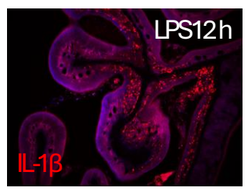
Nasal inflammation-induced lymphangiogenesis in the nasal mucosa

Suzuho Komaki[1], Ryuichi Imai[1], Rei Settsu[2], Aki Obara[2], Atsuyoshi Shimada[1, 2], and Sanae Hasegawa-Ishii[1, 2]

1. Grad Sch Health Sci, Kyorin Univ 2. Fac Health Sci, Kyorin Univ

Introduction

- Patients with chronic nasal inflammation have a higher risk of depression and anxiety.
- Earlier resolution of nasal inflammation would reduce the risk of such psychiatric disorders.
- Recently it is getting clear that the lymphatic vessels are newly generated in inflammatory tissues (lymphangiogenesis) and may contribute to the resolution of inflammation.
- Lymphangiogenesis can occur through proliferation of lymphatic endothelial cells or through transdifferentiation of macrophages.
- Our previous studies have indicated that the administration of lipopolysaccharide (LPS) into the nasal cavity induces nasal inflammation.



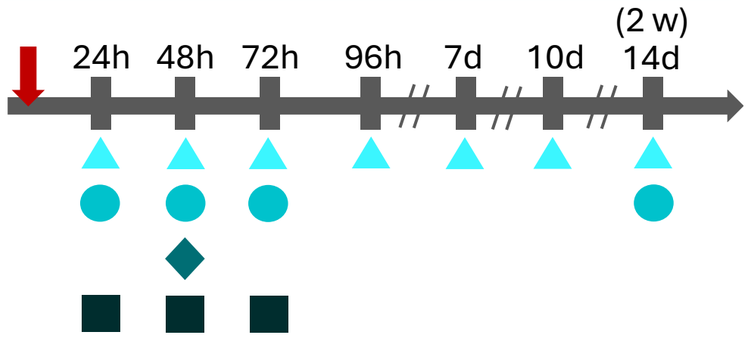
Hypothesis

Nasal inflammation causes lymphangiogenesis in the nasal mucosa that contributes to inflammatory resolution.

Aim

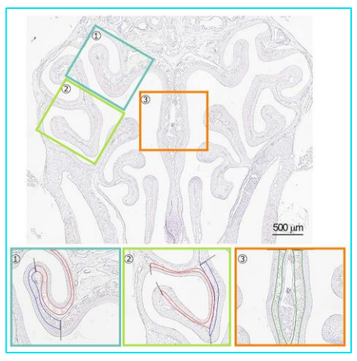
Clarify whether and how the nasal inflammation causes lymphangiogenesis in the nasal mucosa.

Methods



Eight-week-old male C57BL/6JJmsSlc mouse

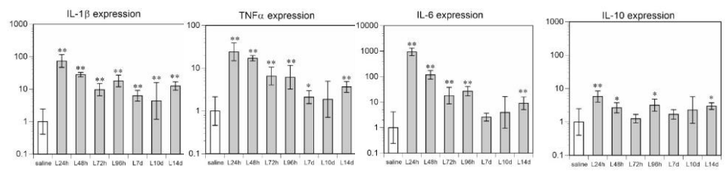
- Intranasal administration
  10μl of saline or LPS (1mg/mL)
- Quantitative RT-PCR
- Immunohistochemistry
- Double immunofluorescence
- ELISA



- Immunohistochemistry
  - Analyzed in three parts
    - ① 1st turbinate
    - ② 2nd turbinate
    - ③ Septum
  - ① and ② were further analyzed with the Inner vs Outer separation

Results

1. Quantitative RT-PCR（cytokines）

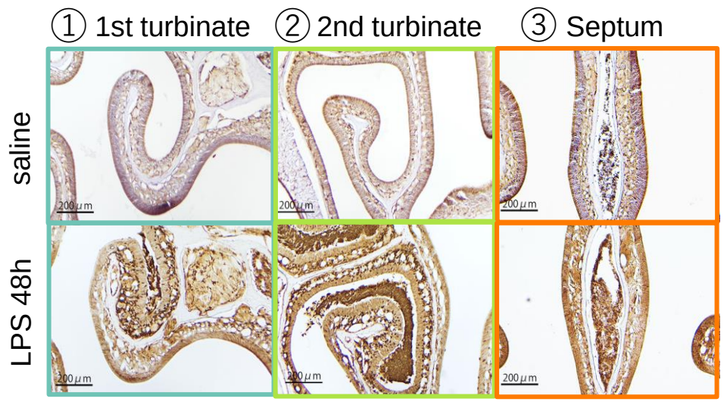


IL-1β, TNF-α, IL-6, and IL-10 were upregulated significantly from 24 h which lasted even until 14 days after the LPS treatment.

