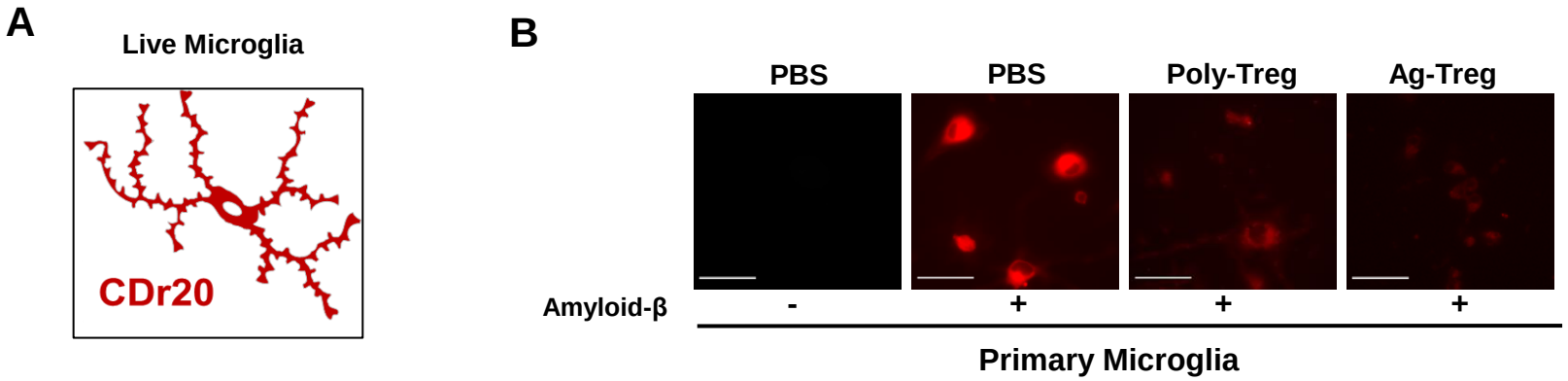

A-beta intensity in CA1 (% of Tg)
150
100
50
0
***
***
**
***
***
WT
Tg
Treg
DT/ 1day
DT/ 1week
DT/ 1month

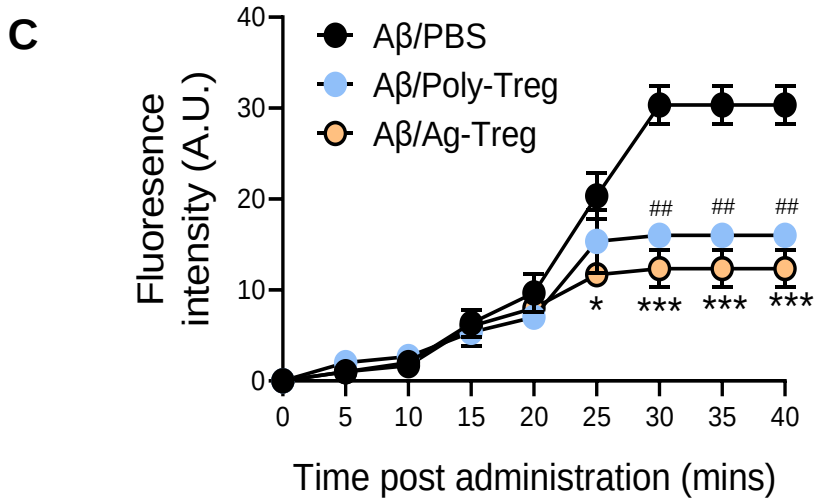

p-tau intensity (% of Tg)
150
100
50
0
***
**
***
WT
Tg
Treg
DT/ 1day
DT/ 1week
DT/ 1month


WT
Tg
Treg
1day
1week
1month
NOS2
Merge
NOS2 intensity (% of Tg)
150
100
50
0
***
***
*
***
WT
Tg
Treg
DT/ 1day
DT/ 1week
DT/ 1month

