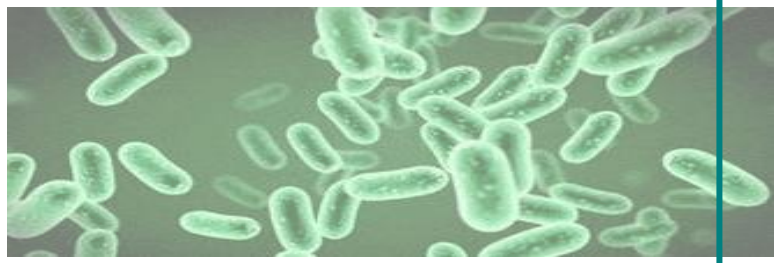
Microbiome and IC/BPS: Is there any connection?



Joonbeom Kwon
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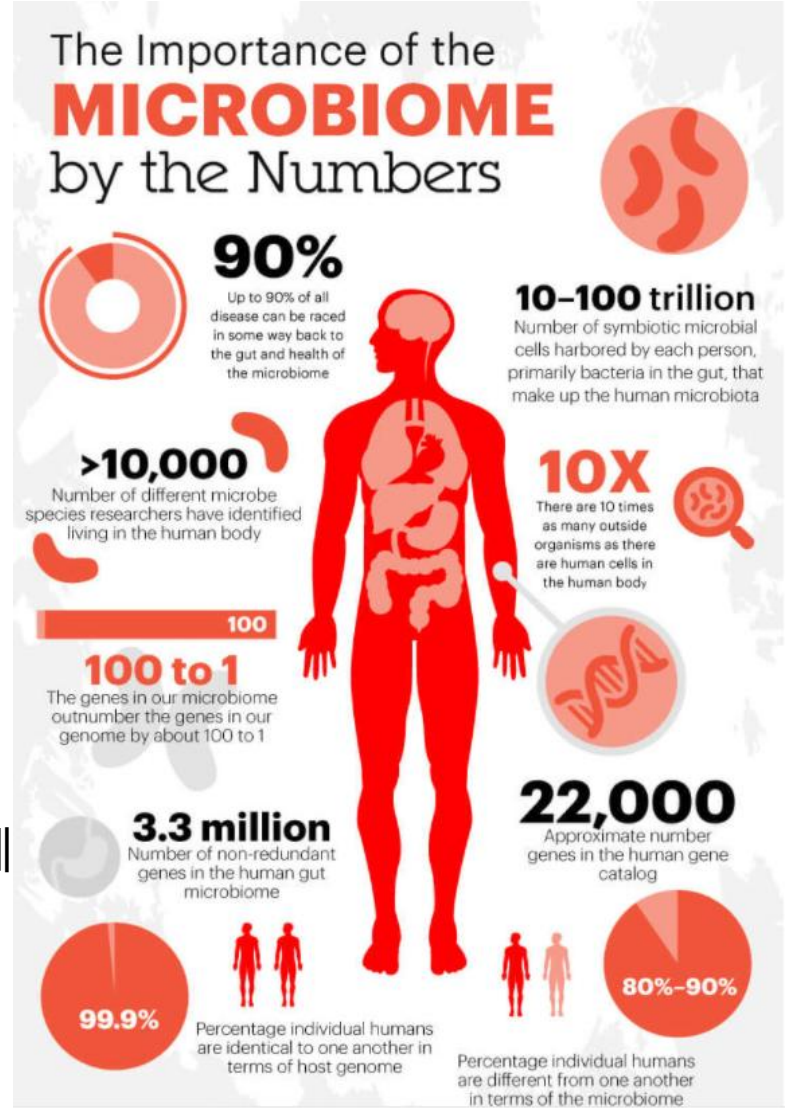
Microbiome & 16S rRNA gene sequencing

Human microbiome

- x10 vs. human cells
- 배양이 가능한 microbes는 전체의 1% 정도
- Next generation sequencing (NGS)이 소개된 후 이제껏 확인하지 못했던 미생물 유전체의 분석(metagenomics)이 활발히 진행
- 16S rRNA는 prokaryotic cell의 rRNA의 부분으로 타종간에 염기서열의 다양성을 보이므로 target sequencing을 하는데 적합



- 2006년 gut-microbiota와 obesity와의 관련성이 알려진 후 이에대한 관심이 폭발
- 아직까지 urobiome에 대한 연구는 시작단계
- Cancer, OAB, Incontince, IC/BPS 등 다양한 urologic disorder와의 관련성에 관한 연구가 밝혀지고 있으나 아직 그 기전에 대한 정확한 증거를 제시하지 못하는 상태

How does microbiome affect human system?

1. 다양한 인체환경에 서식하며, 인체와 상호 적응하며 진화해 옴.

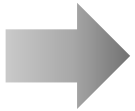
2. 공생하기 위해 가장 필요한 것은 서로를 공격하지 않는 immune tolerance, 그리고 서로 공생을 허용하는 한 이득이 있어야 할 것임. (which advantages?)

3. 따라서 면역체계가 host와의 공생관계를 유지하기 위한 수단으로 진화해 왔고, 이를 위해 host의 immune system에 적극적으로(긍적적인 역할로) 관여함. (evidences?)

4. pathogen에 대해서는 협력하여 protective immune response를 , 평소에는 host immune system을 training 하고 innocuous antigen에 대해서는 tolerance를 잘 조절하는 방향으로

5. 항생제 남용, 식이변화 등은 이러한 immune balance 에 요구되는 microbiome 의 diversity를 잃어버리게 되는 결과를 초래하고, 여러 염증성/자가면역성 질환과의 연관성을 보여주는 증거들이 있음.

Dysbiosis: 이러한 공생관계가 깨어진다면?



- **면역체계의 교란**이 일어나지 않을까?
- win-win에서 서로 **해를 주는 사이**로 바뀌지 않을까?

Microbiome and immunologic disorder

Table 1 Administration of AhR ligands or drugs in treatments of autoimmune diseases or models

Autoimmune diseases or models	AhR ligands or drugs	Types of AhR–ligands or drugs	Tested cells or samples	Influence on immune cells or cytokines related to AhR signaling regulation		References
				Proportion or function of immune cells	Expressions of cytokines	
RA	GNF351	AhR–ligand, antagonist	FLS	unknown	IL-1β(mRNA)↓ IL-6(mRNA)↓	Lahoti et al. (2013)
	TCDD	AhR–ligand, agonist	FLS	unknown	IL-1β(mRNA)↑	Tamaki et al. (2004)
	Curcumin	AhR–ligand, agonist	MSCs	unknown	COX2↓ caspase-3↓	Buhrmann et al. (2010)
	Resveratrol	AhR–ligand, antagonist	FLS	unknown	COX2↓ PGE2↓	Yang et al. (2017)
	Quercetin	AhR–ligand, antagonist	FLS	unknown	COX2↓ PGE2↓ MMP-1↓ MMP-3↓	Sung et al. (2012)
CIA	Tetrandrine	Drug, AhR activator	MLNs, spleen	FoxP3$^+$Tregs↑ Th17↓	IFN-γ↓ IL-17A↓ IL-10↑	Yuan et al. (2016, 2017)
	Resveratrol	AhR–ligand, antagonist	DLNs, serum	Th17↓	IFN-γ↓ TNF-α↓ IL-6↓ IL-1↓ IL-4↓ IL17↓	Xuzhu et al. (2012)
SLE	I3C	AhR–ligand, agonist	Macrophages	M1↓ M2↑	TNF-α↓ IFN-γ↓ IL-6↓ IL-23↓	Mohammadi et al. (2018)
	CH223191	AhR–ligand, antagonist	PBDMs	unknown	IL-6(mRNA)↑ IL-12(mRNA)↑ IL-10(mRNA)↓	Shinde et al. (2018)
SLE mice	TCDD	AhR–ligand, agonist	Spleen, thymus, serum	CD4$^+$CD8$^+$ thymocyte↓ splenic CD4$^+$ T cell↓ splenic B220$^+$sIgM$^+$ B cell↑	IFN-γ↑	Li and McMurray (2009)
EAE	Laquinimod	Drug, AhR activator	Spleen	FoxP3$^+$Tregs↑ DCs↑ Th17↓	IL-17↓	Kaye et al. (2016)
	Gallic acid	Drug, AhR activator	Spleen	CD4$^+$IL-17$^+$ T cell↓ CD4$^+$Foxp3$^+$ T cell↑	TGF-β↑ TNF-α↓ IL-6↓ IL-1β↓ IL-17↓	Abdullah et al. (2019)
EAU	TCDD	AhR–ligand, agonist	DLNs, spleen	FoxP3$^+$Tregs↑ Th17↓	IL-17↓ IL-10↑	Zhang et al. (2010)
	ITE	AhR–ligand, agonist	DLNs	FoxP3$^+$Tregs↑ Th17↓	IFN-γ↓ IL-17↓ IL-10↓	Nugent et al. (2013)
	Baicalin	Drug, AhR activator	DLNs, spleen	FoxP3$^+$Tregs↑ Th17↓	IFN-γ↓ IL-17A↓ TNF-α↓ IL-10↑	Zhu et al. (2018)
NOD mice	ITE	AhR–ligand, agonist	Spleen	DCs↑	IL-6(mRNA)↓ IL-10(mRNA)↑ IL-12(mRNA)↓	Tukpah (2016)
BD	ITE or FICZ	AhR–ligand, agonist	Peripheral blood	Th17↓	IL-1β↓ IL-6↓ IL-17↓ IL-23↓ IFN-γ↓ TNF-α↓ IL-10↑ IL-22↑	Wang et al. (2014, 2014)

