PLASMA AND THE IMMUNE SYSTEM

Vandana Miller, MD
Immune System

- Host defense system
- Protects against disease

HEALTHY IMMUNE SYSTEM

- Distinguish self from non-self
  - Tolerate self
  - Reject non-self (infectious agents)
    - What about normal flora?
  - Reject altered self (diseased cells)
Disease is a rare phenomenon!

Maintains homeostasis!!!
Immune System

https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/347/immune-system
Cells of the Immune System

The Immune System Our Ultimate Line of Defence

Immune System

External (Skin, mucus membranes, nasal islet) Barriers

1st Line Defence

Phagocytes

Natural Killer Cells

2nd Line Defence

Innate Immunity

Adaptation Immunity

Infection Controlled

3rd Line Defence

Exposure to Microbe

First Line of Defence
Physical and Chemical Barriers (skin, mucus membranes, stomach acid)

EFFECTIVE

Second Line of Defence
Innate Immune Response (phagocytes, soluble immune mediators)

INEFFECTIVE

Third Line of Defence
Adaptive Immune Response (B cells, T cells)

INEFFECTIVE

Disease and Spread

https://www.stemcellimmuneregenerative.com/what-is-the-immune-system
Communication Between Immune Cells

- Direct cell-to-cell contact
- Secreted molecules
  - Cytokines
  - Chemokines
  - RONS
    - Byproducts of cell metabolism
    - Cause cell stress and death
    - Balanced by antioxidants to fine tune RONS concentrations in space and time
    - Low level RONS act as intracellular signaling molecules in steady state and during antigen presentation

**RONS of the Immune System**

**Cellular RONS**

- Oxygen radical
- Superoxide/hydroperoxyl radical
- Hydrogen peroxide
- Hydroxyl radical
- Singlet (delta) O$_2$
- Ozone
- Nitrogen radical
- Nitric oxide radical
- Nitrogen dioxide radical
- Nitrite/nitrous acid
- Nitrate/nitric acid
- Peroxynitrite/peroxynitrous acid
- Hypochlorite/hypochlorous acid
- Peroxynitrate/peroxynitric acid

**Plasma RONS**

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Carl Nathan and Aihao Ding
RONS-Mediated Signaling

- DC maturation
- Inflammatory cytokine secretion
- Cross-presentation
- DC migration
- T cell activation

ROS 
Hypoxia

• Oncotarget 10(8) DOI: 10.18632/oncotarget.26608
Cellular RONS as Effectors
## Measurable Immune Cell Functions

1. Recognition and phagocytosis of foreign targets
2. Pathogen killing
3. Cytokine/chemokine secretion
4. Migration
5. Antigen presentation to T and B cells
6. Recruitment of other immune cells
7. Cancer cell killing
Recognition and Phagocytosis of Foreign Targets

Phagocytosis of 15s NTP treated J-Lat cells (CTV/GFP+)

- Untreated (24h) 2.4%
- 15s (24h) 8.08%
Pathogen Killing – Plasma or Immune?

- NAC
- +NAC

Frequency

% MitoSOX Fluorescence

Mock Exposed 30Hz 45Hz 75Hz

Average Syncytia count (8 fields per well)

Mock Exposed 30 Hz 45 Hz 75 Hz

Frequency

HIV, Mock Exposed

HIV, 30 Hz

HIV, 45 Hz

HIV, 75 Hz
"The initial tests suggest that cold atmospheric plasma kills corona viruses in solution," says Jens Kirsch, CEO of Terraplasma medical.


Delivery?
In vivo toxicity of “inhaled” plasma species?

