DISCOVER

"DNA Damage and Immune System COoperation in VEry low Radiation environment"

Responsabile Nazionale: Antonella Sgura

(Università di Roma 3 – INFN-RM 3)

Durata del progetto: 2 anni (2021 – 2022)

La collaborazione

Università degli Studi Roma 3

INFN Roma 3

Istituto Superiore di Sanità

INFN Roma 1

INFN-LNGS

The background

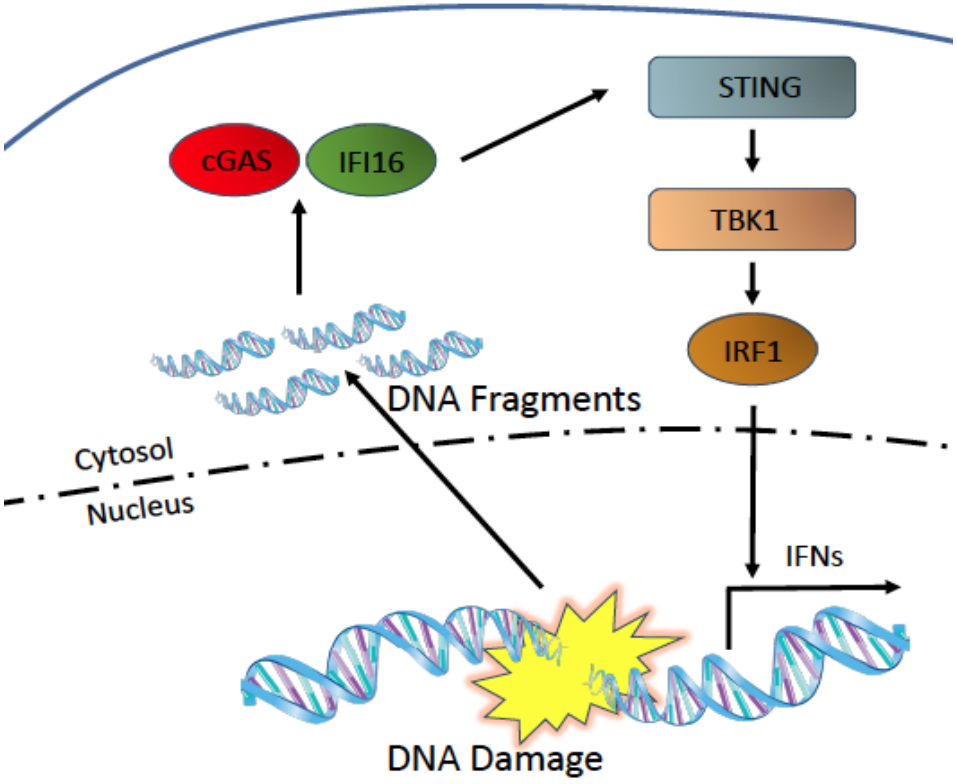
Low-dose radiation (LDR) modulate a variety of immune response processes.

Among biological effects at low doses of radiations, mechanisms that share common mediators with various immunological signaling processes are showed. Investigation in the interactions between ionizing radiation and immune system is leading to the birth of a new interdisciplinary field (i.e. radioimmunobiology)

Investigation of the **immune system response to low radiation doses/dose rates** is also identified as **a priority** by the Multidisciplinary European Low Dose Initiative **(MELODI) European Strategic Research Agenda** (SRA) (http://www.melodi-online.eu/sra. html).

The basic cellular and molecular mechanisms underlying the radiation-induced immune response are still largely unknown and research on this topic has become a hot topic.

