Human peripheral blood mononuclear cells (PBMC) as experimental model for basic and traslation research in the immunology of infectious diseases.

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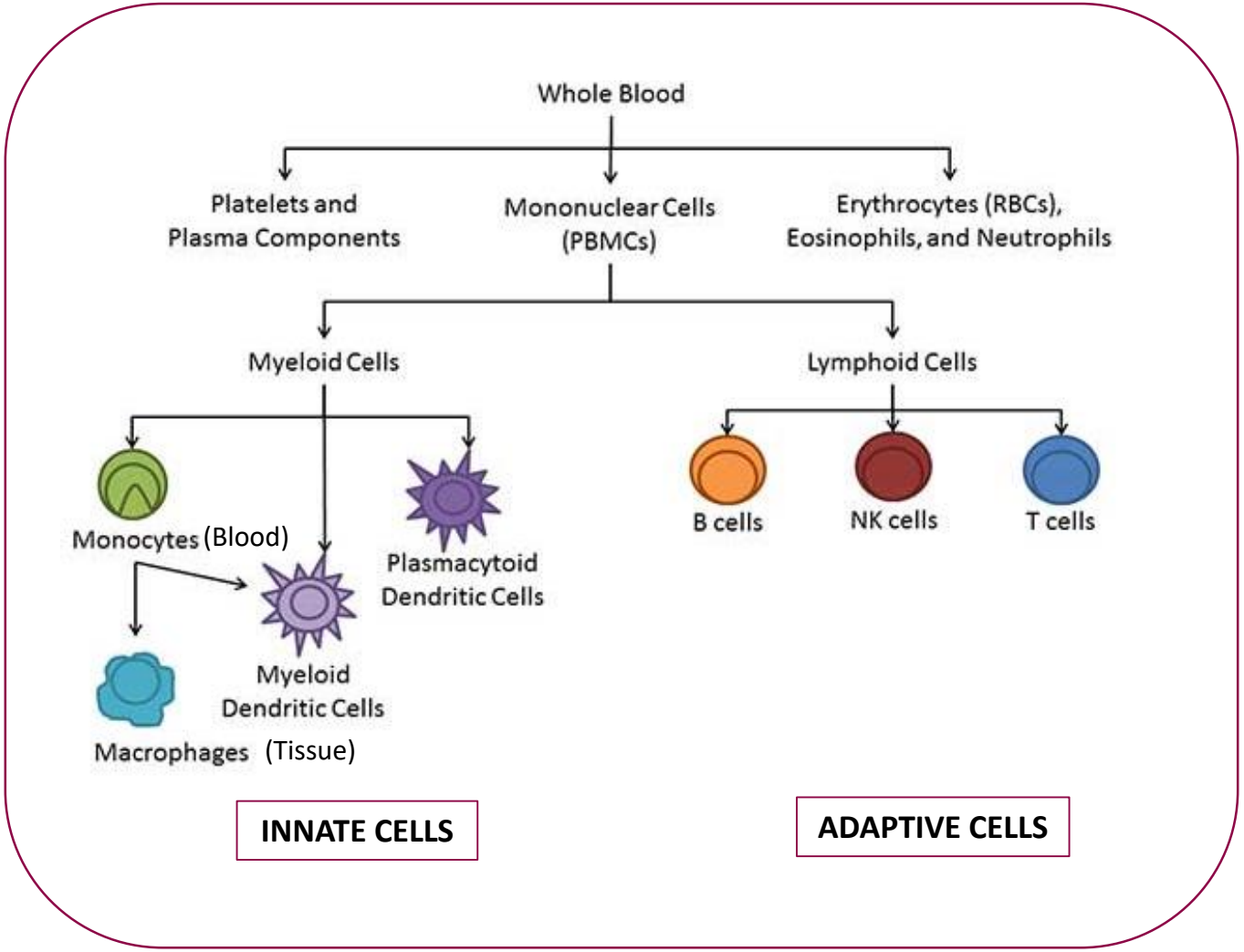
«Presentazione opportunità tesi», 9 Marzo 2023