RA rheumatoid arthritis, *CIA* collagen-induced arthritis, *SLE* systemic lupus erythematosus, *EAE* experimental autoimmune encephalomyelitis, *EAU* experimental model of autoimmune uveitis, *NOD* nonobese diabetic, *BD* Behcet's disease, *TCDD* 2,3,7,8-tetrachlorodibenzo-p-dioxin, *I3C* indole-3-carbinol, *ITE* 2-(19H-indole-39-carbonyl)-thiazole-4-carboxylic acid methyl ester, *FICZ* 6-formylindolo-[3,2-b]-carbazole, *FLS* fibroblast-like synoviocyte, *MSCs* mesenchymal stem cells, *MLNs* mesenteric lymph nodes, *DLNs* draining lymph nodes, *PBDMs* peripheral blood mononuclear cell-derived macrophages, *M1* classic inflammatory M1 phenotype of macrophages, *M2* alternative anti-inflammatory M2 phenotype of macrophages, *COX2* cyclooxygenase-2, *PGE2* prostaglandin E2, *MMP*: matrix metalloproteinase

AhR plays a significant role in both innate and adaptive immune system.

1. regulation of inflammation
   - affect the function of different types of immune cells

2. aberrant AhR signaling may actually be associated with pathogenesis of autoimmune disease

다양한 Experimental autoimmune model들
AhR depletion의 경우 실험적으로 임상상태와 유사한 autoimmune disease 형태가 발현되었고,
AhR ligand (microbiota의 metabolites)등을 보충함으로써 autoimmune pathologic condition의 발현이 호전됨을 확인.

How does Urobiome affect IC/BPS?

여러 편의 study가 있지만,,,

- 대부분 특정 bladder의 microbiota species가 IC/BPS와 관련성이 있느냐에 초점

- 아직까지 정확한 mechanism 을 evidence로 밝힌 study는 없음.

- gut-microbiome이 인체의 면역기전, CNS 등을 통한 neurofunctional alteration에 기여한다는 여러 evidence는 많이 존재함.

- 상상력을 펼치기에는 urobiome에 대한 자료가 부족

- 그럼 역으로,
  IC/BPS의 pathophysiology를 보고 microbiome과의 연관성을 유추해 보자.

Putative pathophysiology of IC/BPS

- **IC와 BPS는 같은 etiology를 가진 질환인가?**

- No (태생은 다르나 생김새가 닮은)

- bladder pain이라는 공통점이 있으나, etiology가 다를 것으로 생각
- chronic pain이라는 관점에서는 일부 mechanisms은 (통증발생의 과정) 유사할 수 있음.

- **HIC와 NHIC는 같은 질환인가?**

- 과거 NHIC는 HIC와 연결선상의 질환으로 추정하기도 (glomerulation 여부로 둘 다 bladder-origined etiology에 의한 것으로 여겨왔음.)

- 최근 연구결과에 따르면, HIC와 NHIC는 gene expression, histology, age distribution이 다름. (NHIC가 bladder-centric etiology가 아닐 가능성)

- NHIC (ESSIC type 2) + BPS (ESSIC type 1, hypersensitive bladder) → BPS로 분류하자는 주장

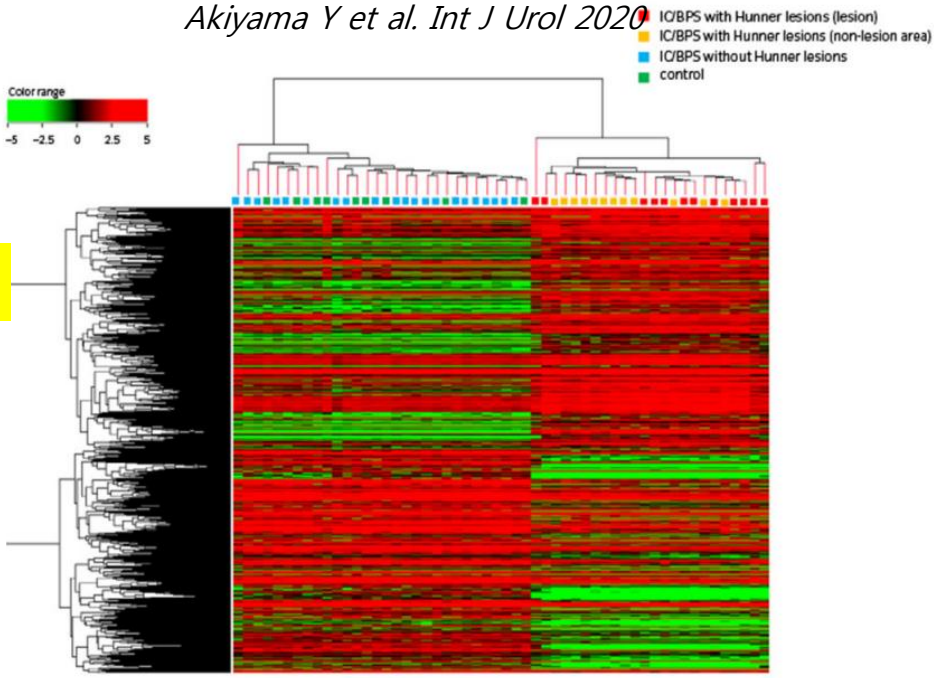
Putative pathophysiology of IC/BPS

Homma Y et al. Current Bladder Dysfunction Reports 2019

guidelines			
Endoscopic findings			
Hunner lesions	+	−	
Glomerulations	+/−	+	−
Terminology			
AUA	IC/BPS		
ESSIC	BPS		
	Type 3	Type 2	Type 1
East Asia	HIC	NHIC	HSB
Proposal	IC/BPS		
	HIC	BPS	

In genomic analyses, IC/BPS with Hunner lesion shows a distint gene expression pattern from IC/BPS without Hunner lesion

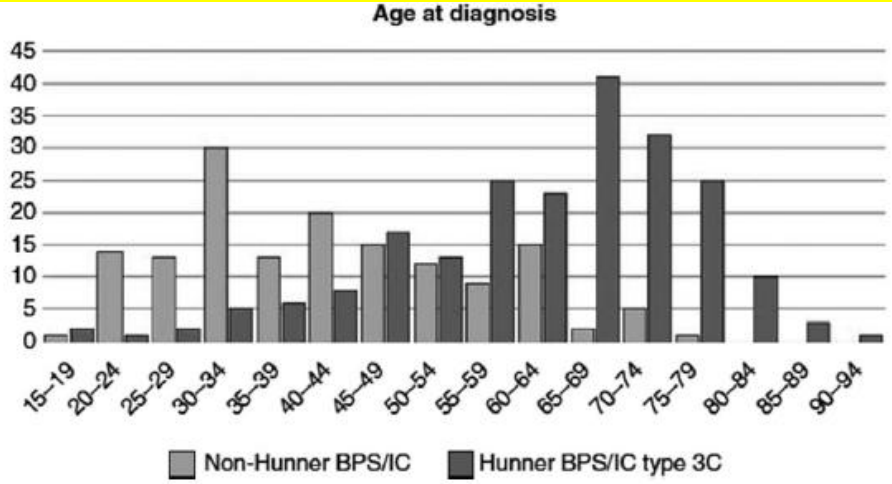
Akiyama Y et al. Int J Urol 2020



Control and IC/BPS without Hunner lesion

IC/BPS with Hunner lesion
- **Hunner lesion**
- **non-Hunner area**

Different age distribution between HIC vs NHIC



Logadottir et al. J Urol Nephrol 2012

Putative pathophysiology of IC/BPS

- Recent clinicopathological and genomic evidence suggests that IC/BPS should be categorized by Hunner lesion, rather than by clinical phenotyping based on sympomatology

