Interleukin-1 role in human Th-17 cell responses, dendritic cell activation, and epithelial cell transformation

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BACK TO THE FUTURE: several new important biological roles of the Interleukin-1 cytokine family emerge from studies of inflammation-dependent carcinogenesis

Human Dendritic Cells
Activation by β-Glucan

Skin carcinogenesis (DMBA/TPA)

Cytokines
Chemokines
Metalloproteases

IL-1α

IL-1β

IL-23
IL-6
TGF-β

(Cytokines, Chemokines, Metalloproteases)

Th17
Activation of human Dendritic Cells by ITAM-signaling receptors

IL-1β

Human Monocyte-derived Dendritic Cells
C-type lectins, Scavenger r.

Toll-like receptors

RIG-I-like Receptors (helicases)

NOD-like Receptors: NALPs, NODs

C-type lectin domain, Immuno globulin-like domain, RNA-helicase, Leucine-repeat, ITAM-like domain, TIR domain, Nucleotide-binding domain, CARD domain, Pyrin domain

Pattern Recognition Receptors (PRR)
Signals from innate receptors and cytokine receptors cooperate and antagonize in inducing pro-inflammatory and immunoregulatory cytokines.

Table 1. Fungal PAMPs and their receptors

<table>
<thead>
<tr>
<th>PAMP</th>
<th>PRR(s)</th>
</tr>
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<tbody>
<tr>
<td>β-1,2 mannosides</td>
<td>Galectin-3</td>
</tr>
<tr>
<td>β-glucan</td>
<td>Dectin-1, SP-D, lactosylceramide</td>
</tr>
<tr>
<td>Chitin</td>
<td>Unknown</td>
</tr>
<tr>
<td>Phospholipomannan</td>
<td>TLR2</td>
</tr>
<tr>
<td>Glucuronoxylomannan</td>
<td>CD14, TLR4</td>
</tr>
<tr>
<td>Mannan</td>
<td>TLR4; SP-A; SP-D, MR, DC-SIGN, Dectin-2, MBL</td>
</tr>
<tr>
<td>Galactomannan</td>
<td>PTX3 (pentraxin-3)</td>
</tr>
<tr>
<td>DNA</td>
<td>TLR9</td>
</tr>
</tbody>
</table>
**Cell Culture and Assays:**

- **Naïve CD4+ T cells**
  - Anti-CD3/28 + 5 days
  - ELISA: IFN-γ (IL-22), IL-17, IL-1, IL-6, IL-12, IL-23

- **DC Culture**
  - Anti-CD3/PMA + 18-h spn
  - ELISA: IL-1, IL-6, IL-12, IL-23

- **Mono-DC**
  - no stimulus, zymosan, β-glucan, LPS + R848
  - ELISA: IL-12, IL-23, IL-6, IL-1β

**Stimuli and Antibodies:**

- **Amyloidosis:**
  - zymosan, β-glucan
  - Anti-IL-12p70, Anti-IL-12p40

- **LPS + R848:**
  - Anti-CD3/PMA

**Diagram:**

- DC (dendritic cell)
- Naïve CD4+ T cells
- ELISA (enzyme-linked immunosorbent assay)
- Anti-CD3/28
- Anti-IL-12p70, Anti-IL-12p40
- IL-1, IL-6, IL-12, IL-23
- IFN-γ, IL-17
Cytokine profile induced by $\beta$-glucan and LPS in human monocyte-derived DCs
Role of endogenous IL-1 in IL-23 production in human monocyte-derived DCs activated by β-glucan

In the presence of the IL-1RA the ability of β-glucan treatment to induce IL-23 protein production (A) and IL-23 p19 and IL-12 p40 mRNA expression (B) in human mono-DCs is decreased.
Role of endogeneous IL-1 in the regulation of the late gene expression induced by β-glucan in human mono-DCs
Microarray analysis of the transcripts induced by b-glucan in human mono-DCs in the presence or not of IL-1RA

“Early genes”

“Late genes”
LPS induces “late genes” expression with a faster kinetic than β-glucan in human mono-DCs

- Both LPS and β-Glucan induce phosphorylation of p38 and ERK1/2 at 30 minutes or earlier.

- LPS induces phosphorylation of IkB-α and NF-κB activation already at 30 minutes whereas β-Glucan induces them only at 4-6 hours.