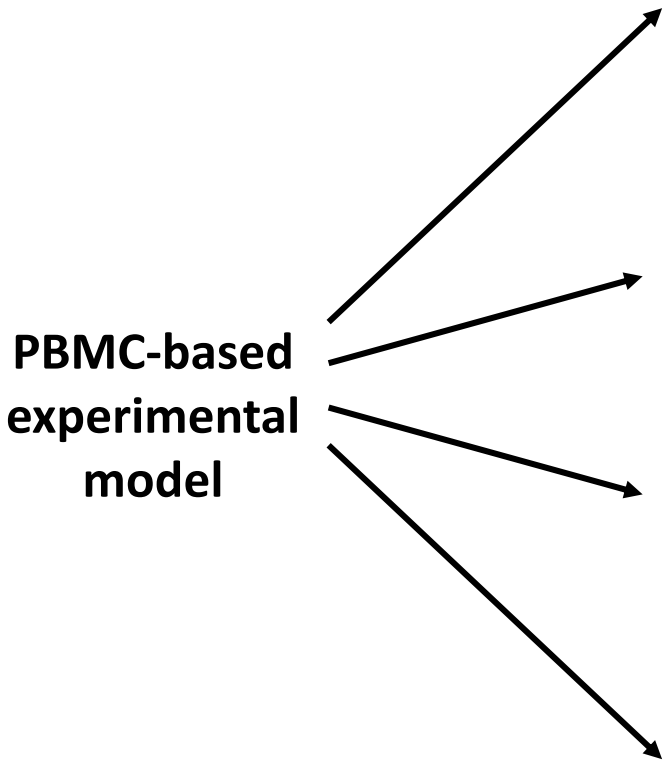
PBMC-based experimental model: one model, many cell types, multiple approaches



A peripheral blood mononuclear cell (PBMC) is any peripheral blood cell having only 1 round nucleus.

httpss/hematopoietic-knowledge-center/pbmcs.aspx
http://varuncnmicro.blogspot.com/2015/11/laboratory-series-8-pbmc-isolation.html

Application of the PBMC-based experimental model: from basic research to translational science



1. Analysis of host/pathogen interaction to study immunity to infections and vaccination (in vitro and ex vivo studies)

2. Analysis of immunopathogenesis of infectious diseases (in vitro and ex vivo studies)

3. Evaluation of altered immune responses in different pathologies, including autoimmune disease (i.e. Multiple Sclerosis) (in vitro and ex vivo studies)

4. Development of cell-based platforms for testing vaccine potency and for drug discovery (in vitro studies)

RELATED PUBLICATIONS

1. ANALYSIS OF HOST/PATHOGEN INTERACTION TO STUDY IMMUNITY TO INFECTIONS AND VACCINATION

2. ANALYSIS OF IMMUNOPATHOGENESIS OF INFECTIOUS DISEASES

> PLoS Pathog. 2021 Apr 15;17(4):e1009505. doi: 10.1371/journal.ppat.1009505. eCollection 2021 Apr.

Human plasmacytoid dendritic cells at the crossroad of type I interferon-regulated B cell differentiation and antiviral response to tick-borne encephalitis virus

Marilena P Etna [1], Aurora Signorazzi [2], Daniela Ricci [1], Martina Severa [1], Fabiana Rizzo [1], Elena Giacomini [1], Andrea Gaggioli [3], Isabelle Bekeredjian-Ding [4], Anke Huckriede [2], Eliana M Coccia [1]

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Differential plasmacytoid dendritic cell phenotype and type I Interferon response in asymptomatic and severe COVID-19 infection

Martina Severa [1], Roberta A Diotti [2], Marilena P Etna [1], Fabiana Rizzo [1], Stefano Fiore [1], Daniela Ricci [1], Marco Iannetta [3], Alessandro Sinigaglia [4], Alessandra Lodi [3], Nicasio Mancini [2], Elena Criscuolo [2], Massimo Clementi [2], Massimo Andreoni [3], Stefano Balducci [5], Luisa Barzon [4], Paola Stefanelli [1], Nicola Clementi [2], Eliana M Coccia [1]

accepted to Clinical & Translational Immunology

A specific anti-COVID-19 BNT162b2 vaccine-induced early innate immune signature positively correlates with the humoral protective response in healthy and multiple sclerosis vaccine recipients

Severa Martina[1], Rizzo Fabiana[1*], Sinigaglia Alessandro[2*], Ricci Daniela[1], Etna Marilena Paola[1], Cola Gaia[3], Landi Doriana[3], Buscarinu Maria Chiara[4], Valdarchi Catia[1], Ristori Giovanni[4, 5], Riccetti Silvia[2], Piubelli Chiara[6], Palmerini Pierangela[7], Rosato Antonio[7, 8], Gobbi Federico[6], Balducci Stefano[9], Marfia Girolama Alessandra[3], Salvetti Marco[4, 10], Barzon Luisa[2#], Coccia Eliana Marina[1#§]

accepted to International Immunopharmacology

Novel evidence of Thymosin α1 immunomodulatory properties in SARS-CoV-2 infection: effect on innate inflammatory response in a peripheral blood mononuclear cell-based *in vitro* model