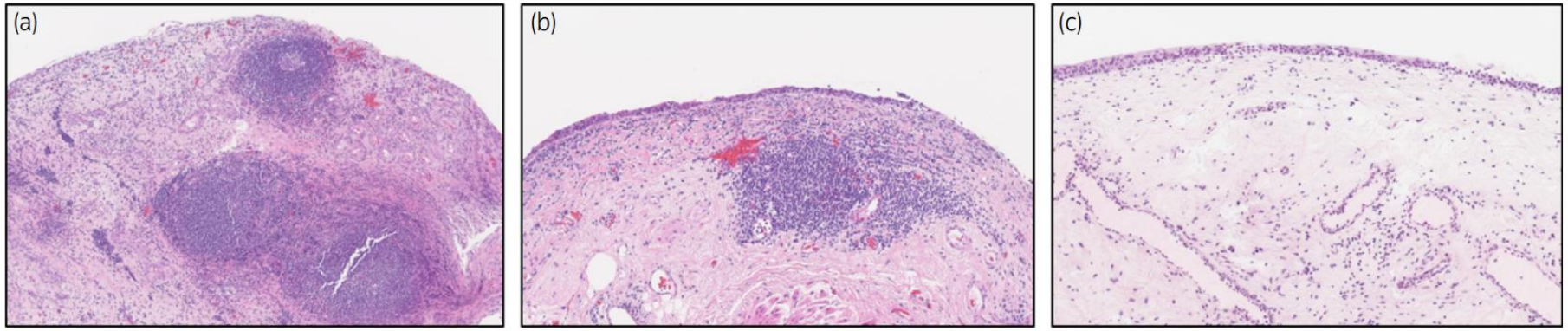
- **Hunner IC**: distinct inflammatory disease with proven bladder etiology characterized by epithelial denudation and enhanced immune responses

- **Non-Hunner IC**: non-inflammatory disorder with little evidence of bladder etiology. Potentially associated with urothelial malfunction and neurophysiological dysfunction. Presentes with somatic and/or psychological symptoms, which commonly results in central sensitization