low doses radiation → Immune response

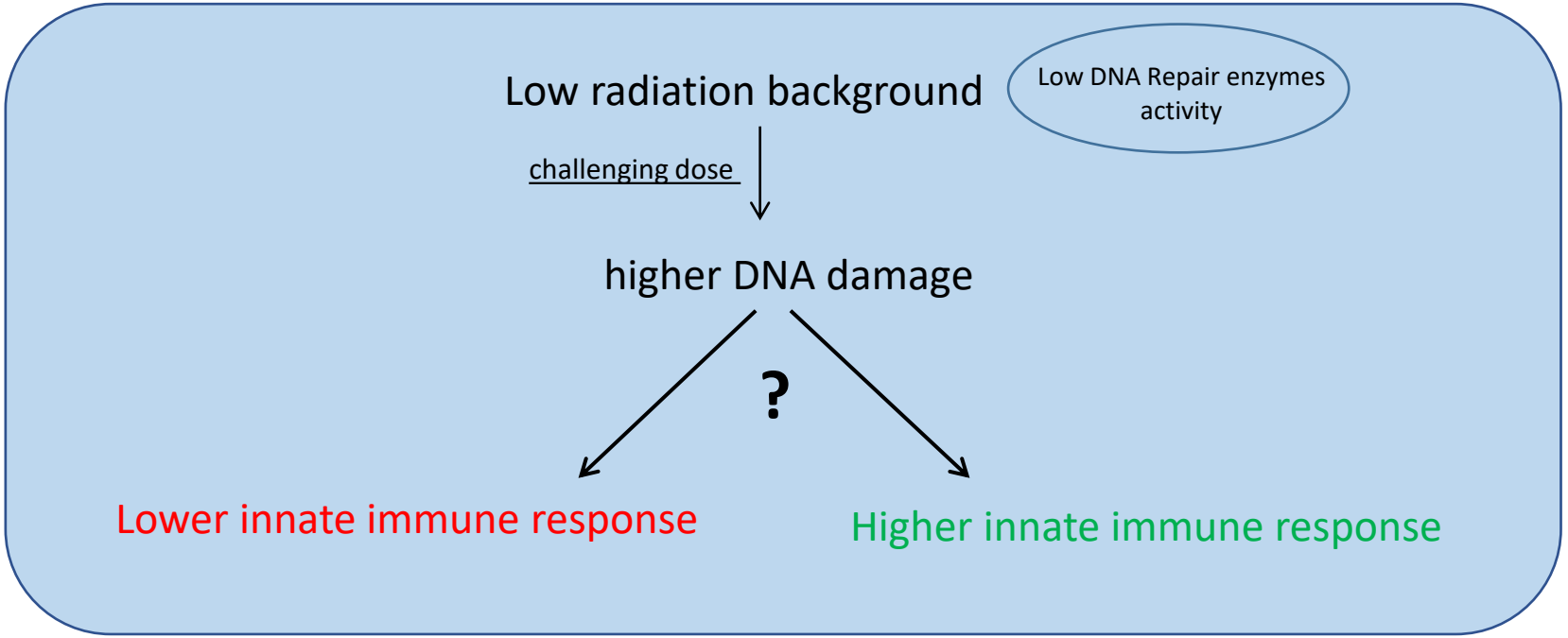


Long term experiments on in vitro models:

Cells cultured in reduced environmental radiation conditions for several months are:

- ✓ Less tolerant to radiation-induced DNA damage
- ✓ Less efficient in scavenging reactive oxygen species

Question: Does low-radiation background influence the response of innate-immune systems to DNA damage?



The availability of cell culture facilities outside and inside the **Gran Sasso National Laboratory (LNGS)** of the Italian Institute of Nuclear Physics (**INFN**) represents a great opportunity to investigate both the influence of doses as low as the environmental ones and even lower in triggering immune response(s).

The proposal

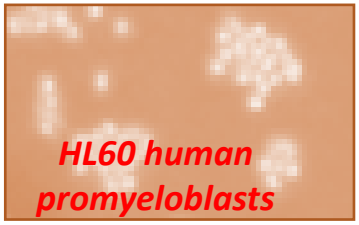
Being the first time that the immune response is studied in reduced environmental radiation, we are proposing a **pilot experiment** with the aim to test the response in different biological systems (*in vitro* and *in vivo* system)

The present proposal has **the following main aims**:

1. To investigate whether low radiation background influences the innate response of the immune system following radiation-induced DNA damage in human fibroblasts.

hTert-Immortalized human fibroblasts

2. To investigate whether low radiation background influences the ability of immature immune cells to differentiate into macrophages and monocytes (cells types that deal with the innate response of the immune system) and/or to maintain their biological function.