Daniela Ricci[a, b#], Marilena Paola Etna[a#], Martina Severa[a#], Stefano Fiore[a], Fabiana Rizzo[a], Marco Iannetta[c], Massimo Andreoni[c], Stefano Balducci[d], Paola Stefanelli[a], Anna Teresa Palamara[a] and Eliana Marina Coccia[a*]

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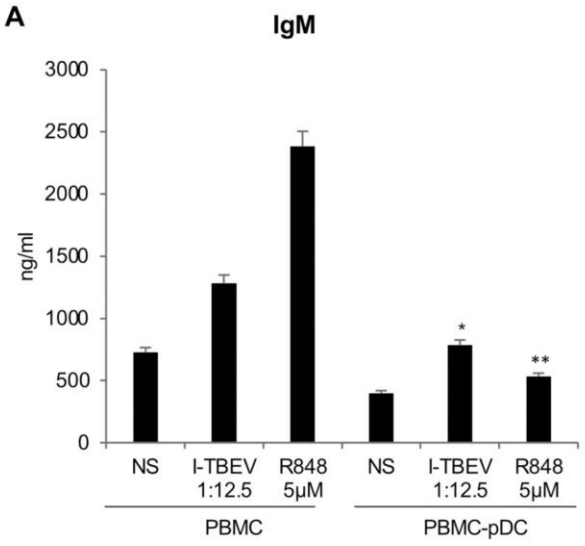
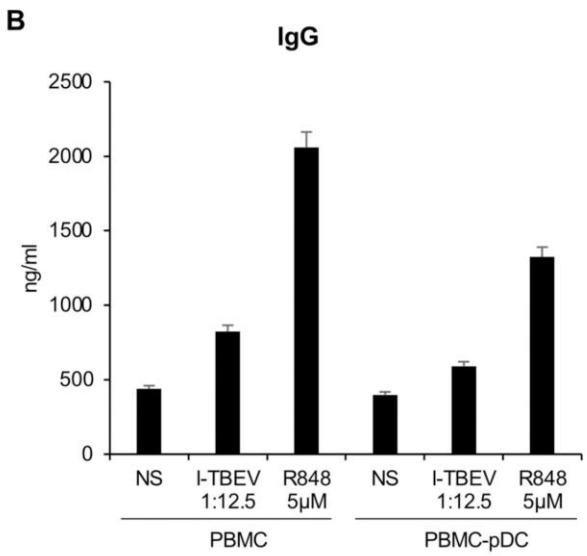
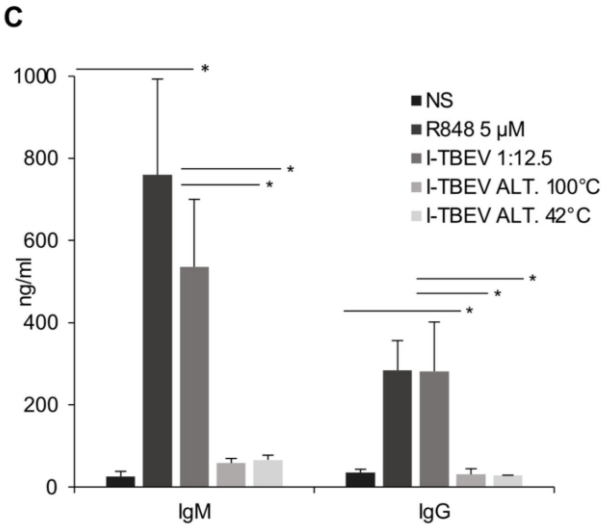
- I-TBEV generates differentiation of a sub-population of plasmacytoid dendritic cells (pDC) that is specialized in type I interferon (IFN) production
- I-TBEV-induced type I IFN together with Interleukin 6 and BAFF to be critical for B cell differentiation to plasmablasts as measured by immunophenotyping and immunoglobulin production.