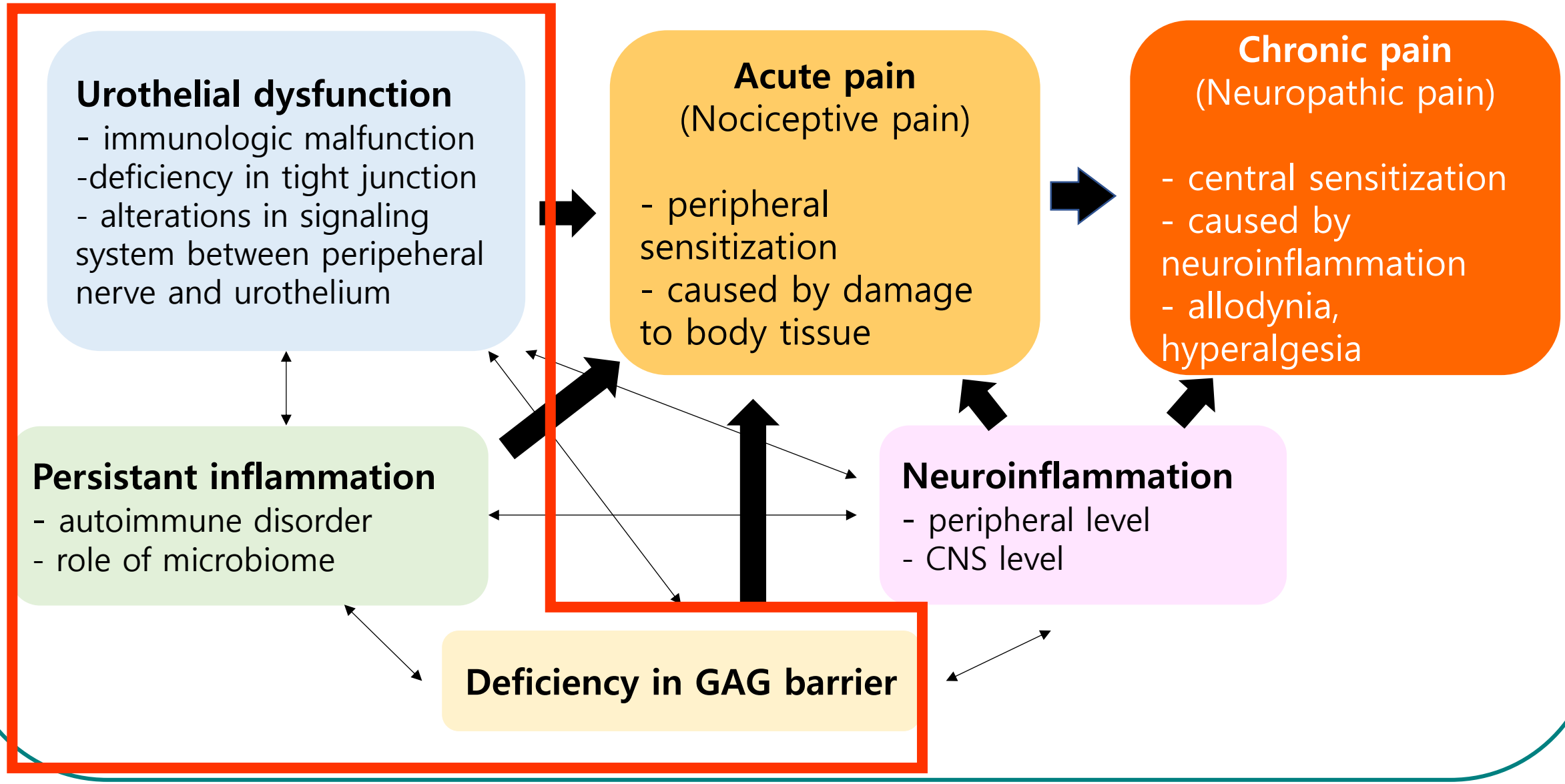
Histologic characters of IC/BPS

Akiyama Y et al. Int J Urol 2020

HIC (Hunner lesion) | HIC (non-Hunner lesion) | NHIC or BPS

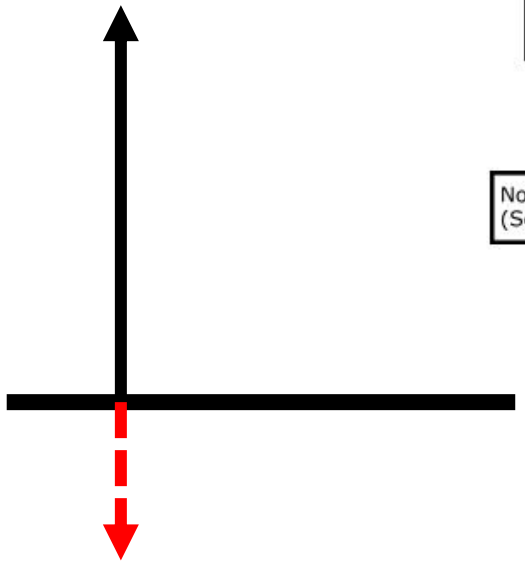


Putative pathophysiology of IC/BPS

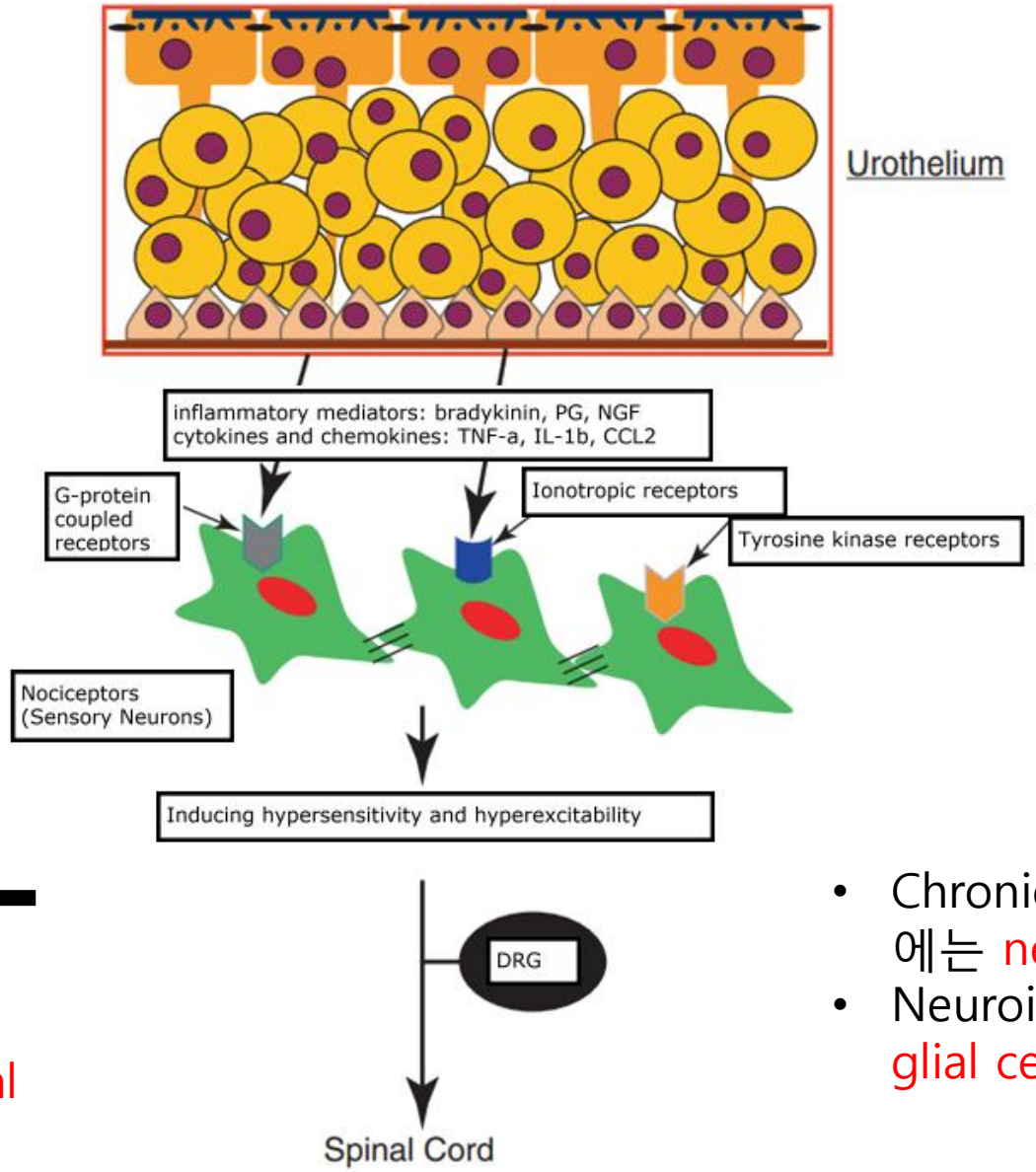


Mechanism of chronic bladder pain

Spontaneou
s resolution



Persistent and chronical progression



Activated nociceptors produces **neurogenic inflammation** via releasing neuropeptides such as **substance P, CGRP**, prostanoids

- Chronicity / centralization 과정에는 neuroinflammation이 중요
- Neuroinflammation 과정에서 glial cell이 중요한 역할

Immune modulation in periphery

Macrophage가 epithelial layer 바깥에 존재하는 microbiota를 인식하고 적절한 면역 반응을 유지하여 침입을 막고 거리를 유지하는 공생관계

(적절한 거리두기, **적절한 면역반응**을 유도)

J. Li et al.

Immunology Letters 232 (2021) 39–44

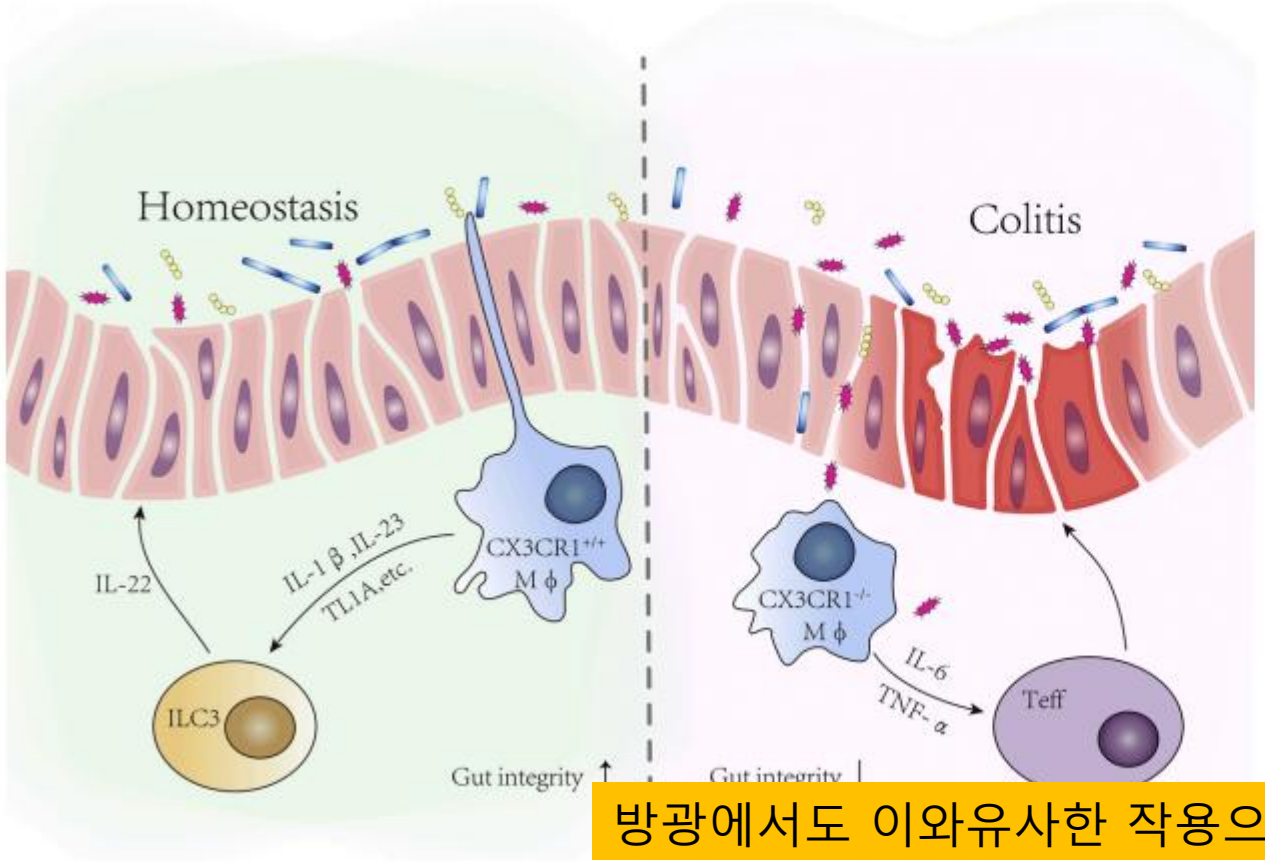
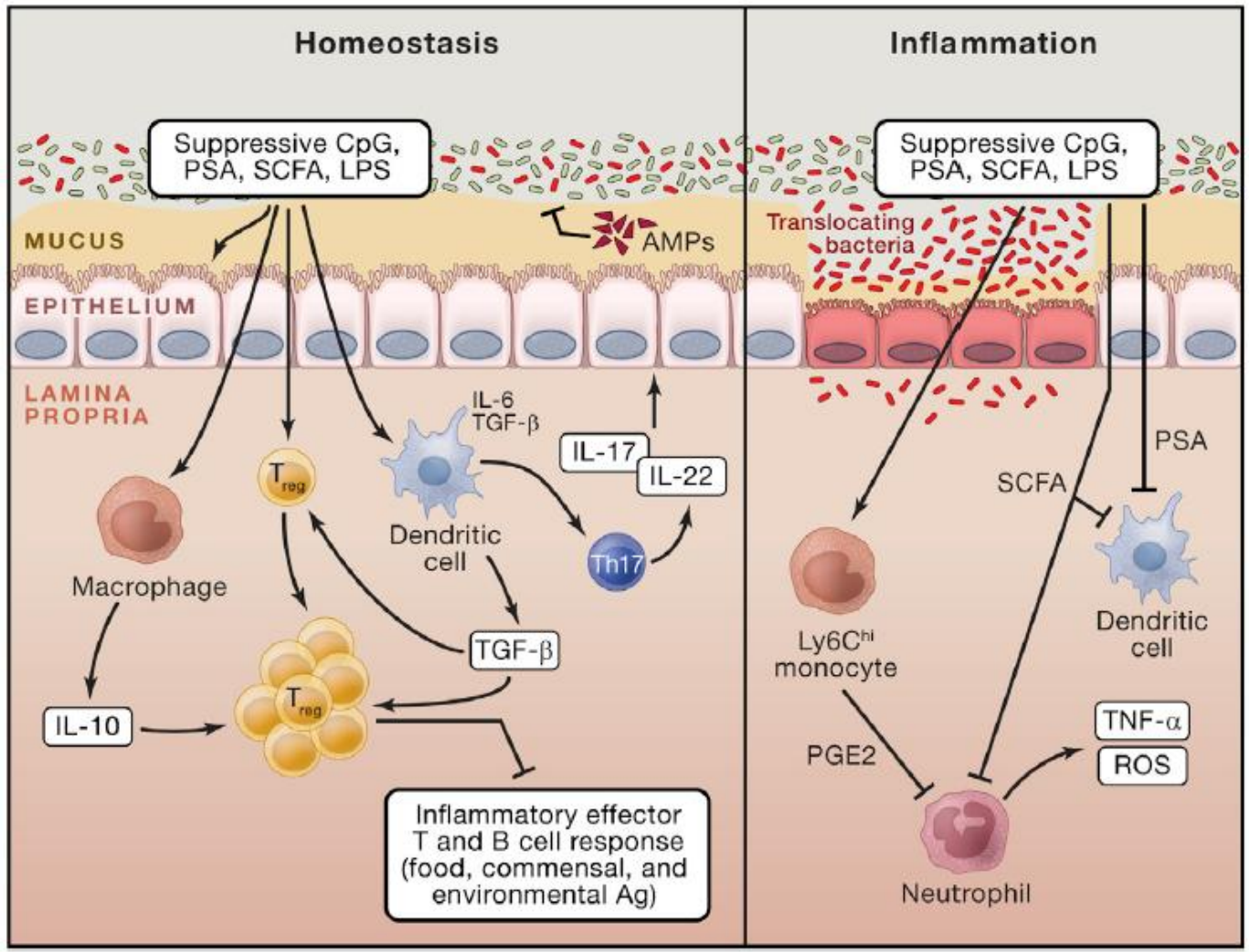