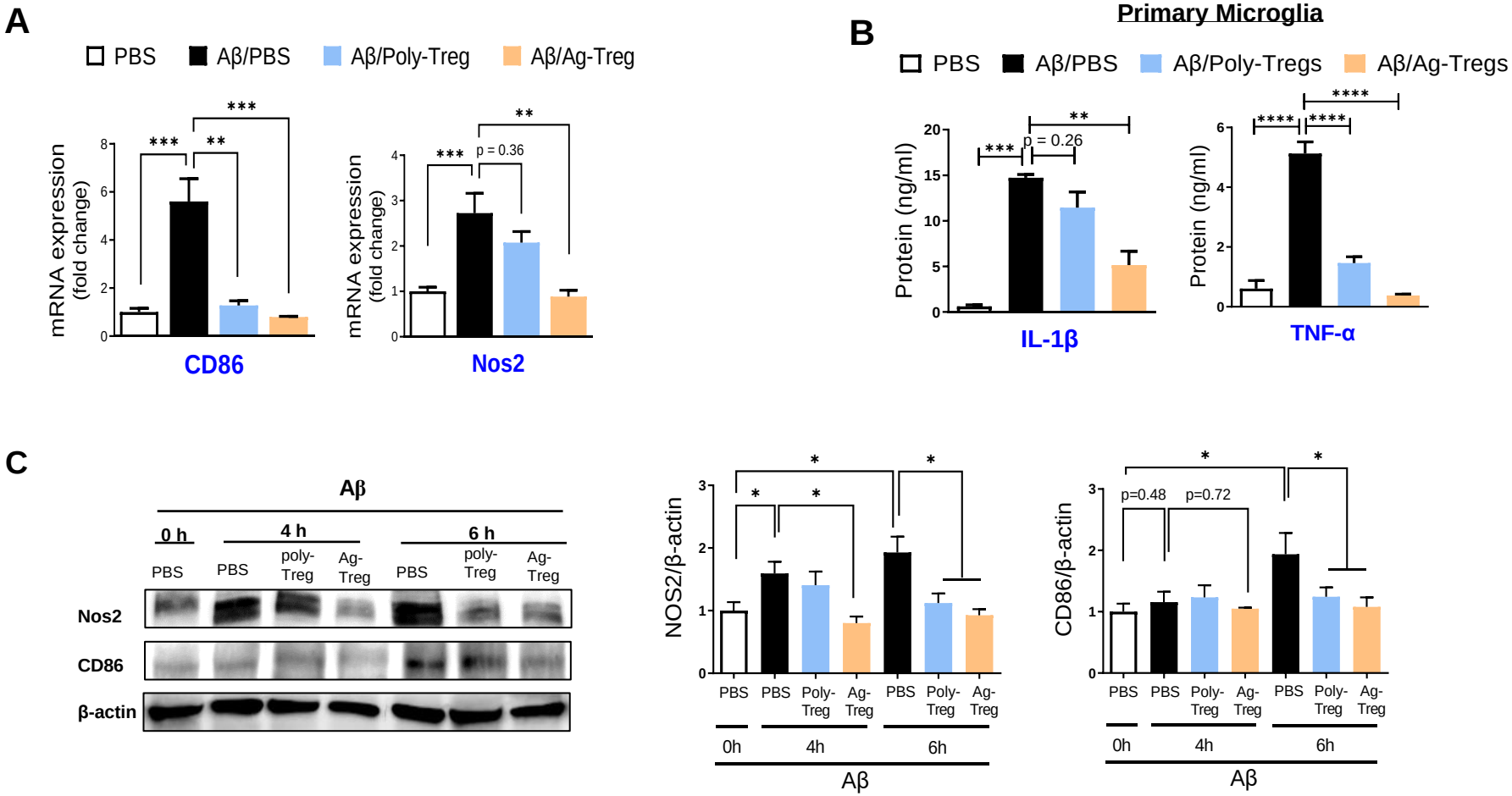
Suppressive Effects of mouse Tregs on the Aβ-treated primary microglia