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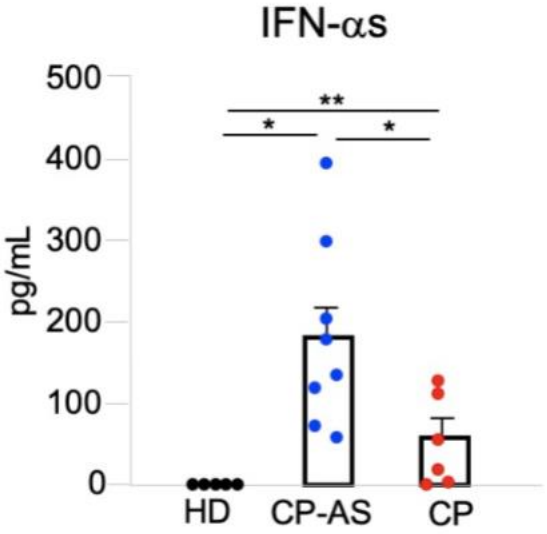
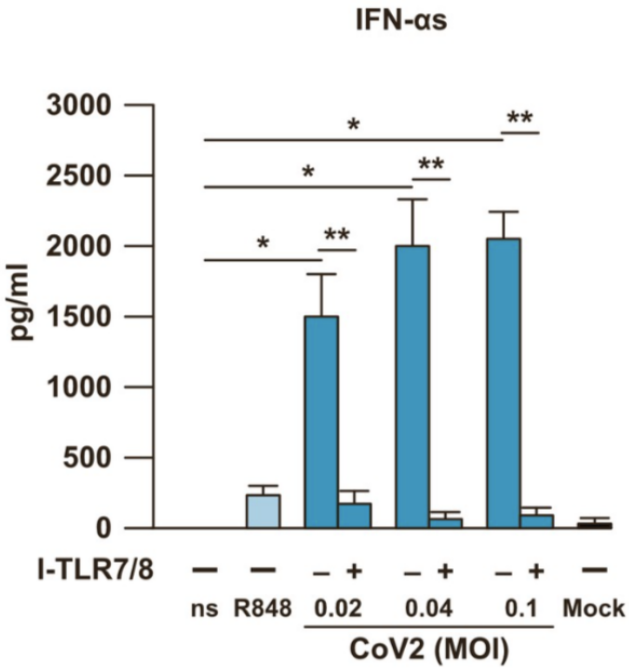
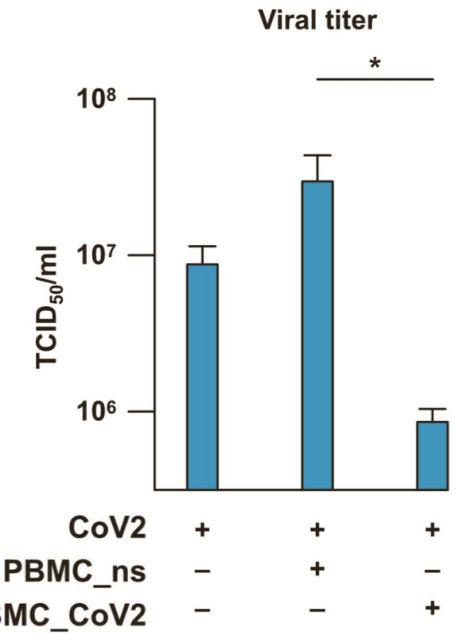
- Even in absence of a productive viral replication, the virus mediates a robust production of IFNs and inflammatory cytokines and che-mokines, known to contribute to the cytokine storm observed in COVID-19.
- Virus-induced type I IFN secreted by PBMC enhances anti-viral response in infected lung epithelial cells, thus, inhibiting viral replication.
- Coherently to what observed in vitro, asymptomatic SARS-CoV-2 infected subjects displayed a similar pDC phenotype associated to a very high serum type I IFN level and induction of anti-viral IFN-stimulated genes in PBMC.







RELATED PUBLICATIONS

3. EVALUATION OF ALTERED IMMUNE RESPONSES IN DIFFERENT PATHOLOGIES, INCLUDING AUTOIMMUNE DISEASE (I.E. MULTIPLE SCLEROSIS)

› Immunol Cell Biol. 2016 Oct;94(9):886-894. doi: 10.1038/icb.2016.55. Epub 2016 Jun 6.

Interferon-β therapy specifically reduces pathogenic memory B cells in multiple sclerosis patients by inducing a FAS-mediated apoptosis

Fabiana Rizzo [1], Elena Giacomini [1], Rosella Mechelli [2], Maria Chiara Buscarinu [2], Marco Salvetti [2], Martina Severa [1], Eliana Marina Coccia [1]

› Mult Scler. 2018 Feb;24(2):127-139. doi: 10.1177/1352458517695892. Epub 2017 Feb 1.