Fig. 1. CX3CR1^{+} macrophage is indispensable for the maintenance of gut integrity. Intestinal resident CX3CR1$^{+/+}$ macrophages generate cytokines including IL-1β and IL-23 to enhance IL-22 production of ILC2 cells. IL-22 plays crucial roles in maintaining gut integrity through the induction of epithelial cell proliferation, goblet cell hyperplasia and anti-microbial peptide (AMP) secretion. On the other hand, CX3CR1$^{-/-}$ macrophages fail to keep pathogenic microbes at bay; otherwise, a colitogenic T cell (Teff) response is elicited to accelerate colitis progression.

Gut-microbiota가 ILC3 cell을 통해 IL-22를 유도하여, gut integrity를 유지:
(1) epithelial cell proliferation,
(2) goblet cell hyperplasia,
(3) antimicrobial peptide (AMP) 분비

방광에서도 이와유사한 작용으로 urothelial integrity를 유지하는 것이 아닐까?

Immune modulation in periphery

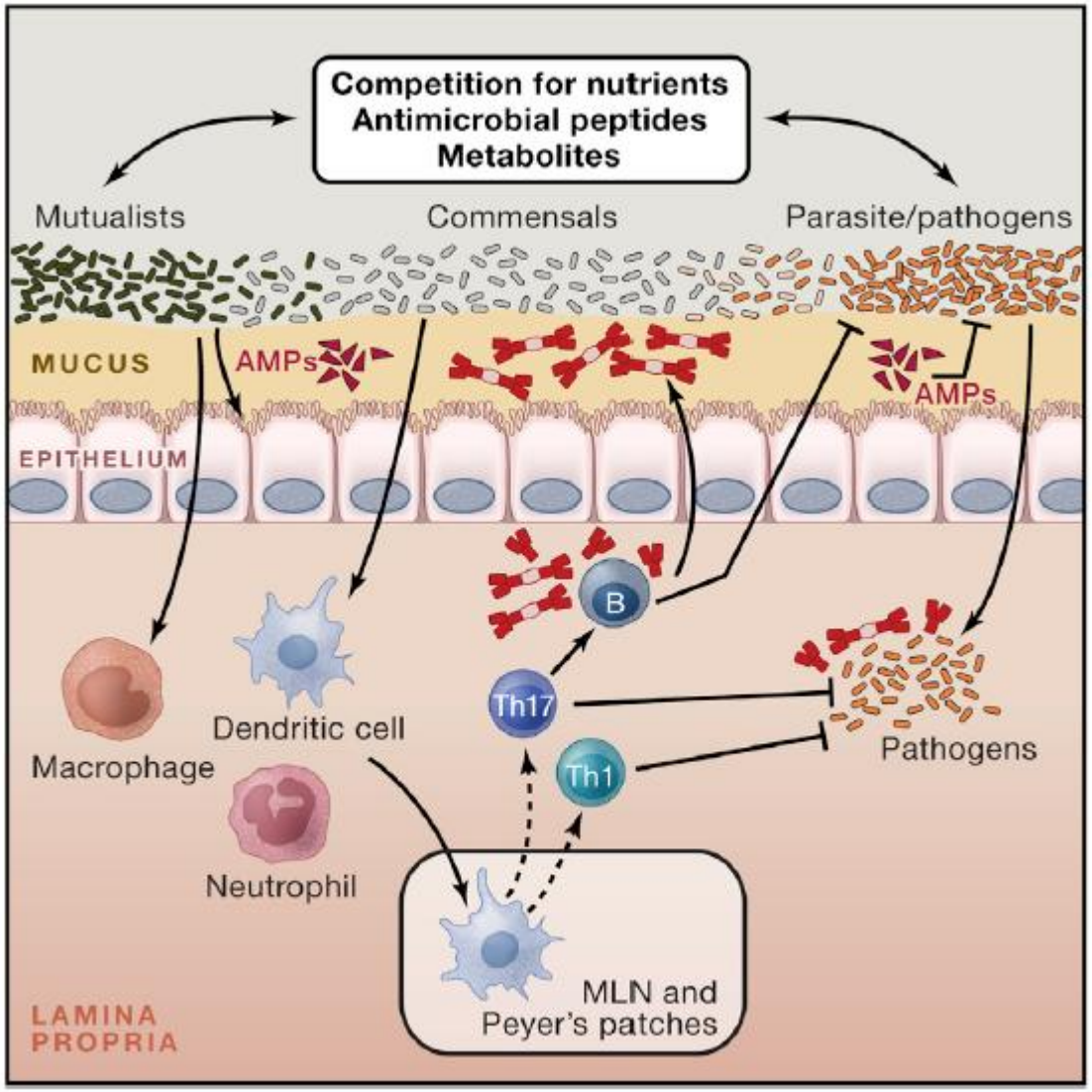


Immune regulations:
Homeostasis vs Inflammation

1. Homeostasis
- microbial metabolite는 T or DCs 로 하여금 commensal specific Treg cell (Th17) 및 IgA producing B cell 유도를 촉진
- Th17은 epithelial cell 기능과 homeostasis를 조절