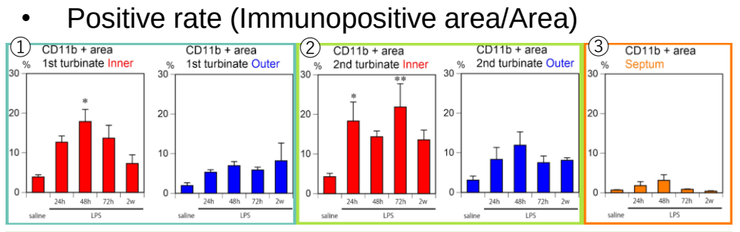
2. Immunohistochemistry

◆ **Nasal inflammation**

➢ **CD11b**



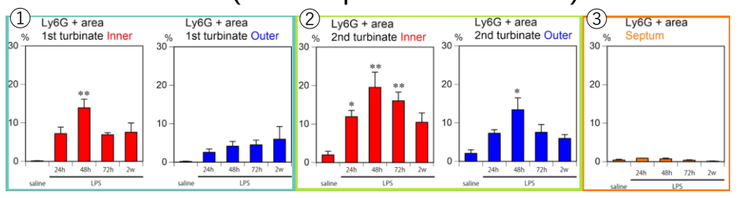
- Positive rate (Immunopositive area/Area)



CD11b+ cells increased in number in the nasal turbinates, but not in the septum, 24-72 h after the LPS administration.

➢ **Ly6G**

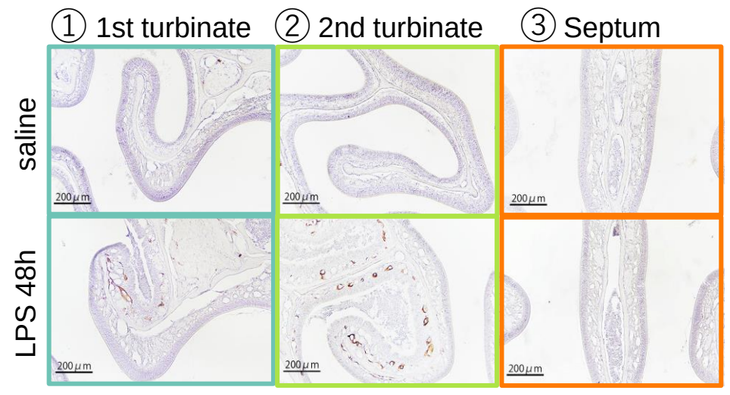
- Positive rate (Immunopositive area/Area)



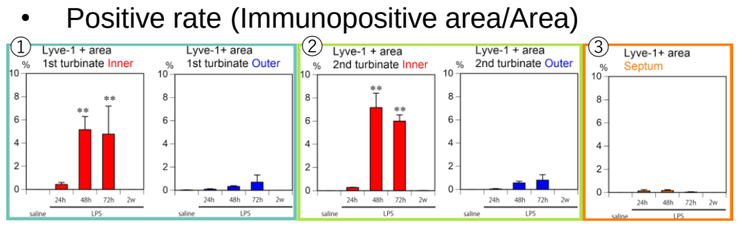
Ly6G+ cells increased in number in the nasal turbinates, but not in the septum, 24-72 h after the LPS administration.