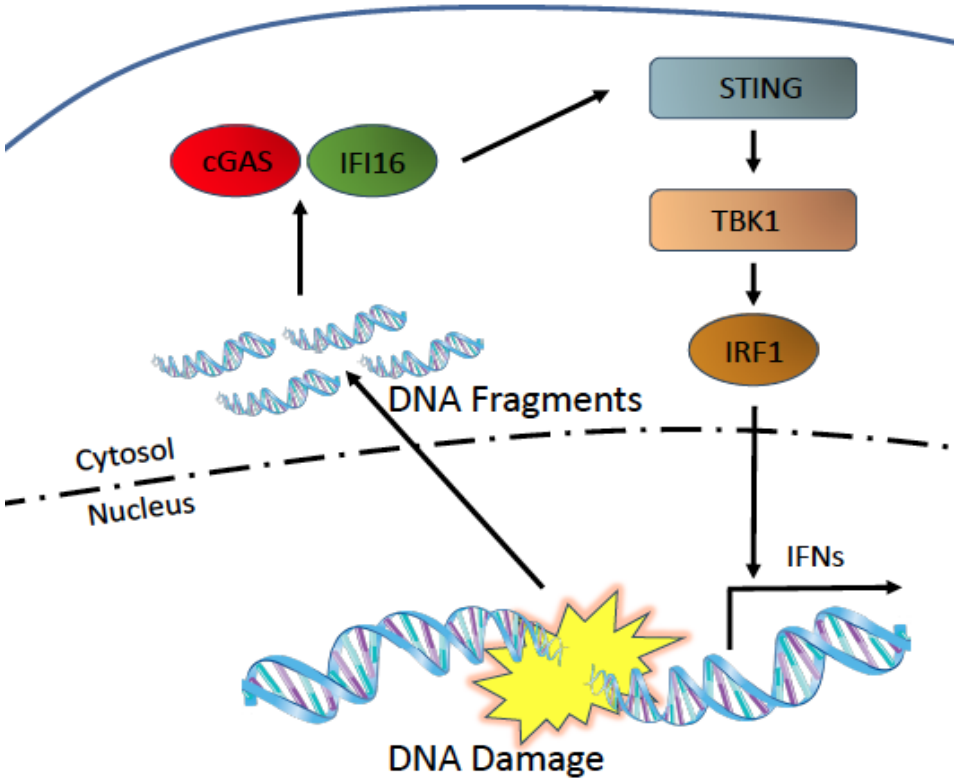
HL60 human promyeloblasts

3. To get information on the expression of genes related to the immune response in Drosophila melanogaster, taking advantage from the transcriptomic analysis planned in the framework of the **RENOIR experiment**.

Drosophila melanogaster

Methodology

1) Immortalized human fibroblasts will be grown in *Low Radiation Environment* and *Reference Radiation Environment* for the 0.5, 1 and 2 months. Afterwards, the cells will be brought to Rome, irradiated with an X-ray challenging dose of 2 Gy. DNA damage and the signal of cGAS-STING -a measure of innate immune system activation- will be analyzed.



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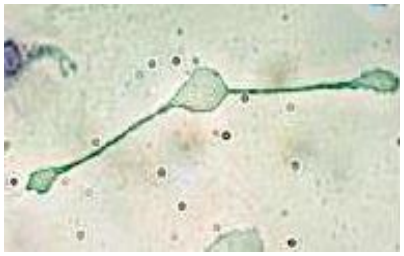
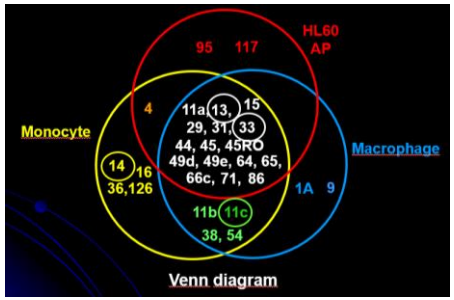
2) human promyeloblasts (**AP cells**) will be grown in LRE and RRE for the 0.5, 1 and 2 months. Afterwards, the cells will be induced to differentiate in: (A) **monocytes** by addition of 1-alpha-25-dihydroxyvitamin-D3 (vit D3) and (B) **macrophages** by addition of 12-O tetradecanoyl-phorbol-13-acetate (TPA).

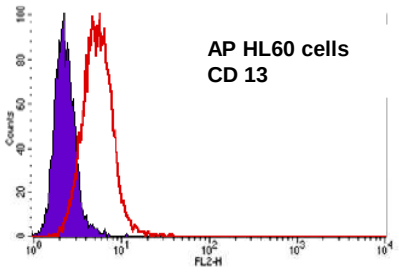
(A)

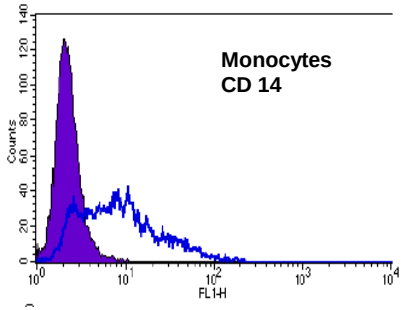
(B)

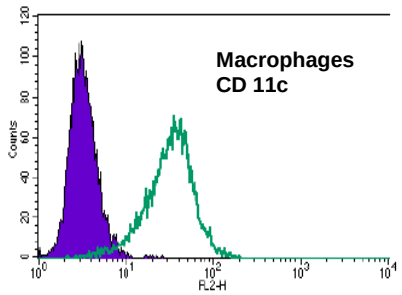


Flow cytometry will be used to study the expression of specific membrane antigens (CDs) as markers of differentiation: CD13 e CD33; CD11c; CD14;