Thymosin-α1 expands deficient IL-10-producing regulatory B cell subsets in relapsing-remitting multiple sclerosis patients

Elena Giacomini [1], Fabiana Rizzo [1], Marilena P Etna [1], Melania Cruciani [1], Rosella Mechelli [2], Maria Chiara Buscarinu [2], Francesca Pica [3], Cartesio D'Agostini [4], Marco Salvetti [2], Eliana M Coccia [1], Martina Severa [1]

› J Autoimmun. 2019 Jul;101:1-16. doi: 10.1016/j.jaut.2019.04.006. Epub 2019 Apr 30.

A cell type-specific transcriptomic approach to map B cell and monocyte type I interferon-linked pathogenic signatures in Multiple Sclerosis

Martina Severa [1], Fabiana Rizzo [2], Sundararajan Srinivasan [3], Marco Di Dario [3], Elena Giacomini [2], Maria Chiara Buscarinu [4], Melania Cruciani [2], Marilena P Etna [2], Silvia Sandini [2], Rosella Mechelli [5], Antonella Farina [6], Pankaj Trivedi [6], Paul J Hertzog [7], Marco Salvetti [8], Cinthia Farina [3], Eliana M Coccia [9]

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Thymosin-α1 expands deficient IL-10-producing regulatory B cell subsets in relapsing-remitting multiple sclerosis patients

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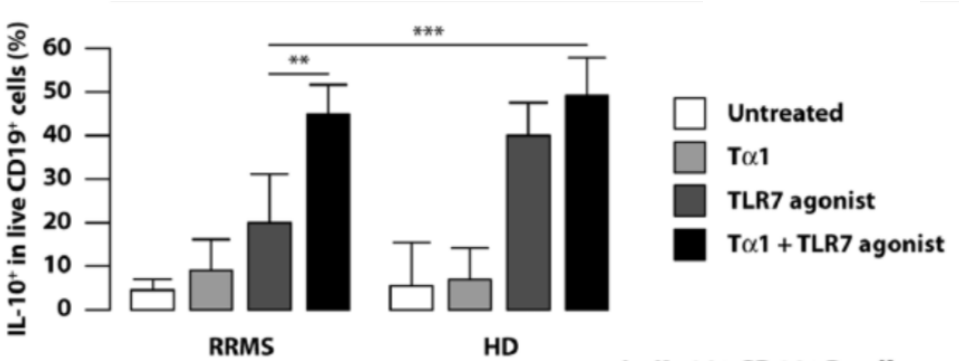
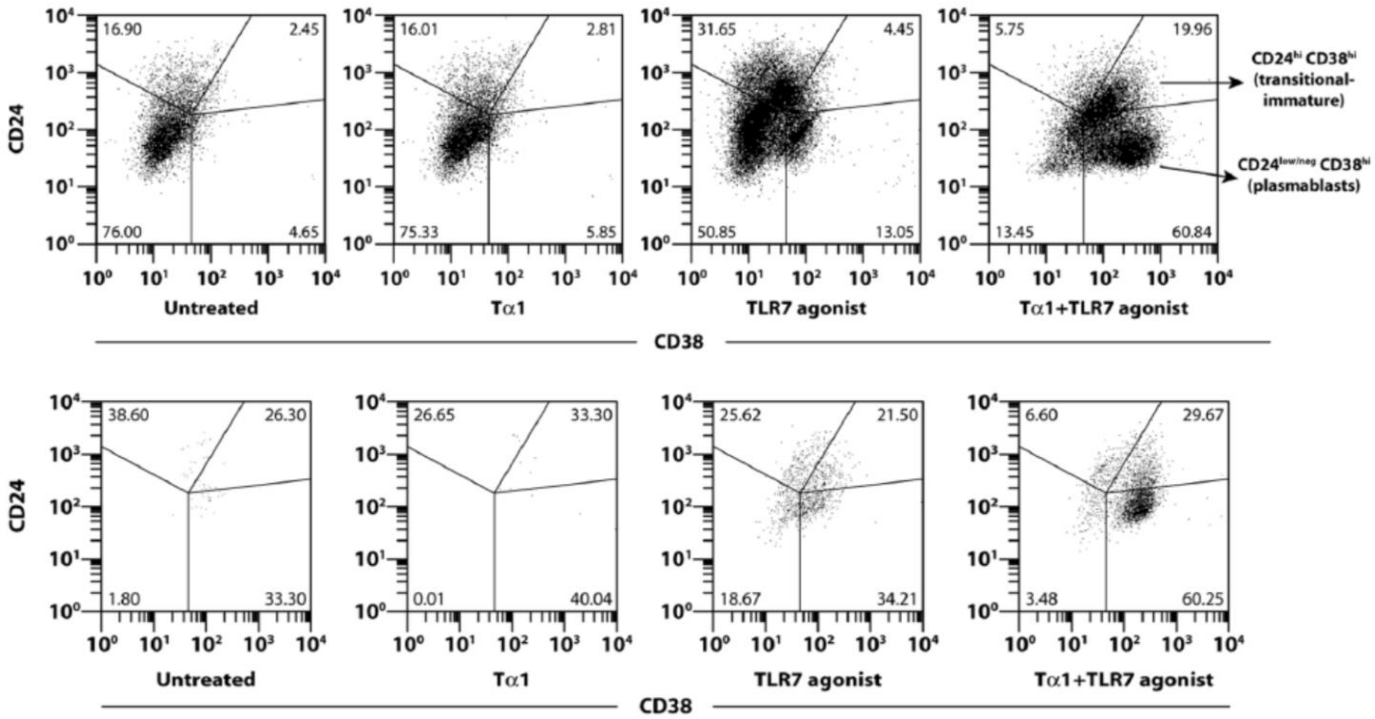