- IkB-α phosphorylation and degradation by β-glucan but not by LPS is reversed by Interleukin-1RA.
The expression of “Late genes” in human DCs stimulated by β-Glucan is dependent on IL-1β and, to a lesser extent, IL-1α.

β-glucan but not LPS activates Caspase-1 in mono-DC

Caspase-1 inhibitors decrease IL-1β and also IL-23 and IL-10 secretion by β-glucan-activated mono-DC

Anti-IL-1β is more effective than anti-IL-1α to inhibit IL-23 production by β-glucan-activated mono-DC

Production of IL-1β and IL-1α by β-glucan-stimulated mono-DC

IL-23

Untreated β-Glucan
IL-1β has a central role in the Th17 response by being involved in an autocrine way to increase production of the Th-17 inducing cytokine IL-6 and IL-23 from DC and by directly acting on the CD4+ T cells in the induction of IL-17 production.
Role of IL-1α in skin carcinogenesis
MyD88 is required for DMBA-induced Mouse Skin Tumorigenesis

DMBA
1 application
25 µg in 200 µl acetone

TPA
biweekly application
200 µl of 10^{-4}M solution in acetone

% of mice with papillomas

weeks

% of mice with papillomas

weeks
Bone marrow chimera experiments indicate that optimal skin carcinogenesis requires MyD88 expression both in hematopoietic cells and in recipient cells.
v-ras\textsuperscript{Ha} transformed keratinocytes from wild type mice produce CXCR2 ligands and other cytokines.
Upregulation of CXCR2 ligands by oncogenic v-ras\textsuperscript{Ha} in primary keratinocytes requires EGFR expression

Real-time PCR analysis

- Mock transduced
- v-ras\textsuperscript{Ha} transduced

v-ras\textsuperscript{Ha} transformed keratinocytes from MyD88-/- mice are able to produce EGFR ligands

M: mock-transduced
R: v-ras\textsuperscript{Ha}-transduced
Ras target genes affected by MyD88 deficiency are NF-κB regulated

- **mcmv**: mock, control Ad; **mikb**: mock, IκBα SR Ad
- **rcmv**: v-ras, control Ad; **rikb**: v-ras, IκBα SR Ad

Ras induction of IL-1α is EGFR-dependent but only partially MyD88 and NFκB dependent
v-ras^Ha transformed keratinocytes from IL-1R-/− mice are defective in production of CXCR2 ligands

**CXCL1**

- **GM-CSF**

- **TNF**

- **MMP-9**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>v-ras^Ha</th>
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<tbody>
<tr>
<td>IL-1R +/+</td>
<td></td>
<td></td>
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<tr>
<td>IL-1R -/-</td>
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IL-1RA inhibits the production of pro-inflammatory chemokines and cytokines by v-ras<sup>Ha</sup> transformed keratinocytes.
Gene expression (microarray analysis) in v-ras\textsuperscript{ha} transduced mouse keratinocytes treated with IL-1RA

Fold gene expression IL-1RA-treated vs untreated

<table>
<thead>
<tr>
<th>Gene Expression</th>
<th>Control v-ras\textsuperscript{ha}</th>
<th>Anakinra v-ras\textsuperscript{Ha}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anakinra</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>v-ras\textsuperscript{Ha}</td>
<td>-</td>
<td>+</td>
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Ca++ (mM) 0.05 0.12

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<thead>
<tr>
<th>v-ras\textsuperscript{Ha}</th>
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<th>+</th>
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<tr>
<td>v-ras\textsuperscript{Ha}</td>
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<td>-</td>
<td>-</td>
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<td>+</td>
<td>+</td>
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K1

Actin

Ca++ (mM) 0.05 0.12

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<tr>
<th>v-ras\textsuperscript{Ha}</th>
<th>-</th>
<th>+</th>
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<td>+</td>
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</table>

K10

Actin (high)
Nude mouse grafting of v-ras$^{Ha}$ transduced keratinocytes

v-ras$^{Ha}$ transduced primary keratinocytes (4 x 10$^6$)

C57/B16

or

MyD88$^{ko}$

+ primary dermal fibroblasts (SENCAR) (6 x 10$^6$)

MyD88$^{+/+}$

MyD88$^{-/-}$

Tumor Volume (mm$^2$)