CDr20- Microglia Specific Fluorogenic Probe



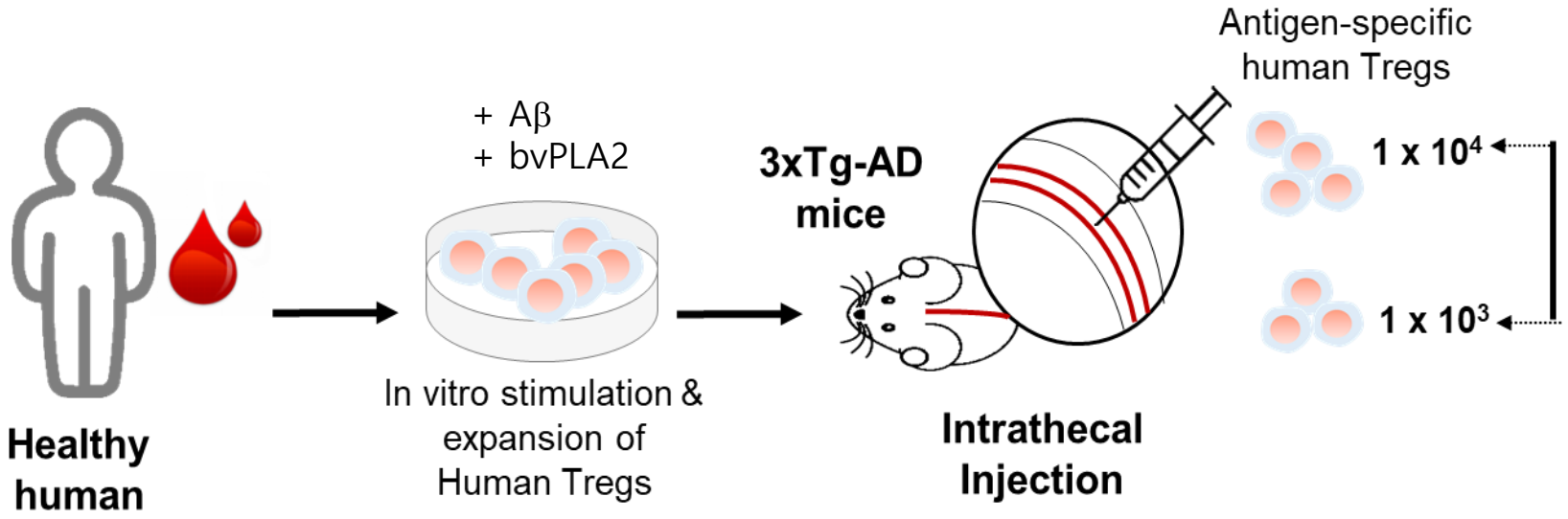


Effect of Treg on Primary (M1) microglia expression

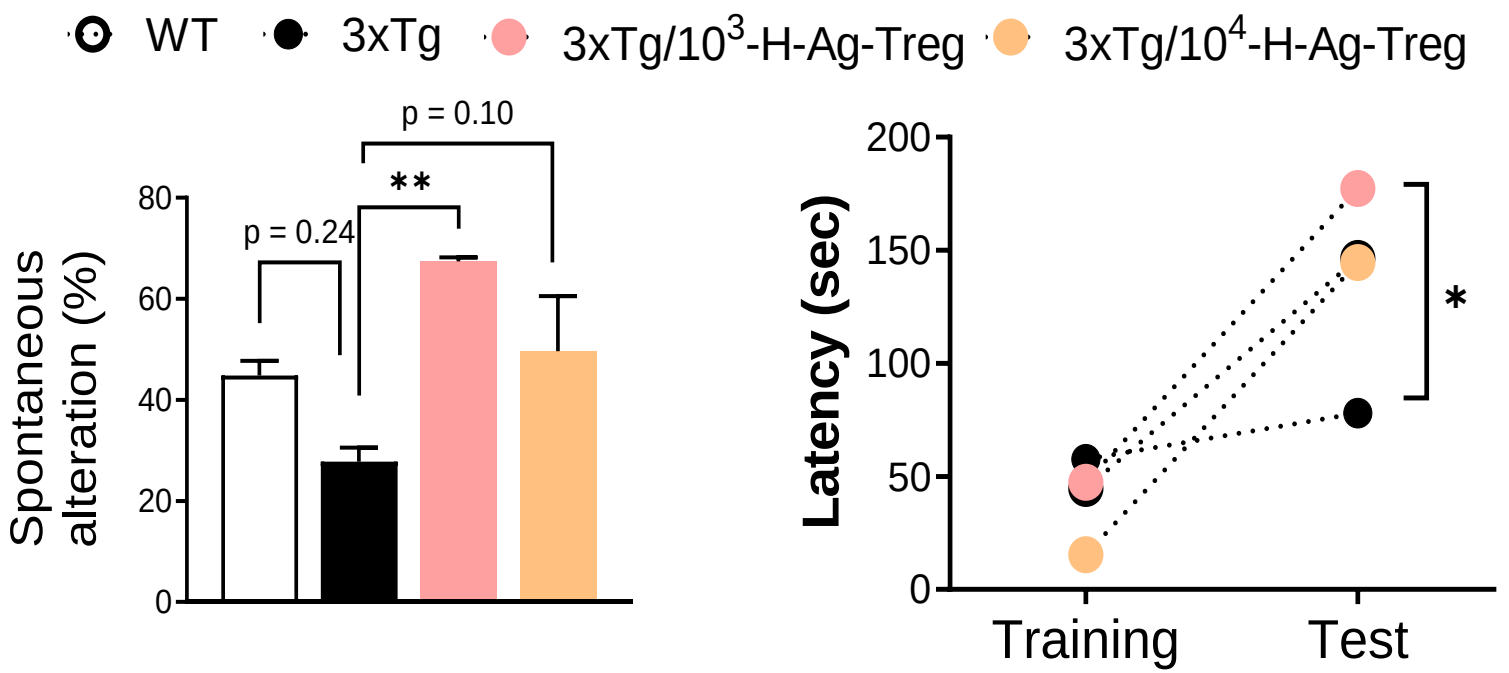


Effects of human Tregs on 3xTg-AD