2ème édition des journées scientifiques sur les maladies infectieuses dans le Sud-Ouest de l'Océan Indien.

PROGRAMME RUN-EMERGE

Mouse Macrophage Innate Immune Response to Chikungunya Virus Infection

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Chikungunya virus

- Member of Alphavirus
- Lipid enveloped studded with glycoprotein E1 and E2
- Positive sense single strand RNA genome
- Approximately 12 Kb

(Zhang, Mukhopadhyay *et al.*, 2002).

Non structural protein

Structural protein

(Strauss and Strauss, 2001).
CHIKVD and chronicity

Chikungunya

Post-Chikungunya Chronic conditions

Post-CHIKV encephalitis

Encephalitis

Post-CHIKV arthritis

Arthritis

Infection
Previous work on Human

• Previously it has been describe for RRV (alphavirus) that the virus hijack the soldier of the immune system i.e resident tissue macrophages (S. Mahalingam et al., 2007).

• It was true for the CHIKV, as persistent of the virus was seen in synovial tissue macrophages (J-J. Hoarau et al., 2010).

Synovial tissue section, (JJ. Hoarau et al., 2010)
Objective: Characterize macrophage response to CHIKV

- Cell death (Apoptosis ?)
- Innate immune response following contact with CHIKV ?
- Polarization (pro-inflammatory M1 type)
- Polarization (anti-inflammatory M2 type)
- RAW264.7 (mouse macrophage cell line)
- Viral replication ?
- Can be infected by CHIKV ?
Mouse macrophage 264.7 cells are infected by CHIKV (but % of infected cells is limited and in form of clusters)
Viral replication in RAW264.7
Production of positive and negative strand of CHIKV
Expression of structural and non structural protein in RAW264.7

600x magnification

400x magnification
Innate-Immune response against CHIKV

Virus

Cell

Nucleus

PRR

TLR, RIG-I

IFNAR

Type-I IFN

Inflammatory cytokines - TNF-\(\alpha\), IL-6, TGF-\(\beta\)….

ISG-56
IFNα4 and ISG-56 mRNA expression (RT-qPCR) after CHIKV infection

RAW264.7 mounts a rather poor anti-CHIKV Innate-Immune response in contrast to CLTT
At 24hr PI, RAW264.7 is showing a robust expression of TNF-α (associated with an elevated expression of GM-CSF and IL-6, TGF-β but no IL-1, IL-10 and CCL2).
Apoptosis

Extrinsic and Intrinsic signaling cascade for apoptosis

Cell death by Apoptosis

Death receptor

Extrinsic pathway

Caspase-8

Mitochondria

Intrinsic pathway

Caspase-9

Caspase-3

Cell

Nucleus

Extrinsic and Intrinsic signaling cascade for apoptosis
Infected RAW264.7 cells were negative for cleaved caspase 3 whereas all infected CLTT cells were positive for this apoptotic marker.
Intrinsic pathway is not engaged

48hr PI

RAW264.7

CLTT

400x magnification
Aptoptotic and innate immune response in RAW264.7 macrophage after SINV infection

48hr PI

RAW264.7

DAPI

ALPHAVIRUS

Cleaved caspase3

400x magnification

ISG-56

IFN alpha4

Fold change over control

Time

8hr 24hr
Conclusion:

- The virus could interfere with the innate immune response.

- Low infectivity of macrophage cells can also be a reason of poor innate immune response.

- Polarization towards pro-inflammatory cytokines (TNF-α, IL-6, TGF-β and GM-CSF) may contribute to arthritis.

Immune response in macrophage was different among the virus of same family.
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