Days after Grafting

v-ras$^{Ha}$-MyD88$^{+/+}$

v-ras$^{Ha}$-MyD88$^{-/-}$
Nude mouse grafting of v-ras$^{Ha}$ transduced keratinocytes
EGFR signaling (Ras/PI3K/STAT pathway)

Pro-inflammatory factors (chemokines, cytokines, growth factors, metalloproteases)
Skin carcinogenesis (DMBA/TPA)

Cytokines
Chemokines
Metalloproteases

IL-1\(\alpha\)

NF-\(\kappa\)B

Human Dendritic Cells Activation by \(\beta\)-Glucan

IL-1\(\beta\)

IL-23
IL-6
TGF-\(\beta\)
(Cytokines
Chemokines,
Metalloproteases)
TNF -/- mice have more lymphocytic infiltration and more pronounced inflammation following 4 cycles of DSS
Cancer and Inflammation

Tumor Necrosis Factor can be an anti-tumor or a tumor promoting factor depending on the producer cell type and likely the location and time of production.

Targeting inflammation for preventing cancer initiation and progression.
William B. Coley (1862-1936)

Coley’s mixed toxins (mixed bacterial vaccine, MBV):
- Heat-killed Streptococcus pyogenes
- Bacillus prodigiosus (Serratia marcescens)


Innate (Natural) Resistance (Inflammation) ↔ Adaptive Immunity

- Natural resistance to tumors
- Non-specific inflammatory anti-tumor effects
- Pro-inflammatory cancer therapy (Coley Toxin, BCG, TLR ligands)
- Tumor-specific Immunity
- Immuno-surveillance
- Antigen-specific immunotherapy
Although the role of inflammation in favoring carcinogenesis has generated much interest in the last 10-15 years, the Greek physician Claudius Galenus already observed almost 2 thousand years ago some similarity among cancer and inflammation.

In 1863 Rudolf Virchow noted leucocytes in neoplastic tissues and made a connection between inflammation and cancer. He suggested that the "lymphoreticular infiltrate" reflected the origin of cancer at sites of chronic inflammation.

The recent upsurge of studies linking cancer and inflammation were inspired by the observation made two decades ago by Harold Dvorak, M.D., of Harvard University, who observed that inflammation and cancer share some basic developmental mechanisms (angiogenesis) and cells (lymphocytes, macrophages, and mast cells), and that tumors act like "wounds that do not heal."

The term cancer was originally applied by Galenus to certain tumors of the breast in which superficial veins appeared much swollen and radiated somewhat like the claws of a crab. Later the name was extended to include all malignant and infiltrating growths.
• Toll-like receptors were originally studied prevalently in hematopoietic cells (primarily dendritic cells, phagocytes, and B lymphocytes) but at least some members of this receptor family are widely expressed on other cell types including tumor cells.

• Although they have been described to recognize products of foreign organisms (pathogenic or not) they also participate in the regulation of inflammation by recognizing endogenous ligands (“alarmins”) that are present in inflamed tissues.

• The cellular response to TLR ligands is not only production of pro-inflammatory mediators but they are also involved in control of tissue homeostasis and regulate cellular differentiation, proliferation, and apoptosis. The balance between MyD88 and TRIF signaling and the production of type I IFN determine proliferation versus apoptosis in tissue and tumor cells and activation versus survival in dendritic cells.

• Epidemiologic/genetic evidence indicates a role of TLRs and signaling molecules (TLR1,2,6,10,3,4, MyD88, IRAK4) in the frequency and progression of human cancer.
Pattern Recognition Receptors (PRR)

C-type lectins, Scavenger r.

Toll-like receptors

RIG-I-like Receptors (helicases)

NOD-like Receptors:

NALPs, NODs

Toll-like receptors
Role of MyD88 in inflammation-dependent carcinogenesis: A tale of three Interleukins-1

Skin carcinogenesis (DM BA/TPA)

IL-1α

Cytokines
Chemokines
Metalloproteases

Colon carcinogenesis (AOM/DSS)

IL-18
(IL-1F4, IL-1γ)

Human Dendritic Cells
Activation by β-Glucan

IL-1β
IL-1α

Th17

IL-23
IL-6
TGF-β
(Cytokines
Chemokines, Metalloproteases)