mouse with intrathecal (i.t.) injection

Experimental Schedule



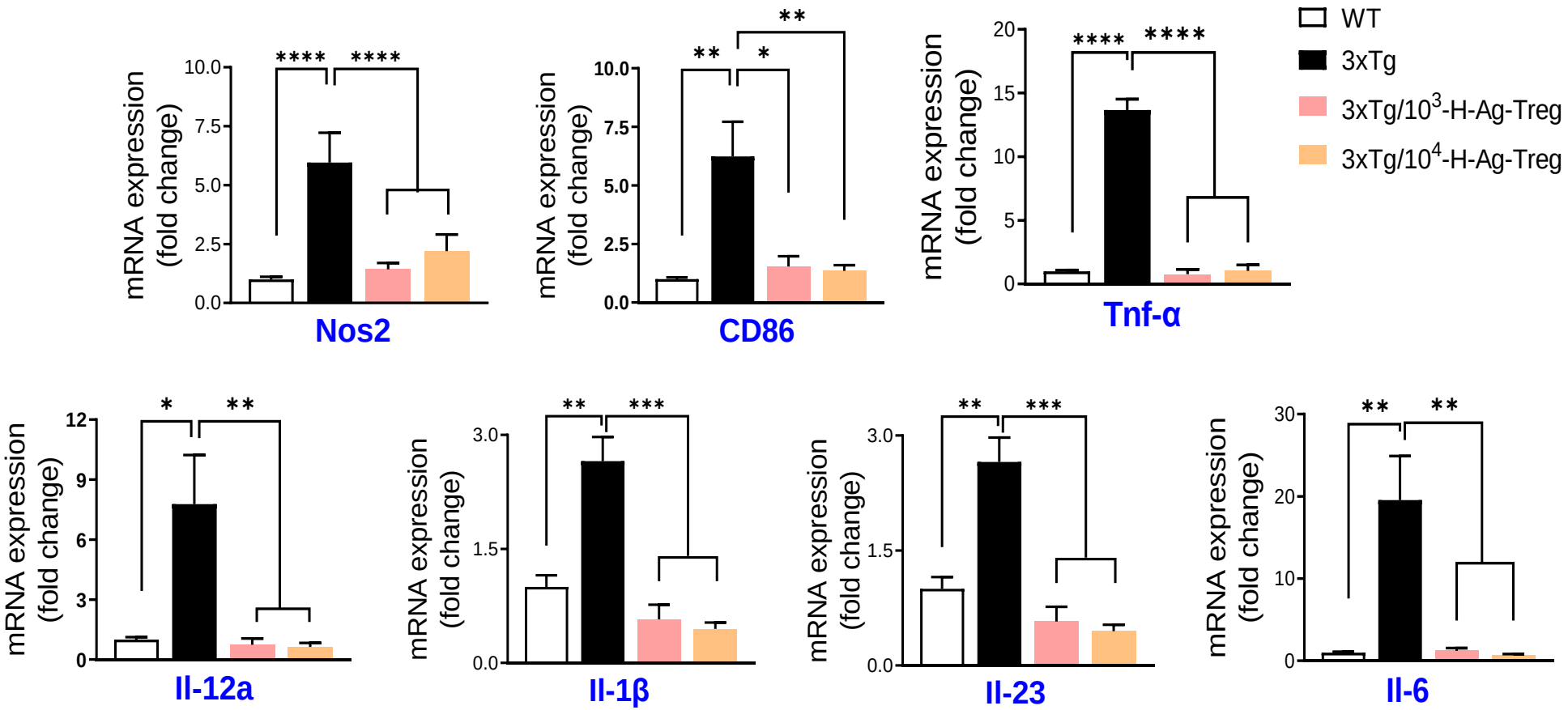
Human Ag-Treg improved the spatial learning and memory of 3xTg-AD mice using the Passive Avoidance and Y-maze test.



