Effects of Tritiated Thymidine on Hematopoietic Stem Cells

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How can we study *in vivo* the long term effects of a cellular contamination by tritium?

Contamination by a high dose of tritium can be easily studied as the biological effects are rapid and dramatic.

However, we must consider the long term effects of a contamination by a small dose of tritium. These long term effects have to be followed during several months and thus the use of somatic cells, which are short term cells *in vivo,* is not relevant.

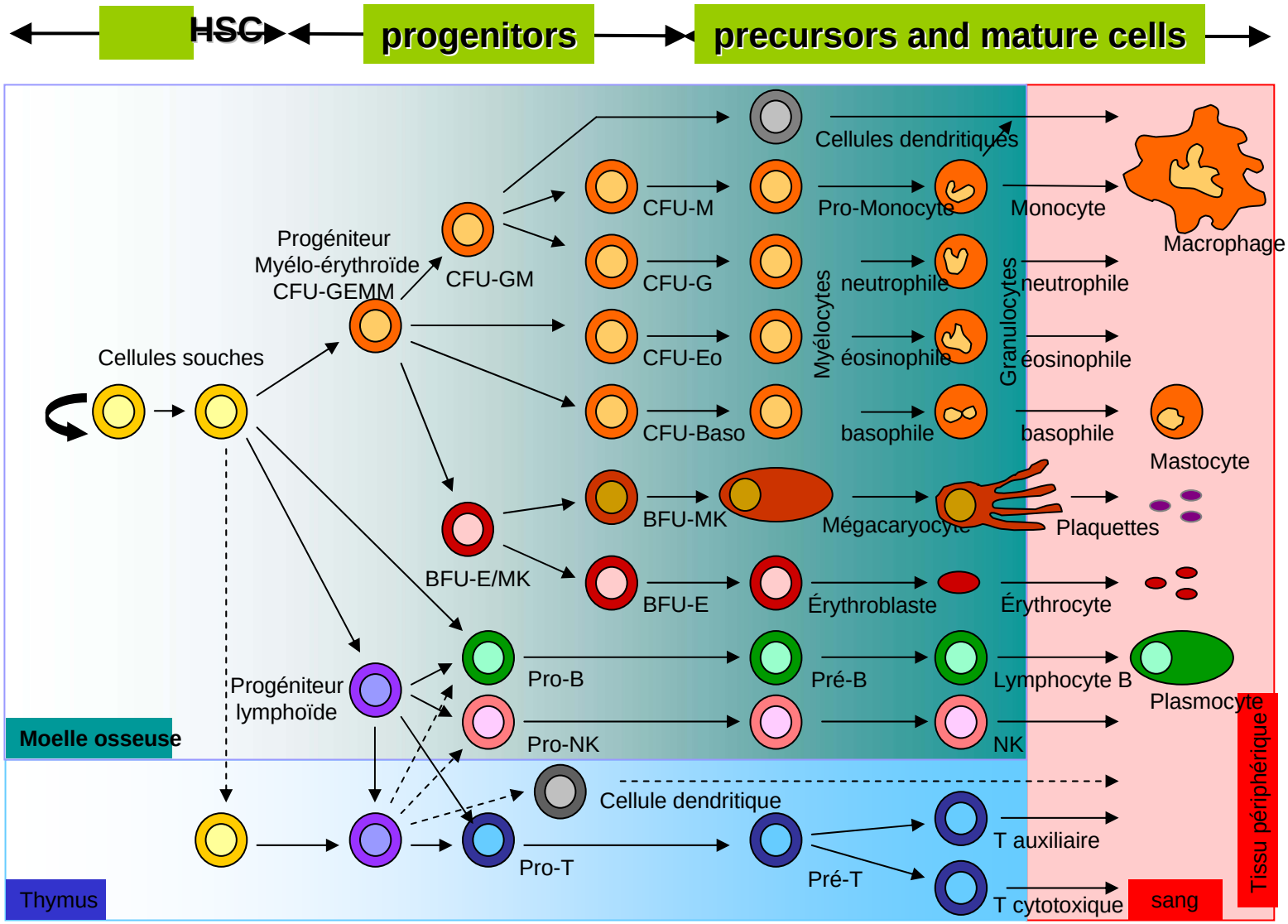
A cellular model that can be used to follow the *in vivo* long term effects of a contamination by a small dose of tritium must be have the following properties, at least when mice are used

1. The biological effects have to be followed during months.

2. The biological effects have to be transplantable in order to extend the observations for periods that exceed the life time of a mouse.

Thus, these effects need the use of somatic stem cells that can reconstitute a tissue or an organ and that can be transplanted.

Hematopoiesis



Hematopoietic Stem Cells (HSCs)

- Hematopoietic Stem Cells (HSCs) can reconstitute life long hematopoiesis of a mouse irradiated at 10Gy.

- Hematopoietic Stem Cells can be purified using a defined set of surface markers antibodies and flow sorting.

- Hematopoietic Stem Cells can be manipulated *in vitro* for a short time (24 to 48 hours) and have the same biological properties.