Thymosin α1 (**Tα1**) is a peptide naturally present in the thymus, that can modulate the immune system depending on the host immune status.
In particular, in vitro treatment of B cells from MS patients with Tα1 promotes an anti-inflammatory phenotype, thus contributing to the development of IL-10 producing B regulatory cells.



RELATED PUBLICATIONS

4. DEVELOPMENT OF CELL-BASED PLATFORMS FOR TESTING VACCINE POTENCY AND FOR DRUG DISCOVERY

> ALTEX. 2020;37(4):532-544. doi: 10.14573/altex.2002252. Epub 2020 May 26.

Optimization of the monocyte activation test for evaluating pyrogenicity of tick-borne encephalitis virus vaccine

Marilena P Etna [1], Elena Giacomini [1], Fabiana Rizzo [1], Martina Severa [1], Daniela Ricci [1], Shahjahan Shaid [2], Denis Lambrigts [2], Sara Valentini [3], Luisa Galli Stampino [3], Liliana Alleri [3], Andrea Gaggioli [4], Christina Von Hunolstein [4], Ingo Spreitzer [5], Eliana M Coccia [1]

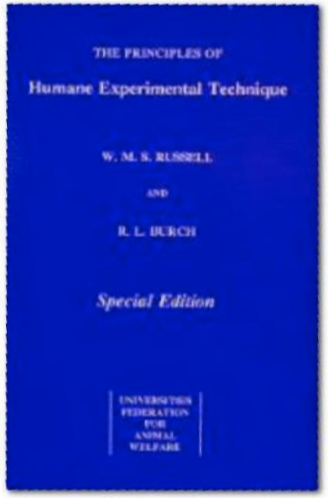
> ALTEX. 2021;38(3):431-441. doi: 10.14573/altex.2010081. Epub 2021 Jan 13.

In vitro assessment of tick-borne encephalitis vaccine: Suitable human cell platforms and potential biomarkers

Aurora Signorazzi [1], Marilena P Etna [2], Eliana M Coccia [2], Anke Huckriede [1]

3Rs PRINCIPLE

The Principles of Human Experimental Technique



Russell & Burch, 1959

	Classical	Contemporary
REPLACE	Non-animal methods	Accelerating development and use of human-relevant tools (based on latest technologies)
REDUCE	Minimum number of animals to obtain scientifically consistent information	Appropriately designed animal experiments that are robust & reproducible
REFINE	Decrease of pain, severity and distress in those animals which still have to be used	New in vivo technologies that can benefit animal welfare