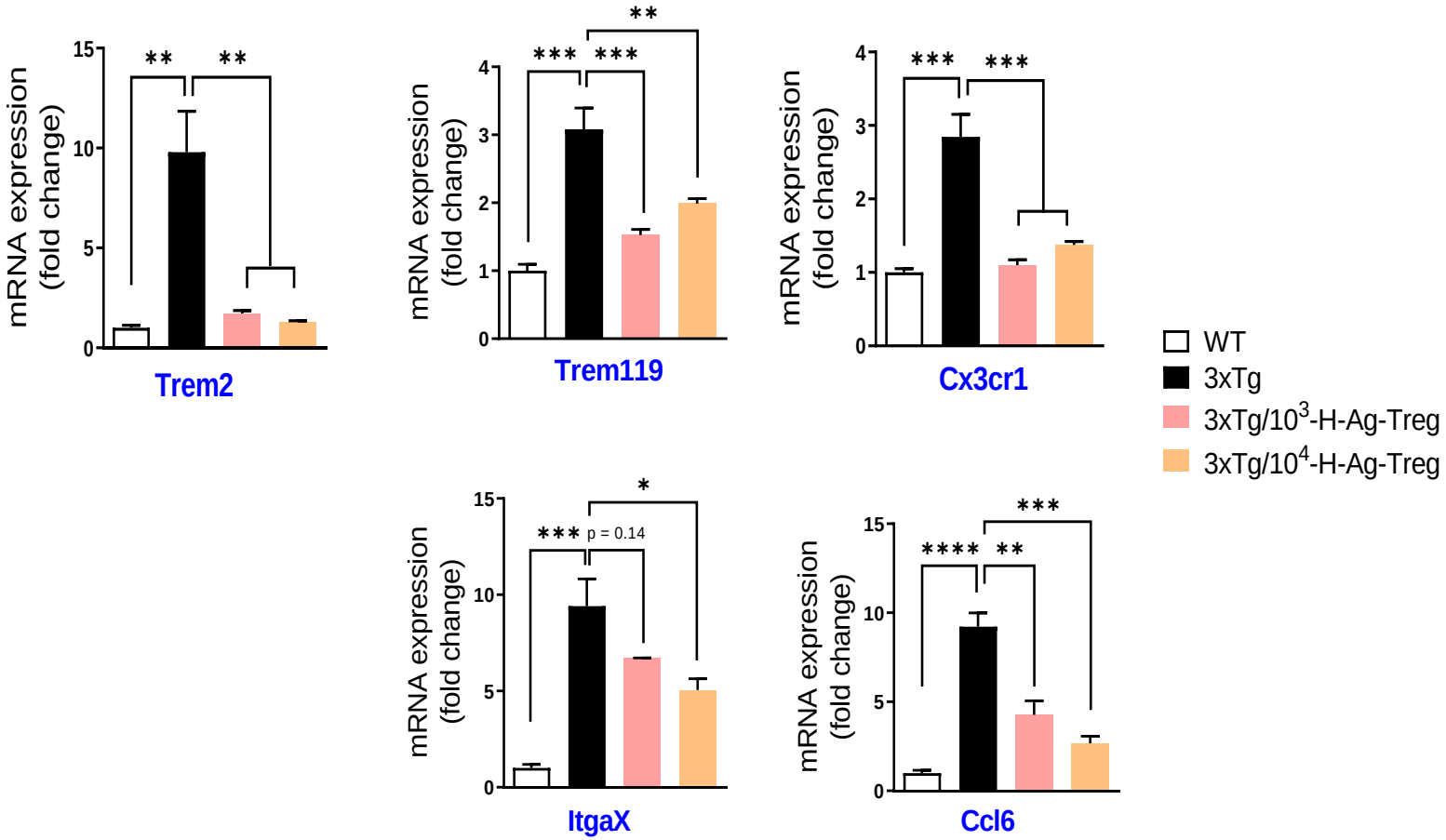
Y-Maze

Passive avoidance

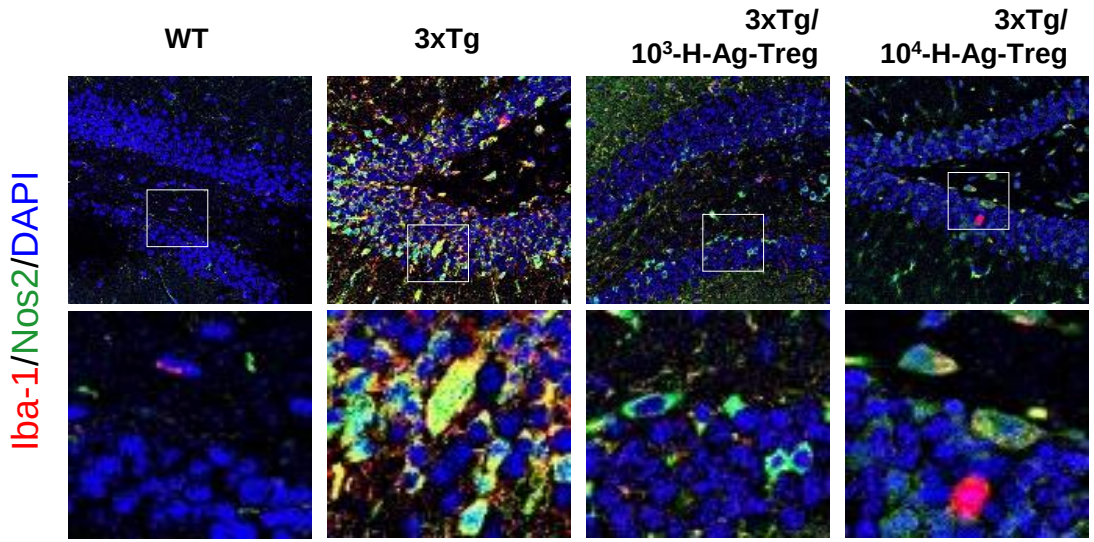
Human Ag-Treg suppressed activated (M1) microglia mRNA expression.

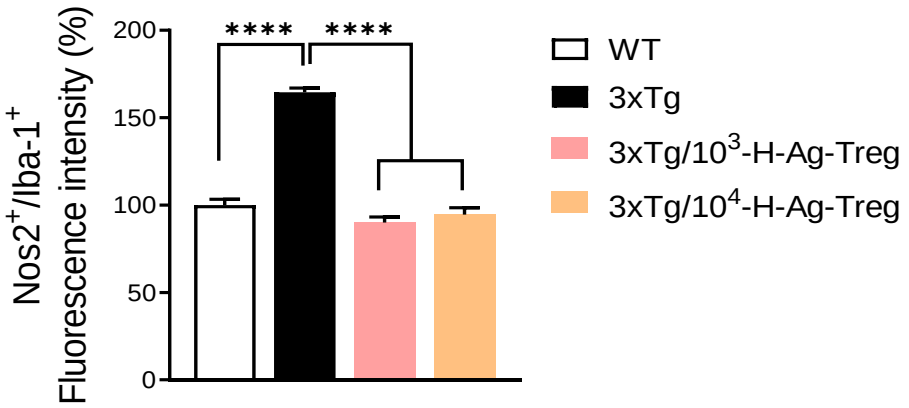


Human Ag-Treg modulated disease associated microglia (DAM) mRNA expression.

