Murine embryonic stem cells as a route towards exploring host-pathogen interactions

By

Raffaella Rossi

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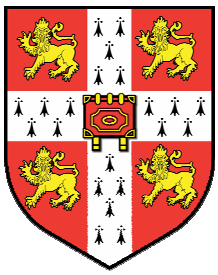

Clare Hall College
Cambridge University

Sanger Institute

Abstract

In the last 20 years embryonic stem (ES) cells have emerged as a new hope for future therapeutic solutions for chronic human diseases such as Alzheimer's, Parkinson's, cancer and cardiovascular disease. They may also pave the way for pharmaceutical drug discovery, minimizing the reliance on animal models. In this study the potential of murine embryonic stem cell technology to probe host-pathogen interactions and infectious diseases is investigated, focusing on the pathogens *Shigella* and *Salmonella* that are able to establish complicated lifestyles within mammalian cells. Murine ES cells were infected independently with *Shigella flexneri* and *Salmonella* Typhimurium and characterized by flow cytometry, microbiology and microscopic observations. It was observed that these pathogens could enter and survive within ES cells in a manner that resembled their interactions with terminally differentiated cells growing in culture. Consequently there is evidence that substantiates the proposal that mouse ES cells could be comparably infected by these bacteria since they also occupied similar intracellular niches. Once the infection protocol was established, the mRNA expression profile of ES cells during infection was investigated, using Affymetrix mouse arrays. The data produced was analyzed by three separate programmes: Bioconductor, GeneSpring and ASCA and they identified a weak immune response. This highlighted the fact that ES cells maintain strict controls on gene expression and that bacterial infection does not induce a characteristic immune response as in specialized cells. Consequently, the potential of ES cells to be differentiated into specific antigen presenting cells like dendritic cell was explored. After differentiation of the ES cells into dendritic cells and their characterization by flow cytometry, the resulting cells were infected with *S.* Typhimurium and purified by FACs sorting in order to perform mRNA expression profiling utilizing Illumina arrays. The expression pattern of surface antigenic markers and the ability of these cells to present antigen and secrete cytokines was also analyzed. The microarray data analysis, cell marker studies and cytokine production profiles emphasized a clear immunological response to bacterial infection in the murine ES derived dendritic cells (esDC). This is the first study of this type performed and further analysis may help to understand both the cell differentiation process and ES cell behavior during infection. In addition, this study revealed the flexibility and the applicability of ES cells as a new *in vitro* model for infectiouse disease research.

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I would like to dedicate this thesis to my family.

Declaration

I declare that this dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration, except where specifically indicated in the text.

The gentamicin assays employing wild type *Shigella* were performed in collaboration with Dr. Jun Yu. Guidance with the bone marrow extraction and bone marrow dendritic cells differentiation was provided by Dr. Petrovska. The microarray analyses using Bioconductor were performed by Dr. Lefebvre, Dr. Andrews from the Wellcome Trust Sanger Institute, and the analysis using ASCA was performed by Dr. Conesa from the Centro de Investigación Príncipe Felipe. Synthesis of cDNA, cRNA and hybridizations on Illumina arrays was performed by Peter Ellis from the microarray facility at Wellcome Trust Sanger Institute. Electron microscopy pictures were taken by David Goulding the microscope officer at the Wellcome Trust Sanger Institute.

All the other techniques including tissue cultures, FACS analyses, confocal characterization, Affymetrix hybridization, GeneSpring analysis, RT-PCR, statistical analysis and optimization of invasion protocols were performed by the author.

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Paper

Interaction of Enteric bacteria Pathogens with Murine Embryonic Stem Cells

Jun Yu*ξ, **Raffaella Rossi***, Christine Hale, David Goulding and Gordon Dougan.

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Abbreviations

Ab : Antibody

Ag : Antigen

APC: Antigen Presentation Cell

Amp: Ampicillin

BM: Bone Marrow

BMDC: Bone Marrow derived Dendritic Cells

BSA : Bovine Serum Albumin

CBA: Cytometric Bead Array

CD: Cluster of Differentiation

ConA: Concanavalin A

Ct: Treshold Cycle

DC: Dendritic Cell

DMEM: Dulbecco's Modified Eagle's Medium

ECACC: European Collection of Cell Cultures

ES: Embryonic Stem

esDC: Embryonic stem cells derived Dendritic cell

FCS : Fetal Calf/Bovine Serum

FITC: Fluorescein isothiocyanate

GFP: Green Fluorescent Protein

GM-CSF : Granulocyte/Macrophage-Colony Stimulating Factor

HI-FBS: Heat Inactivated Foetal Bovine Serum

IL: Interleukin

IMDM: Iscove's modified Dulbecco's Medium

M&M: Materials and Methods

LB: Luria Bertani

LIF : Leukemia Inhibitory Factor

LPS: Lipopolysaccharide

MHC: Major Histocompatibility Complex

MOI: Multiplicity of Infection

OD: Optical Density

OVA : Ovalbumin

PAMP: Pathogen-Associated Molecular Pattern

PRR: Pattern Recognition Receptor

PBS: Phosphate Buffered Saline

PE: Phycoerythrin

QC: Quality Control

RT: Room Temperature

RT-PCR: Real Time Polymerase Chain Reaction

S. Typhimurium: *Salmonella enterica* serovar Typhimuirum

SPI: Salmonella Pathogenic Island

TIIISS: Type Three Secretion System

TLR: Toll Like Receptor

TNFα: Tumor Necrosis Factor alpha

WTSI : Wellcome Trust Sanger Institute

WHOSU: World Health Organization Standard Units