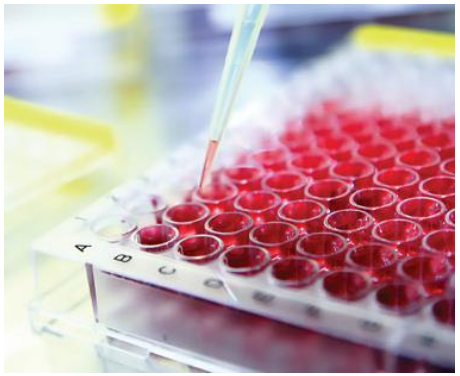
WHY TO CHANGE

Animal welfare	• Large % of animals used in QC exposed to severe pain and distress • Animals are sentient beings • Societal concerns using animals
Science	• In vivo models act as black box • Relevance to human sometimes questionable • High variability, poor robustness
Economic	• *In vivo* test are expensive • Long cycle times • Variability can lead to rejection of safe and efficacious vaccines, delays to market release and vaccine shortage
Legal basis in Europe	• Directive 2010/63/EU on the protection of animals used for scientific purposes: “Member States should ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used”

TO ADHERE TO 3Rs PRINCIPLES...

RPT- Rabbit pyrogen test
(Qualitative measurement of pyrogens)

MAT - Monocyte activation test
(Semi-quantitative/quantitative measurement of pyrogens)



EUROPEAN PHARMACOPOEIA 11.0

07/2017:20630
corrected 11.0

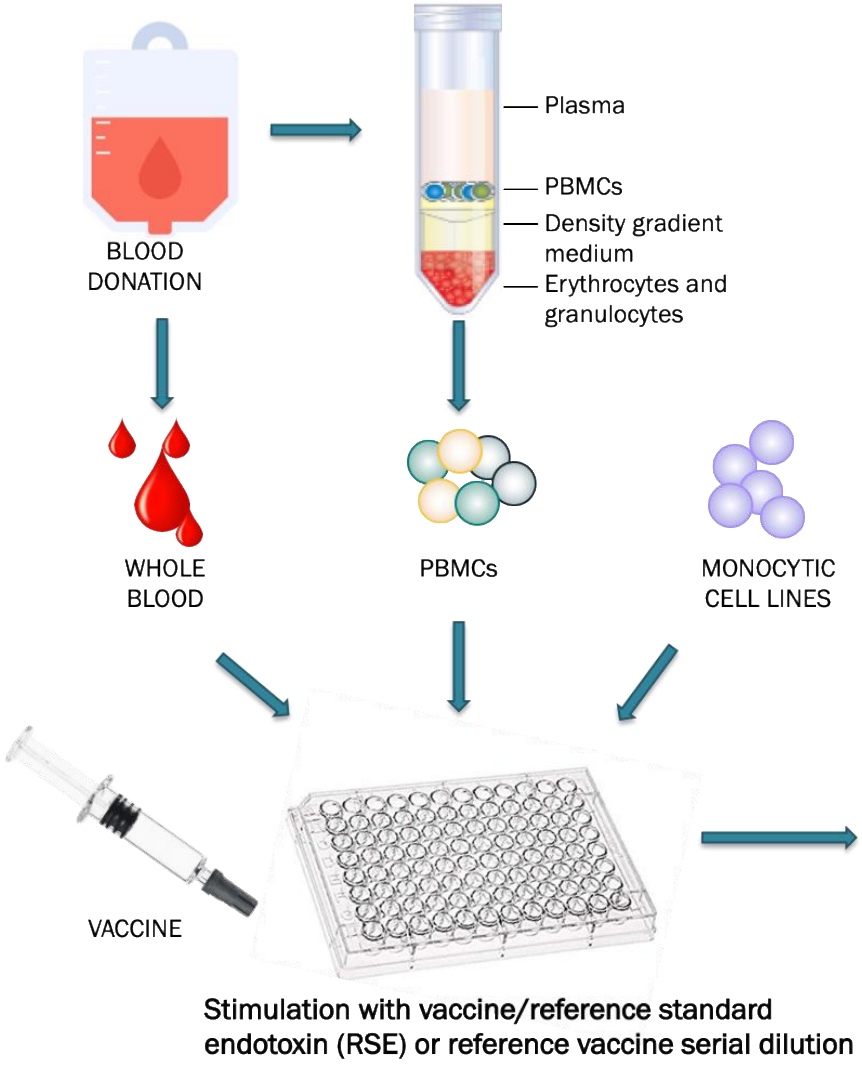
2.6.30. MONOCYTE-ACTIVATION TEST

1. INTRODUCTION

The monocyte-activation test (MAT) is used to detect or quantify substances that activate human monocytes or monocytic cells to release endogenous mediators such as pro-inflammatory cytokines, for example tumour necrosis factor alpha (TNFα), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6). These cytokines have a role in fever pathogenesis. Consequently, the MAT will detect the presence of pyrogens in the test sample. The MAT is suitable, after a product-specific validation, as a replacement for the rabbit pyrogen test.

