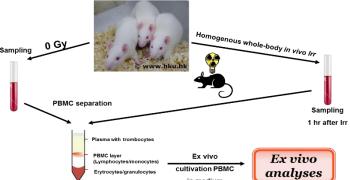


Ex vivo experimental model in biodosimetry

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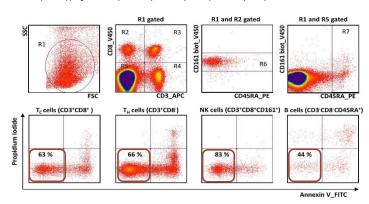
Wistar Rat Rattus sp. strain Wistar



	Antibody against	Clone	Fluoro- chrome	Specificity to
т	CD45RA	1F4	PE	B-lymphocytes
	CD8	OX-8	V450	MHC-I restricted T-cells (suppressor/cytotoxic T cells), NK cells, activated CD4 ⁺ T helpers
	CD3	1F4	APC	T-lymphocytes
	CD161	NKR-P1A	V500	NK cells, small T-lymphocyte subset
	Annexin V		FITC	Apoptotic and dead cells
	Propidium iodide			Dead cells

Six Wistar rats per group were $in\ vivo$ whole-body exposed to homogeneous ionizing irradiation (IRR) of 0 - 7 Gy (dose rate 0.48 Gy/min) from a distance of 1 m. Peripheral blood (5 – 7 ml) of each animal was collected 1 hour or 4 hours after exposition. Non-irradiated animals were served as controls. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation through a Ficoll Histopaque 1077 cushion according to the manufacturer instructions and washed in Iscove's Modified Dulbecco's Medium.

Finally, the suspension density of 1×10^6 PBMC in 1ml of IMDM was prepared and cultivated in cultivation plates 1hr, 3 hrs, 5 hrs, 7 hrs, and 23 hrs, respectively, under specific *ex vivo* conditions (37°C, 5% CO₂). The representations of viable (non-apoptotic) lymphocytes were detected by immunophenotyping and analyzed at Cyan ADP (DakoCytomation) analyzer.



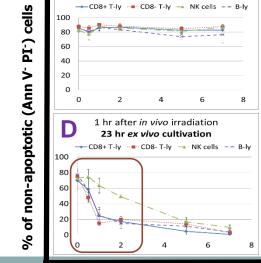
BIODOSIMETRY is a subdiscipline of radiobiology.

Its aim is a quantification of the absorbed dose of ionizing radiation according detection of postradiation changes in the irradiated organism.

- **A.** Apopototic cells are not detectable immediately after sampling (0 hr *ex vivo* cultivation). Apoptotic cells are eliminated from peripheral blood by active scavenging system.
- **B.** The unique decline of B-lymphocytes 5 hrs after irradiation instead of remaining high viability of T-lymphocytes and NK cells is a specific evidence (phenomenon) of the irradiation effect.
- C. T-lymphocyte and B-lymphocyte decreases are dose-dependent 7 hours after ex vivo cultivation. The assessment of their representation allows to distinguish between low (< 1Gy), high (> 5 Gy) and medium value of absorbed dose of irradiation.
- **D. NK cells are the most relative radioresistant** peripheral white blood cells. Their decline is dose dependent 23 hours after *ex vivo* cultivation.
- E. T-lymphocytes collected 1 hour after in vivo irradiation decrease within a linear manner 23 hours after ex vivo cultivation. Their assessment allows the back estimate of low doses of irradiation (0 1 Gy).

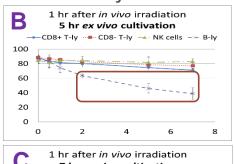
RESULS I

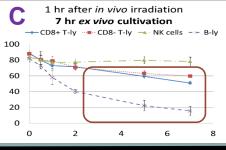
Acute irradiation by 0.5 - 1 - 2 - 5 and 7 Gy



1 hr after in vivo irradiation

0 hr ex vivo cultivation





RESULS II Low dose irradiation by $0.4-0.6-0.8\,$ and 1 Gy