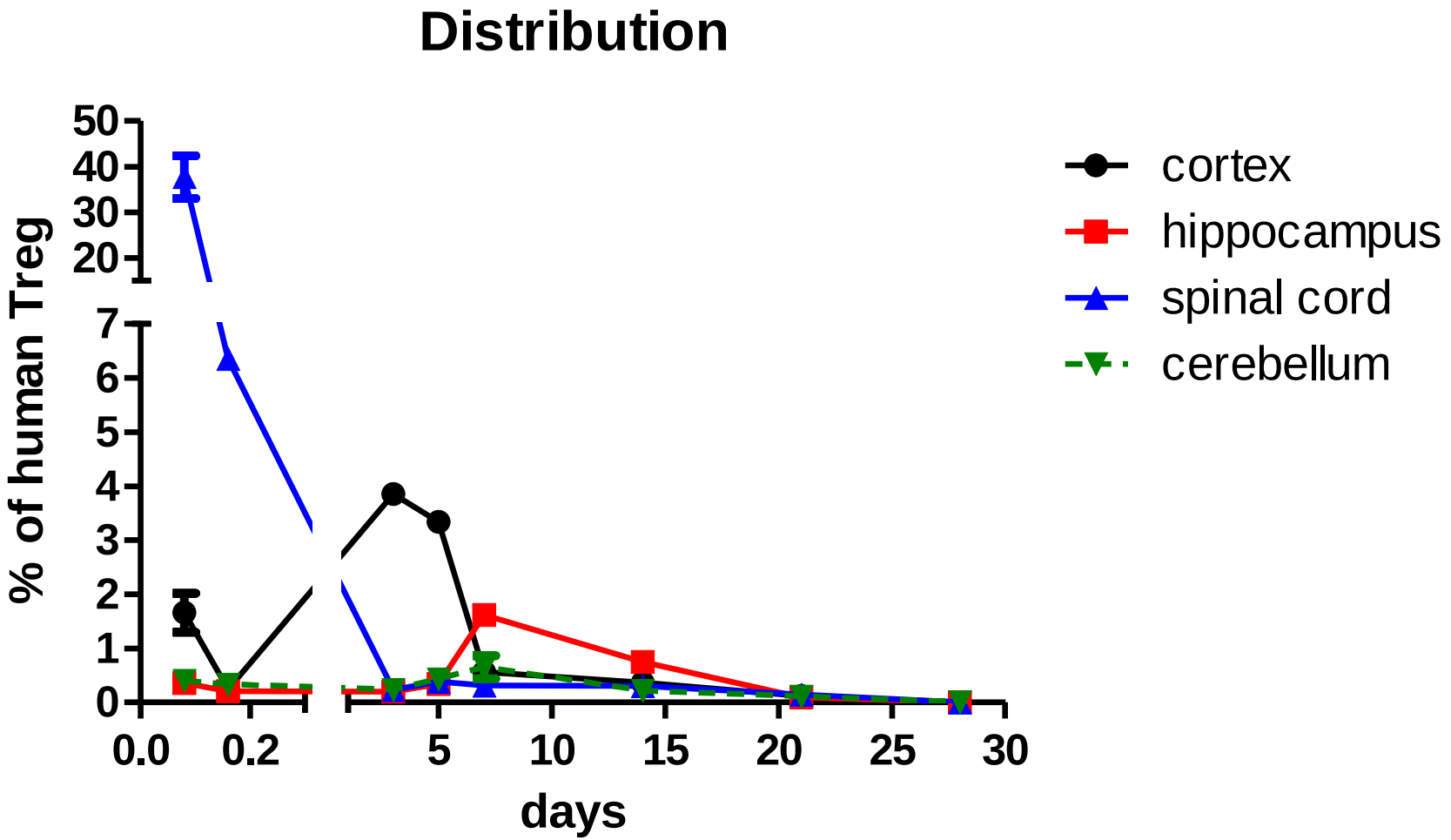


Human Ag-Treg suppressed microglia activation on 3xTg-AD mouse DG region.





Human Ag-Treg Distribution and Duration



Conclusion II

- Single administration of Treg adoptive transfer significantly ameliorates AD pathology in 3xTg mice
- Amyloid beta conditioned (specific) Tregs are more effective on AD progression than polyclonal Treg.
- Treg directly modulate microglia activation status
- Human Treg adoptive transfer (I.T.) significantly alleviates AD-like progression in 3xTg mice
- These findings pave the way for the clinical application of Tregs as cell therapy for treating AD

Acknowlegement

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8 Department of Physiology, College of Medicine, **Kyung Hee University**
9 Center for Self-assembly and Complexity, **Institute for Basic Science (IBS)**
10 Stroke center, **Kyung Hee University**
11 Department of Health Sciences, **The Graduate School of Dong-A University**

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