GESINAS - ImmunoTools Award 2015

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Immunophenotyping of lymphocyte populations in samples from patients with dengue

1. INTRODUCTION

The Word Health Organization defines the dengue disease as the most prevalent arboviruses worldwide [1,2]. In Brazil, from 1990 to 2013 there were more than 8.5 million cases of dengue and of this, 200,000 cases of severe dengue and 8,000 deaths [3,4]. The precise pathophysiologic mechanism for dengue disease was not completely understood. Immunopathological aspects like immune cell response [5-7], can contribute to development of severe cases of dengue. Accordingly, the inflammatory process induced by the response of $CD8^+$ T cells plays a central role in the pathogenesis of dengue.

2. OBJETIVE

Characterize the population of $CD8^+$ T lymphocytes, present in peripheral blood samples from patients infected by DV residents in Cambé city, Paraná, Brazil.

To accomplish this, the following steps are performed:

- Obtain samples from patients with clinically diagnostic of dengue infection;
- Isolate dengue virus from the serum;
- Freeze peripheral blood mononuclear cells (PBMC) present in patients samples;
- Thawing PBMC samples, perform immunophenotyping;
- Treat the PBMC with polyclonal stimuli (PHA, or α-CD3/α-CD28);
- Obtain cultures enhanced for $CD8^+$ T cells cultures;
- Co-cultivate infect cells lineages with DV cells with $CD8^+$ T cells;
- Compare the different response obtained in immunophenotyping of co-cultures and clinical condition of patients;

3. METODOLOGY

3.1 – Samples of PBMC from DV infected patients;

Primary cells will be collected from patients presenting clinical diagnostically of infection by DV by venipuncture surface (CEP - 514/09 / Fiocruz). PBMC from whole blood will be obtained by density separation technique. This cell suspension will by freeze slowly (-1°C/min) and storage in liquid nitrogen until use.

3.2 – Isolation of DV strains;

The samples of serum will by obtained with centrifugation at 3000 x g by 10 min of whole blood collected from patients presenting clinical diagnostically of infection by DV. This serum will be diluted in L15 C6/36 (*Aedes albopictus* mosquito cells) medium, 1:100 v/v, and will be used to inoculate a C6/36 culture for 30 min at 27°C. The virus title will be determined in supernatant of cultures, to confirm diagnostics and will be used to future experiments.

3.3 – Immunophenotype and proliferation of thawing PBMC;

To determine the immunophenotype of PBMC from DV infected patients, cells suspension will by thawed at 37°C in water bath. These cells are divided into two, the first will be used to immunophenotyping determination, as follows: CD3-FITC / CD4-PE (Multicolour combinations anti-human); or CD3-FITC / CD8-PE / CD45 PerCP (Multicolour combinations anti-human) plus CD62L-APC or CD69-APC (Singlecolor anti-human); or CD3-FITC plus CD19-PE (Singlecolor anti-human); or CD3-FITC plus IFN-gamma-PE plus CD8-PercP plus CD62L-APC (These cells are previously permeabilized for IFN-gamma labeling) (Singlecolor anti-human). (ImmunoTools). Control cells will be marked with the respective isotype controls (ImmunoTools).

The second portion of the cell suspension is used for cell proliferation assays. For this, cell suspension will be transferred to flat bottom plates of 96 wells previously treated or untreated with anti-CD3/anti-CD28 (ImmunoTools). After incubation, immunophenotyping as described in the previous paragraph will be performed.

3.4 – Co-culture of DV-infected lineages cells with $CD8^+$ T cells;

Pre-stimulated cells (PHA or anti-DC3/anti-CD28), will be separated by a cell-sorting technique. Positive populations for labeling with anti-CD3-FITC/anti-CD8-PE (ImmunoTools, multicolour anti-human combinations) plus anti-CD62L (positive and negative) will be isolated by cell-sorting. These $CD8^+$ T cells isolated will be co-cultivated with Huh7.5 (human hepatoma) cells lineages, previously infected with DV. After 24 hours of co-culture, the cells will be immunolabeled, as described above, for

DV presence (mAb anti-envelope protein of *Flavivirus* group), plus CD3-FITC plus IFN-gamma-PE plus CD8-PercP. Additionally, the presence of cytokines in the supernatant of cultures will be evaluated. Using the ELISA set for human IFN-gamma and TNF-alpha (ImmunoTools).

4. EXPECTED RESULTS

Defining the $CD8^+$ T cells population, as well as their activation profile, may be accessed to the initial conditions of the host response to infection. Accessing the $CD8^+$ T cells activation during infection and observing the general condition of the response, will be possible to delimit a basal host immune cell response against DV infection.

5. REFERENCES

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For the GESINAS award:

The initiative to contribute to institutions that work on assistance to people in social vulnerability came from personal experience working for seventeen years in public health in Brazil.

During this period, I witnessed situations where people are affected by a variety of illnesses: accident, infection disease, violent situations that increased social vulnerability, lead to extreme suffering condition.

Touched with this scenario, I decided to contribute financially to institutions aimed at alleviating the suffering of these people, including health care, disability and domestic violence.

At present, I make monthly financial donations to the three institutions.

CASPC - House of Social Support for People with Cancer.

The CASPC is a non-profit organization that operates in sponsorship and administration of funds from donations only. The objective of the institution is to contribute to a life with more dignity to families and people with cancer. The work is aimed at people in social vulnerability, focusing on the material support (medication,

hygiene equipment) and providing professional support: psychological, social work and physiotherapy.

AAFAE - Association for the Support of Special Families
The AAFAE is a nonprofit institution that acts managing and providing material support to the families of people with special requirements. The institution focuses on the collection of food supplies, hygiene products, toys to distribute to families.

Foundation Initiative
The Foundation Initiative is a charitable organization dedicated to the care and support of children and adolescents in personal and social risk situation (abandonment and violence). It has four " casas lares " with social mothers.

GESINAS - ImmunoTools AWARD for

Guilherme Ferreira Silveira includes 50 reagents

FITC - conjugated anti-human CD3, CD4, CD8, CD11b, CD11c, CD14, CD19, CD45, CD45RA, CD62L, CD69, Control-IgG1

PE - conjugated anti-human CD3, CD4, CD8, CD19, CD62L, CD69, IFN-gamma, Control-IgG2a

PerCP - conjugated anti-human CD3, CD4, CD8, CD45RA, Control-IgG1

APC - conjugated anti-human CD3, CD4, CD19, CD69, Control-IgG2b, Annexin V,

CD3 FITC / CD4 PE

CD3 FITC / CD8 PE

CD4 FITC / CD3 PE / CD8 PerCP

CD3 FITC / CD8 PE / CD45 PerCP

CD3 FITC / CD4 PE / CD19 APC

human ELISA-set for 96 wells, human IFN-gamma, human TNF-a (each 3 reagents),

recombinant human cytokines: rh IL-2, rh IL-4, rh GM-CSF

soluble human receptors: rh sCD40L / CD154, rh CTLA-4 / CD152, rh FAS-Ligand / CD178, rh IL-6rec, rh sTNFrec / CD120b DETAILS more AWARDS