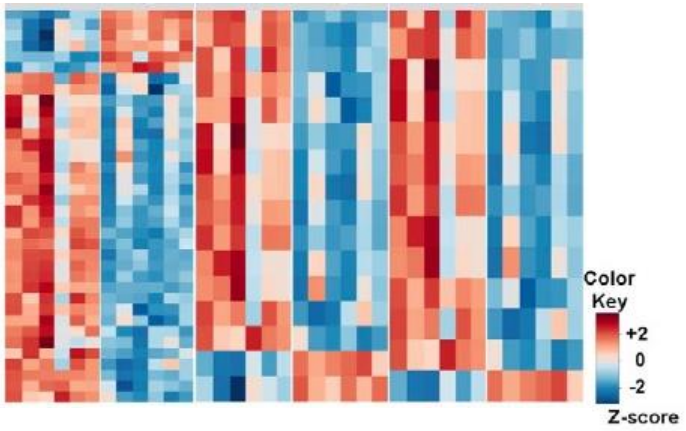




3) *Drosophila melanogaster* is the ***in vivo* model** used in RENOIR experiment, where one of the aims is to analyze the modulation of the genomic expression, by RNA-Seq analysis, to understand the molecular mechanisms underlying the influence of low-radiation environment.

In the framework of DISCOVER, we will analyze a subset of results of the transcriptional profiling experiments from RENOIR to find a possible modulation specifically in the **expression of immune system genes related to low radiation enviroment**.

Drosophila melanogaster



gene expression profiling

Impact and possible implications of this study :

- implications in low dose radiation therapy;
- influence of cosmic rays on the immune system may be relevant for deep space exploration
- Studies on adaptation of life on earth during the evolution of living beings.

Milestones

M1: data for the choose of the adaptation time (*0-**6** months*)

M2: Preliminary results on immune system response (*6-**12** months*)

M3: evaluation of *in vitro* LRE effect on immune system response
(*12-**24** months*)

M4: evaluation of *in vivo* LRE effect on immune system response
(*0-**24** months*)

Anagrafica

Roma 3		FTE 1.5
Antonella Sgura	Professore associato	**60**
Francesco Berardinelli	Ricercatore	**30**
Ion Udroiu	Assegnista UNI Roma 3	**50**
Antonio Antoccia	Professore associato	**10**

Roma 1		FTE 1.0
Valentina Dini	Ricercatore	**70**
Maria Antonella Tabocchini	Primo Ricercatore	**20**
Giuseppe Esposito	Ricercatore	**10**
Pasquale Anello	*Tecnico TD*	

LNGS		
Patrizia Morciano	Assegnista INFN	**10**
Dottorando GSSI (per bioinformatica)		

Altri collaboratori
Gianni Cenci (SAPIENZA)
Alessandra Tessitore (UNI AQ)
Carlos Pena-Garay (Canfranc)

La richiesta finanziaria copre tutta la durata del progetto (2 anni)

INFN - Sezione di Roma3 (15 kEuro)

Consumo: 9 kEuro

- Test biologici:
  - Reagenti per test di immunofluorescenza: **4 kEuro**
  - Altro (Plastiche monouso speciali; reagenti chimici/biochimici): **2 kEuro**
  - Siero specifico dedicato per colture cellulari: **3 kEuro**

Missioni: 6 kEuro

- da Roma ai LNGS (30/anno 2 persone)

INFN - Sezione di Roma1 (15 kEuro)

Consumo: 9 kEuro

- Test biologici:
  - CD per lo studio del differenziamento: **4 kEuro**
  - Reagenti per test di attività fagocitaria dei macrofagi: **0.5 kEuro**
  - Prodotti chimici/biochimici per differenziamento e citofluorimetria, plastiche speciali monouso: **4.5 kEuro**

Missioni: 6 kEuro

- da Roma ai LNGS (30/anno per 2 persone)

INFN – LNGS (4.5 kEuro)

Consumo: 4 kEuro

- Kit e reagenti per PCR (validazione dati trascrittomica)

Missioni: 0.5 kEuro

- da LNGS a Roma (1/anno per 1 persone)

Cofinanziamento:

- come previsto nell'ambito della **convenzione operativa tra ISS e INFN**
- Istituto Superiore di Sanità
- Dipartimento di Scienze dell'Università «Roma Tre»