◆ **Lymphatic vessels**

➢ **LYVE-1**

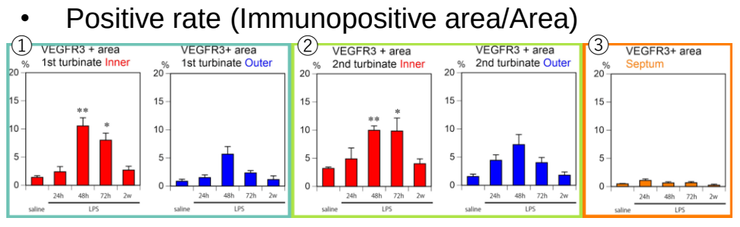


- Positive rate (Immunopositive area/Area)



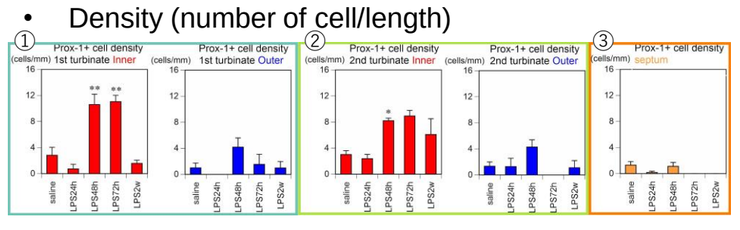
➢ **VEGFR-3**

- Positive rate (Immunopositive area/Area)



➢ **Prox-1**

- Density (number of cell/length)

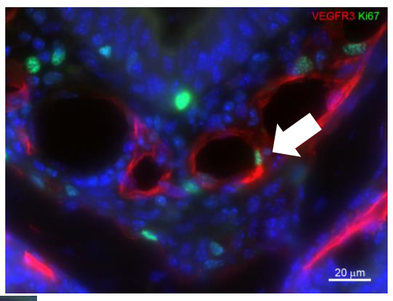


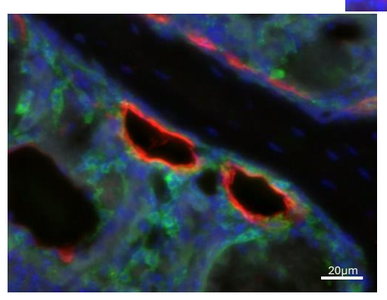
Lymphatic vessels increased in marker staining in the nasal turbinates, but not in the septum, 48-72 h after the LPS administration.

3. Double immunofluorescence

VEGFR-3 / Ki-67

The lymphatic endothelial cells were proliferating.

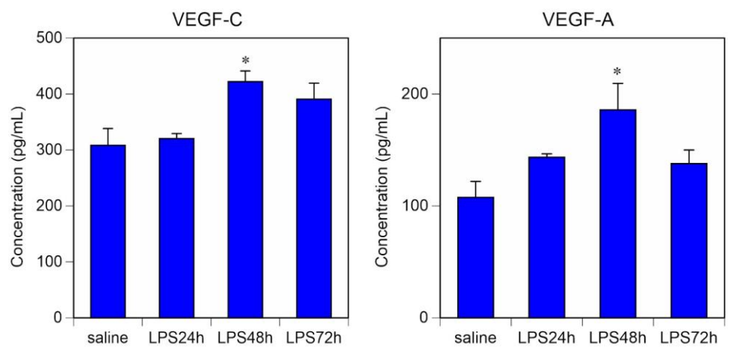




Macrophages do not express lymphatic markers.
(not transdifferentiation)

VEGFR-3 / CD11b

4. ELISA



VEGF-C and VEGF-A proteins that promote lymphangiogenesis were elevated in the nasal tissue 48 h after the LPS administration.

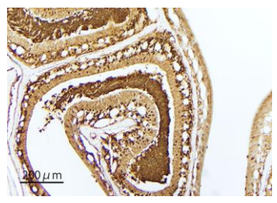
Conclusion

Administration of LPS

↓

Nasal inflammation 24h~72h

- ➢ Increase in cytokines
  - ◆ lasted even until 14 days
- ➢ Infiltration of Immune cell
  - ◆ especially at the turbinates
  - ◆ not in the septum



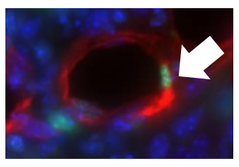
↓

Increase in VEGF-C and VEGF-A 48h

↓

Proliferation of the lymphatic endothelial cells 48h

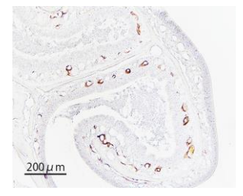
- ➢ Not transdifferentiation



↓

Lymphangiogenesis 48h~72h

- ➢ Increase in the positive rates for lymphatic vessel marker
  - ◆ especially at the turbinates
  - ◆ not in the septum



↓

Resolution of nasal inflammation?