2. Inflammation (**억제하는 방향**으로)
- commensal derived metabolites가 inflammatory cell에 영향
- SCFA는 neutrophil 혹은 DCs activation을 저해
- microbial ligand에 의해 monocyte가 PGE2를 생성하여 neutrophil activation을 억제

Immune modulation in periphery



Promotion of protective immunity
(commensals control pathologic microbes as below)

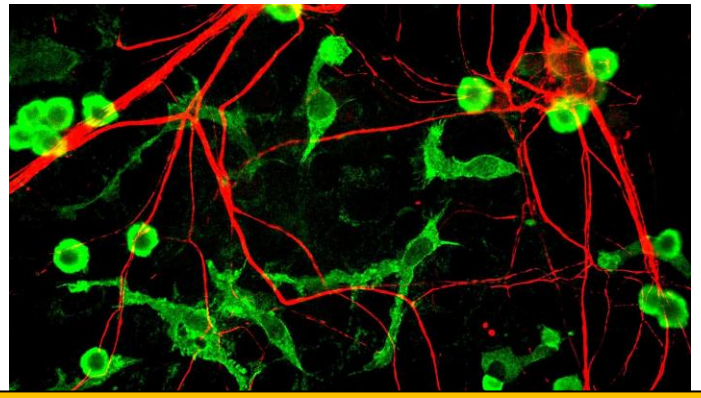
1. compete for nutrients
2. epithelial cell을 통해 AMP 생성을 촉진
3. reinforce tight junction
4. effector T, B cell의 유도를 촉진하여, DCs이나 innate immune cell의 국소/전신반응을 조절
5. commensal에서 생성된 metabolite 가 pathogen의 생존이나 독성에 영향을 주기도

6. 이러한 mechanism이 억제되지 않을 경우,
Microbiota의 이러한 면역조절기능은 inflammatory and autoimmune disorder를 유발/촉진할 수

Immune modulation in CNS: glial cells in CNS

Microglia

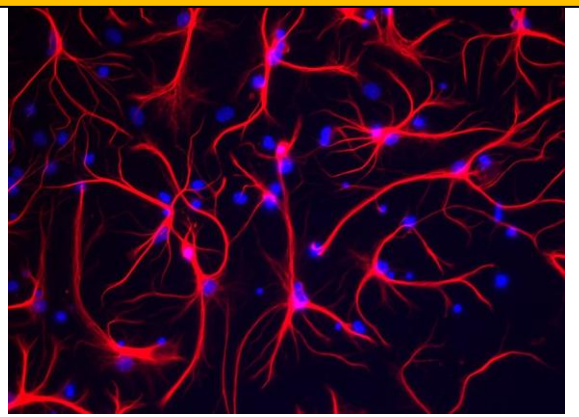
- CNS에서 immune response와 homeostasis의 유지에 관여 (primary immune cell in CNS like macrophage in peripheral)
- phagocytosis/clearance of debris and infectious antigens
- signaling: antigen presentation/cytokine production
- rapid immune surveillance/activation of inflammatory responses
- AD, PD, MS 등과 같은 neurodegenerative disease와도 관련
- 신경발달과정에서 신경분화를 유도하는 역할



CNS glial cell의 대표적인 microglia, astrocyte는 CNS에서 phagocytosis 같은 immune response, long term potentiation 등과 같은 neural remodeling에 깊이 관여하는 중요한 cell들이며 neuron-glial interaction을 통해 이러한 작용을 나타낸다.

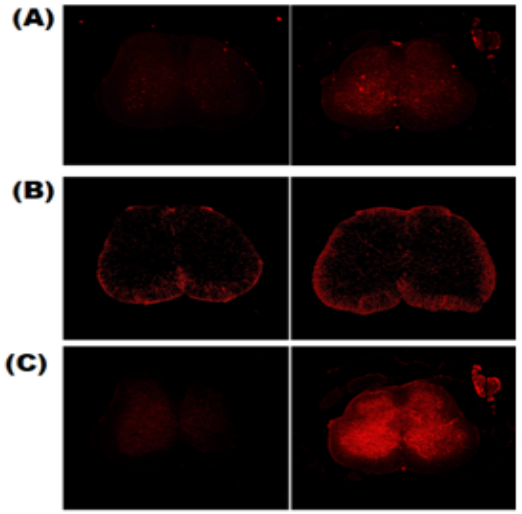
Astrocyte

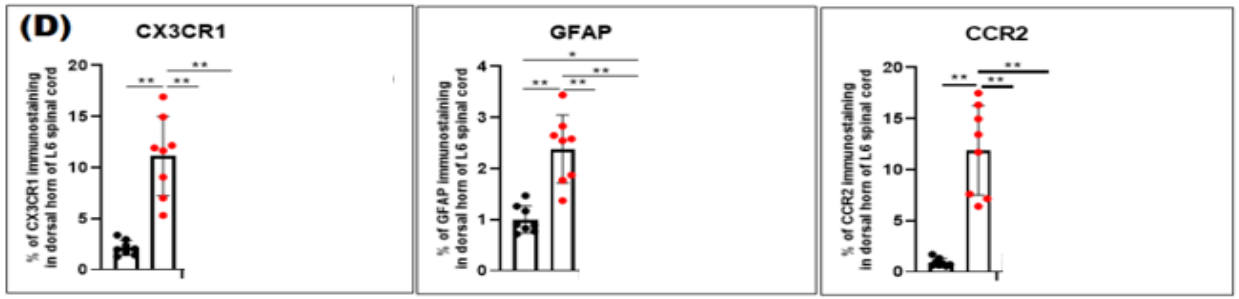
- regulation of BBB integrity
- secretion or absorption of neural transmitters (glutamate, ATP, GABA)
- regulate neural excitability and synaptic transmission
- repair nervous system
- express PRR(pattern recognition receptors) for detection of MAMPs (microbe-associated molecular patterns)
- modulate neuroinflammatory response through cytokine production and antigen presentation (MHC II)

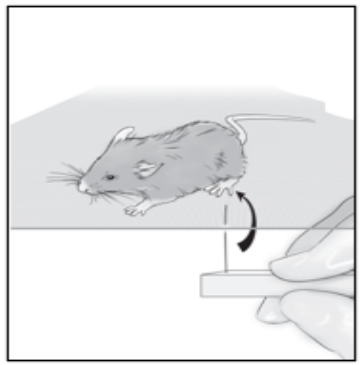


Expression of microglia/astrocyte in experimental IC model

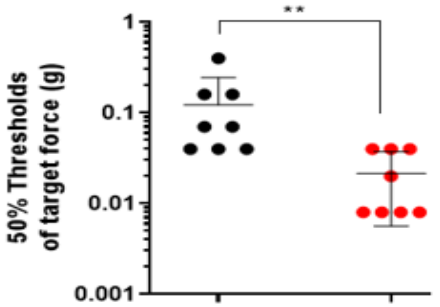
Kwon J. 2021 ICS, 2022 SUFU Basic Science Essay Award



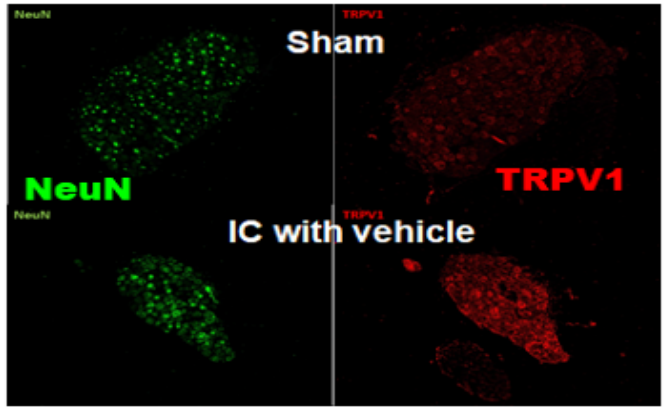


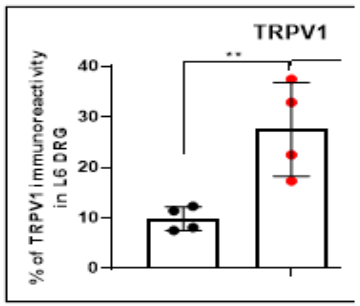


(Fig. Ref) Mulder GB and Pritchett K. Rodent analgesiometry: The hot plate, tail flick and Von Frey hairs. Contemp Topic Lab Anim Sci. 2004;43(3):54-5.