MONOCYTE ACTIVATION TEST

-Workflow-



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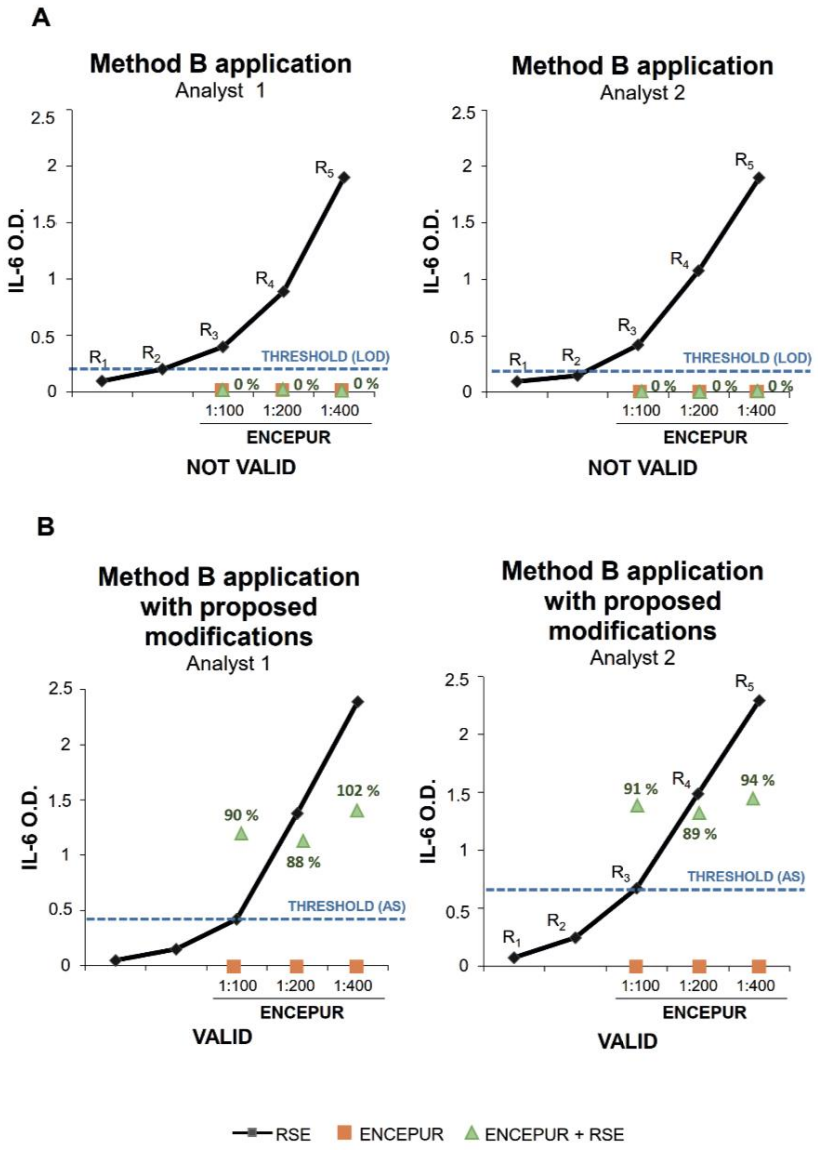

- Replacement of RPT with MAT for assessing the pyrogenicity of TBEV vaccine batches ➔ *Implementation of MAT for routine testing*

- Contribution to the definition of critical parameters of the assay for its application to not-intrinsically pyrogenic vaccines ➔ *New guidelines for MAT application to vaccine testing are under evaluation by Pharmacopoeia Experts*

FUTURE OBJECTIVE:

Human primary cell-based settings for the identification of biomarkers of innate immunity predictive of vaccine potency (since PBMC posses a wide repertoire of receptors for studying host-pathogen interaction)

Three-Dimensional cell culture systems/organoids as reliable models for studying mechanism of pathogenicity for those microbes for which animal model of infection does not exactly reproduce what happen in humans (i.e.: mouse model for Tuberculosis, COVID-19…)

Assay Validation: fulfillment of statistical and regulatory requirements

Techniques:

PBMC isolation
Purification of leukocyte populations
Cytofluorimetric analysis
Cytokine determination
RNA and protein extraction
Real Time PCR
Western blotting