What about the immune system?
Pathogen Killing COVID-19

Predicted host immune responses to SARS-CoV-2

Upon entry into the alveolar epithelium, the virus is recognised by innate immune receptors - namely, the S protein, RIG-I, MDA-5, and the inflammasome sensor, NLRP3. This leads to the activation of NF-κB and IRF3/7 and the subsequent production of pro-inflammatory cytokines (e.g., IL-1β and IL-6) and type I IFNs, respectively. The antiviral activity of type I IFNs is essential in limiting the propagation of the virus and is further amplified by the expression of a plethora of ISGs such as RNase L. Cytokines released by infected cells modulate the adaptive immune response by recruiting and activating immune cells such as macrophages, B cells, and T cells to orchestrate the elimination of the virus. However, an unbalanced immune response can lead to hyper-inflammatory conditions causing some of the severe clinical symptoms of COVID-19.
Cytokine/Chemokine Secretion

Clinical Plasma Medicine, Volume 11, September 2018, Pages 1-9
Maturation/Migration of Monocytic Cells

Mitochondrial ROS enhance monocytic maturation and migration

\[ e \text{ mitochondrial oxidation} \]

\[ \text{THP monocytes} \]

\[ \text{I: control, II: plasma, III: PMA} \]


Untreated

141mJ: 15Hz

Untreated

Plasma Treated

APC (CD163)

doi/10.1002/ppap.201400168

Plasma Medicine: 10.1615/PlasmaMed.2017019883
Cancer Cell Killing

Indirect Effect of Plasma on Immune Cells

- More practical approach because immune cells are not easily accessible

- Evidence from studies in cancer treatment and wound healing that diseased cells can be triggered to signal to immune cells and recruit their help
Immunogenic Cell Death

Dying tumor cell

HSP70/90

ATP

P2RX7

TLR4

CRT

HMGB1

Antigen engulfment

CD91

Immature DC

Mature DC

Tumor antigen-MHC complex

CD80

CD86

HLA-DR

CD40

IL-12

NIR light

Near infrared phototheraputery (NIR-PIT)

Cell swelling and rupture

Receptor-expressing tumor cells

Immunogenic cell death (ICD)

Plasma

DOI: https://doi.org/10.1038/mt.2013.220

Oncotarget. 2017; 8:10425-10436
Indirect Effect of Plasma on Immune Cells

Induction of immunogenic cell death

Active Plasma Species?

Graphs showing the CRT intensity and concentration of 
active plasma species under different treatments.

References:

- Appl. Sci. 2020, 10(6), 2025; https://doi.org/10.3390/app10062025
Enhanced Antigen Presentation?

Oncotarget. 2017; 8:10425-10436

Plasma


DOI: https://doi.org/10.1038/mt.2013.220

Oxidative Medicine and Cellular Longevity https://doi.org/10.1155/2017/4396467
Indirect Effect of Plasma on Immune Cells

Development of protective adaptive responses

ONCOIMMUNOLOGY 2018, VOL. 7, NO. 9, e1484978


Indirect Effect of Plasma on Immune Cells

Development of memory

<table>
<thead>
<tr>
<th></th>
<th>Mice with tumors &lt; 850 mm³</th>
<th>Mice without tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>40%</td>
<td>0%</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>40%</td>
<td>10%</td>
</tr>
<tr>
<td>Plasma</td>
<td>90%</td>
<td>30%</td>
</tr>
</tbody>
</table>

Graph:
- Untreated
- Plasma

Days after injection

Tumor Volume (mm³)

ONCOIMMUNOLOGY 2018, VOL. 7, NO. 9, e1484978
H$_2$O$_2$ concentration at the wound margin, starting $\sim$3 min after wounding and peaking at $\sim$20 min and this gradient is required for rapid recruitment of leukocytes to the wound.

Immune Function in Wound Healing

A. Hemostasis

B. Inflammation

C. Proliferation

D. Remodeling
Plasma Triggers Immune Mediators of Wound Healing

Dec 2015 · Physics of Plasmas, DOI: 10.1063/1.4933403
## Measurable Immune Cell Functions

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<th>Status</th>
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<tbody>
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<td>✔️</td>
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</table>
Immune System – Information Management

Information

Sensing

Storage

Response

Interpretation

Adapted from: doi: 10.3389/fimmu.2016.00125, doi.org/10.1681/ASN.2007020151