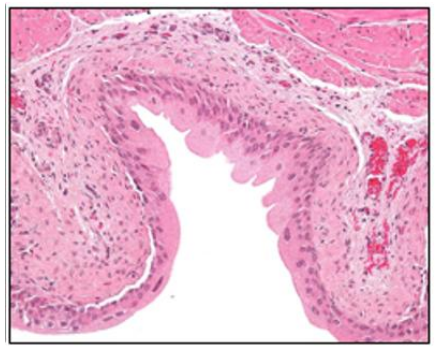
Kwon J. 2022 SUFU

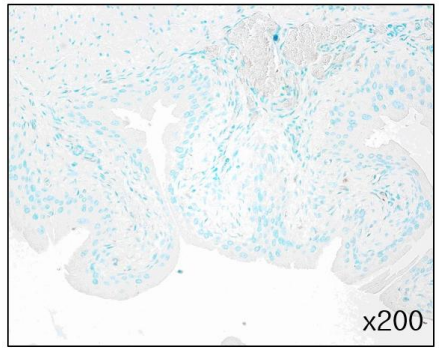




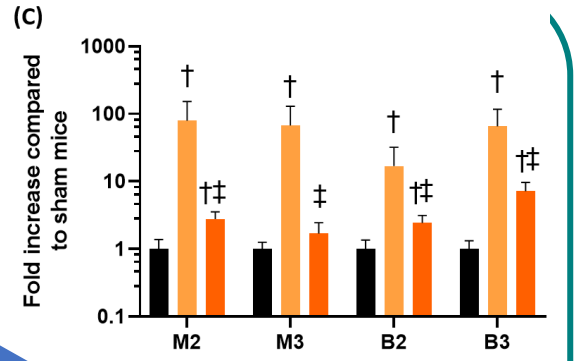
experimental IC model 의 경우 3주 이후에도 지속되는 만성방광통증, mucosal denudation
증가된 염증소견: lymphocyte, monocyte, macrophage, dendritic cell 증가, proinflammatory cytokine 증가
bladder afferent hyperexcitation: bladder M-receptor, P2X purinergic receptor 발현증가, L6-S1 DRG에서 TRPV1가 과발현
방광의 통각수용체 과발현: TACR2 과발현, P2X4, P2X7 증가
Central sensitization: L6 spinal cord에서 glial activation의 증가를 보여주는 CX3CR1, GFAP, CCR2 등의 marker 들이 증가
- 이는 urothelial barrier disrruption과 이로인한 만성염증상태가 bladder afferent hyperexcitation을 통해 CNS의 neural remodeling, 즉 central sensitization을 유발하였고 이러한 CNS의 변화는 국소염증반응이 개선된 후에도 지속되는 만성통증과 관련이 있음.

Immune modulation in periphery





Bladder afferent hyperexcitation

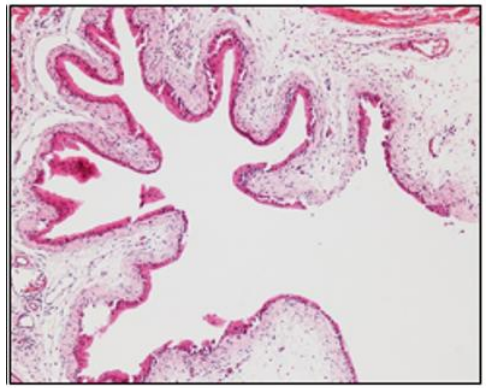


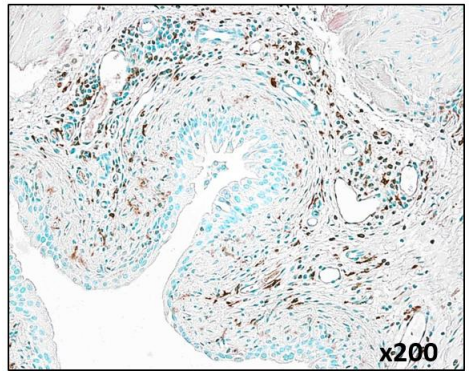
Urothelial barrier function의 변화

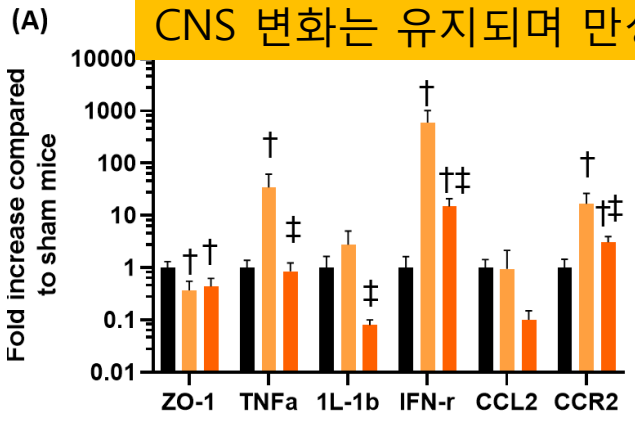
Bladder내 면역세포의 활성화 -

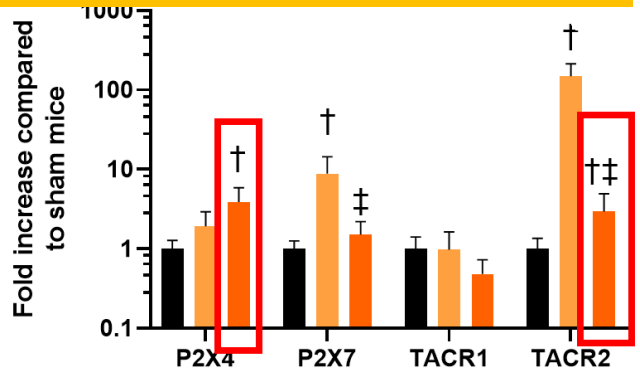
CNS level에서 neurologic change (Neuroplasticity)

