Macrophages mediate colon carcinoma cell adhesion in the rat liver after exposure to lipopolysaccharide

Nuray Gül, Simran Grewal et. al.
Colorectal Cancer

- Incidence rate is 1 million per year

- Patients dying from CRC every year: half a million

- 20-50% develop liver metastases within 5 years after resection

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<table>
<thead>
<tr>
<th>Toll-like receptor</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR-1:TLR-2 heterodimer</td>
<td>Lipomannans, Lipoproteins Cell-wall β-glucans, lipoteichoic acids, Zymosan</td>
</tr>
<tr>
<td>TLR-2:TLR-6 heterodimer</td>
<td>Ligands of TLR-1:TLR-2 heterodimer</td>
</tr>
<tr>
<td>TLR-3</td>
<td>Double-stranded RNA</td>
</tr>
<tr>
<td>TLR-4</td>
<td>LPS, Lipoteichoic acids</td>
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<tr>
<td>TLR-5</td>
<td>Flagellin</td>
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<tr>
<td>TLR-7</td>
<td>Single-stranded RNA</td>
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<tr>
<td>TLR-8</td>
<td>Single-stranded RNA</td>
</tr>
<tr>
<td>TLR-9</td>
<td>DNA with unmethylated CpG</td>
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<tr>
<td>TLR-10</td>
<td>Unknown</td>
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</tbody>
</table>
Macrophages

• Monocytes develop from myeloid progenitor cells
• Monocytes migrate into tissues and differentiate into macrophages
• Main task is the production of cytokines and chemokines (e.g. IL-1β, TNF-α, IL-6, IL-8, IL-12)
• Phagocytosis and production and release of reactive oxygen species ROS

ROS

- Fusion of phagosome and lysosome forms the NADPH oxidase complex
- NADPH oxidase transfers an electron to molecular oxygen forming the superoxide ion $\text{O}_2^-$
- Superoxide Ion is converted by superoxide dismutase to $\text{H}_2\text{O}_2$
- ROS can be released into extracellular environment

Surgery can paradoxically contribute to tumor recurrence and liver metastases development.

Bacterial spillage during surgery may lead to the growth of metastases.

Concentration of Lipopolysaccharide in peripheral blood increases 1h after surgery and normalizes 24 h later.

• LPS activates macrophages through TLR-4
• Activated macrophages can release reactive oxygen species (ROS)
• ROS disrupts the endothelial barrier, leading to vascular permeability in tissues and enhanced tumor-cell adhesion in the liver
• Intraperitoneal injections with PBS (control group) or LPS in rats
• Followed by the inoculation of tumor cells (rat colon carcinoma cell line CC351s- moderately differentiated and immunogenic)
• LPS injections resulted in decreased expression of the thight junction protein zonula occludens 1 (ZO-1)
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Effect of LPS-stimulated macrophages on tumor cell adhesion by disrupting sinus endothelial barriers

Endothelial cell monolayers (of human umbilical vein endothelial cells; HUVECs) were cultured on the upper side of transwell membranes.

Macrophages on the lower side.

Addition of LPS (and LPS inhibitor polymyxin B; PMB)

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• Next step: Adding of tumor cells

• The intercellular gaps in the endothelial monolayer, that were formed upon LPS administration, contained a high number of adherent tumor cells

• No tumor cells attached to endothelial monolayer in absence of LPS
• Role of LPS-activated macrophages in tumor-cell adherence **in vivo**

• liver macrophages (Kupffer cells) had been depleted by injection of clodronate-containing liposomes
The absence of Kupffer cells and newly recruited monocytes was confirmed by ED2 (marker for tissue resident macrophages) and ED1 (marker for newly recruited monocytes).

Less tumor cells were observed in the livers of the KC-depleted rats, pre-treated with LPS and tumor cells.

Higher expression levels of ZO-1 in KC-depleted rats.
• Endothelial layer damage induced by macrophage-produced reactive oxygen species (ROS)

• ROS-scavenging enzymes:
  • Superoxide dismutase (SOD)
  • Catalase
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• Role of ROS in LPS-induced tumor-cell adhesion in vivo

• Rats were treated with the anti-oxidant edaravone (which is used to treat ischemic stroke)

• This resulted in lower numbers of tumor cells and the expression levels of ZO-1 did not decrease
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Discussion

- Polymorphonuclear cells (PMNCs) also produce ROS
- In both cancer patients and tumor-bearing mice, an impairment of ROS-scavenging systems has been found
- Rats that received LPS developed less liver metastases, compared with PBS treated mice
- LPS may stimulate immune responses against immunogenic CC351s cells

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Thank you for your attention!