시간 경과에 따라 proinflammatory cytokine은 다소 호전되나 CNS 변화는 유지되며 만성방광통증의 원인으로 보임



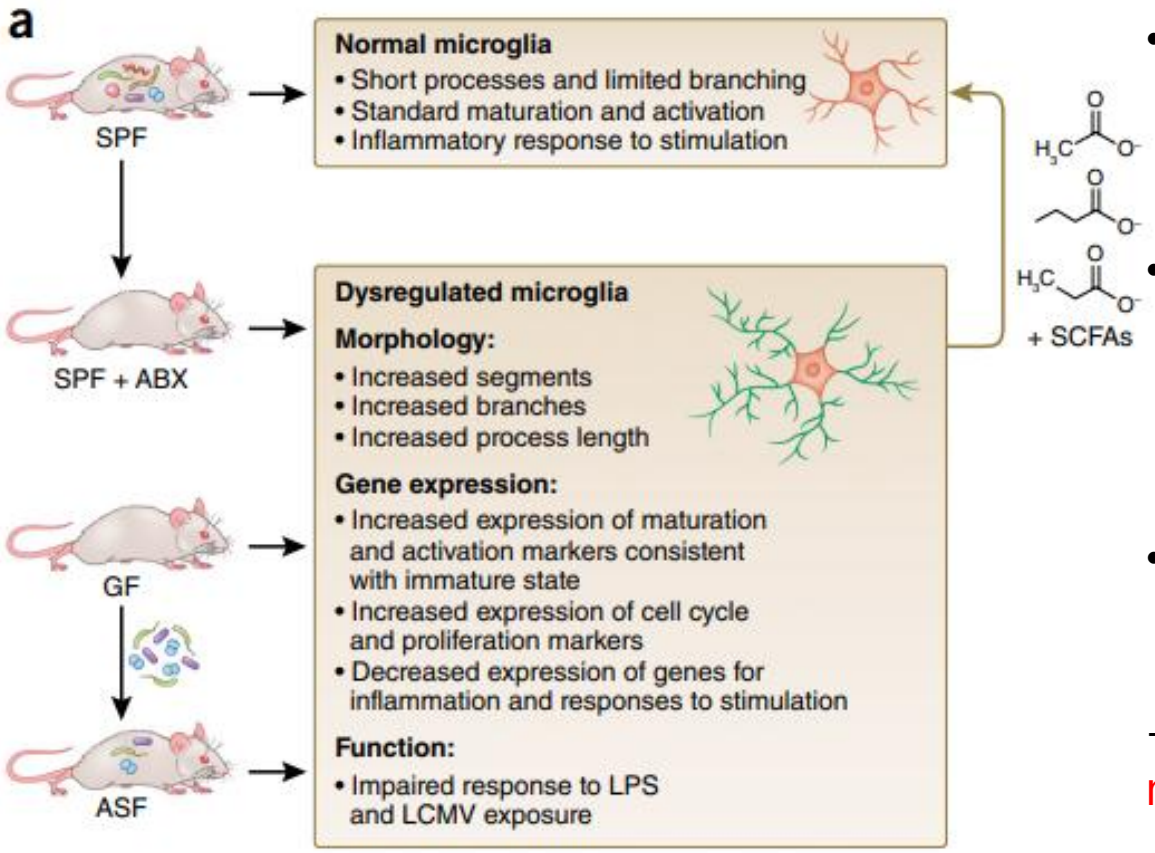






Immune modulation in CNS

GF 혹은 ABx treated mouse에서



Affect neural function through **microglia**

- GF에서 SPF와 비교해 immature microglia가 더 많이 발현하였고, 이는 gut-microbiota가 microglia의 maturation에 관여한다는 의미.
- Adult GF mouse 혹은 SPF + Abx mouse에서 microglia에서 impaired immune function을 보여줌 (attenuated immune activation & impaired proinflmmatory cytokine induction)
- Microbiome의 metabolite인 SCFAs를 보충하자 microglial morphology나 function이 정상화됨.

- 이러한 증거들은 gut-microbiota가 CNS에서 immune modulation에 관여한다는 의미.

- SPF (specific pathogen-free): conventional microbial colonization 을 보임
- GF (germ free): absence of microbial colonization

Fung TC 2017 Nature Neuroscience

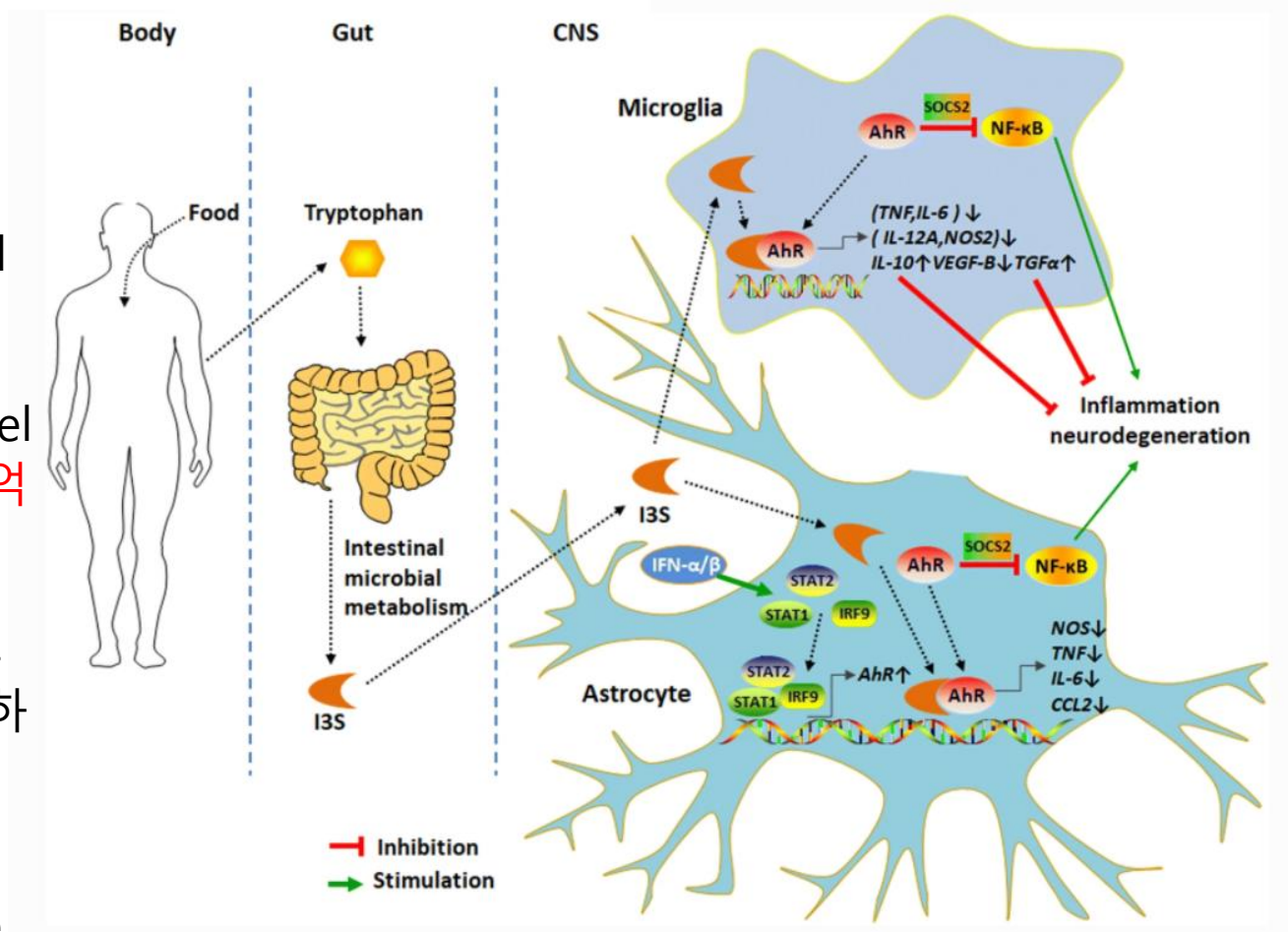
Immune modulation in CNS

Affect neural function through **astrocyte**

- Gut-microbiota는 tryptophan을 대사하여 indole-3-aldehyde와 indole-3-propionic acid를 생성. plasma를 통해 흡수된 metabolite는 CNS에서 astrocyte의 AhR signaling을 activation - neurodegenerative disease model에서 clinical score 및 neuroinflammation을 억제하는 효과

- 항생제사용으로 microbiota depletion인 경우 AHR ligand인 indoxyl-3-sulfate (I3S)가 부족하여 score가 악화됨.

AHR (aryl hydrocarbon receptor) in astrocyte



Wang XS 2020 Inflammopharmacology

Putative mechanisms of microbial effect (dysbiosis) on IC/BPS

- microbiome은 gut에서처럼 방광에서도 host와의 symbiosis를 위해 적절한 정도의 peripheral immune modulation을 할 것으로 추정된다.

- bladder내의 microbiome의 균형이 깨어질 경우 (dysbiosis) 국소면역체계의 교란을 야기할 수 있고, 이러한 면역체계의 교란은 altered barrier function, 그리고 만성적으로 지속되는 억제되지 않는 국소염증반응을 야기할 수 있다.

- 이러한 peripheral damage는 peripheral sensory nerve를 통해 만성적인 신경자극을 CNS로 전달할 것이며, 이는 만성통증의 원인이 되는 CNS level에서 neural remodeling을 초래할 것이다. (HIC의 가설)

- 또한 microbiome이 생성한 metabolite는 plasma 흡수를 통해 CNS에서 glial activation에 깊이 관여를 하는데, microglial maturation/function, 그리고 AHR을 통한 astrocyte의 neuroinflammation 억제에 관여한다.

- 따라서, microbiome이 적절한 역할을 못할 경우 CNS에서 neuroinflammation을 조장하여 만성통증과 관련된 central sensitization을 발생시킬 수도 있다. (BPS의 가설)

Putative mechanisms of microbial effect (dysbiosis) on IC/BPS

1. Altered barrier function by UTI or immunologic changes in bladder due to dysbiosis

2. Urothelial dysfunction affected by dysbiosis

3. Persistant inflammation by pertubated immune responses by dysbiosis

4. Neuroinflammation induced by failure to regulate neuron-glia interaction

경청해주셔서 감사합니다.