- Hematopoietic Stem Cells can differentiate *in vitro* to most of the hematopoietic lineages

Effects of ^{3}HTdr on Hematopoietic cell lines

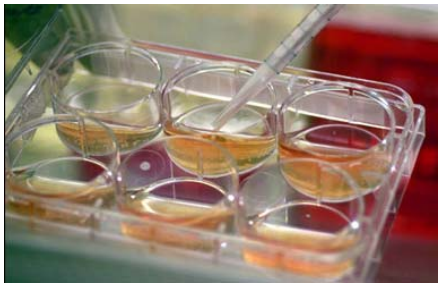
- ^{3}H-Tdr (methyl-^{3}H Thymidine) incorporation in five different human hematopoietic cell lines induces a dose dependent cell death.
  - ✓ scarse influence ----> 0,2 μCi/ml ^{3}H-Tdr
  - ✓ cell proliferation suppression and decreased cell viability ------> 2-5 μCi/ml ^{3}H-Tdr
- Two different cell death pathways are activated after tritium incorporation:
  - ✓ with DNA fragmentation
  - ✓ without DNA fragmentation

---------> There are no data about the effects of ^{3}H-Tdr incorporation on HSC

HSCs ^{3}H-Tdr DNA incorporation

1. In Vitro Experiments

Purification of HSCs from bone marrow



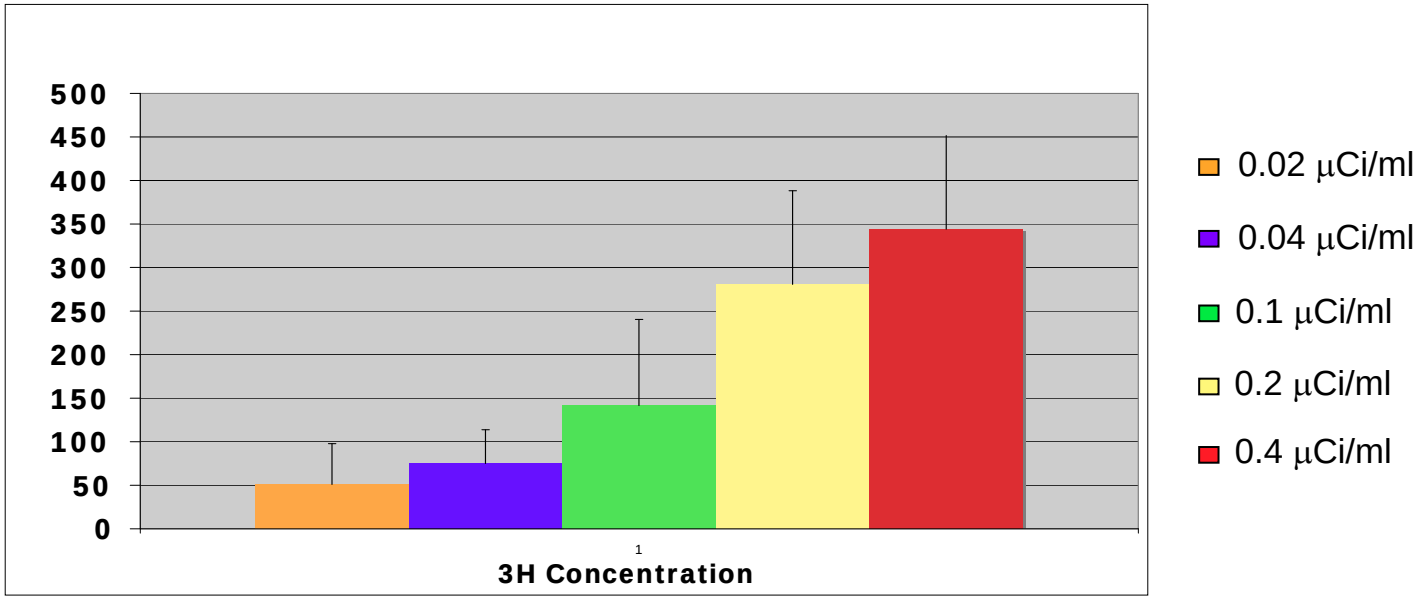
24h and 48h ^{3}H-Tdr incorporation

^{3}H-Tdr DNA incorporation

Cell viability

Effects of ^{3}H-Tdr on HSCs

1- Quantification of ^{3}H-Tdr incorporation

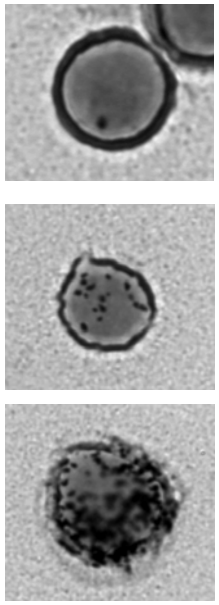


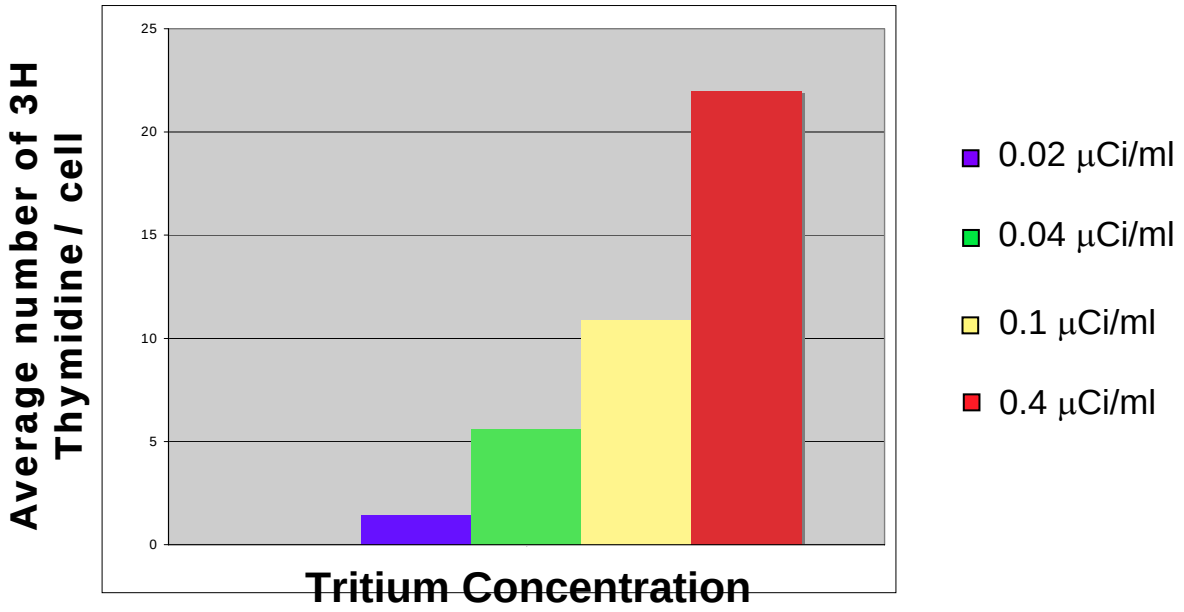
^{3}H-TdR can be incorporated in HSCs DNA

Good correlation between ^{3}H-Tdr concentration in the cell medium and ^{3}H-Tdr incorporation in HSCs DNA

Effects of ^{3}H-Tdr on HSCs

2.Autoradiography

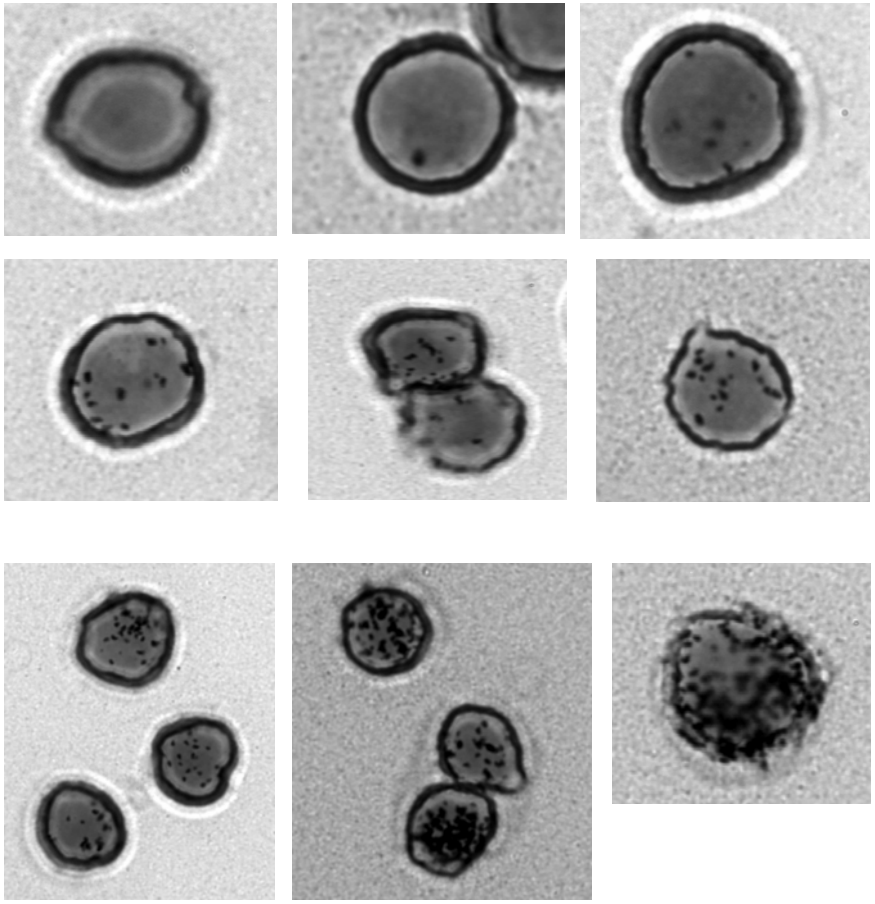


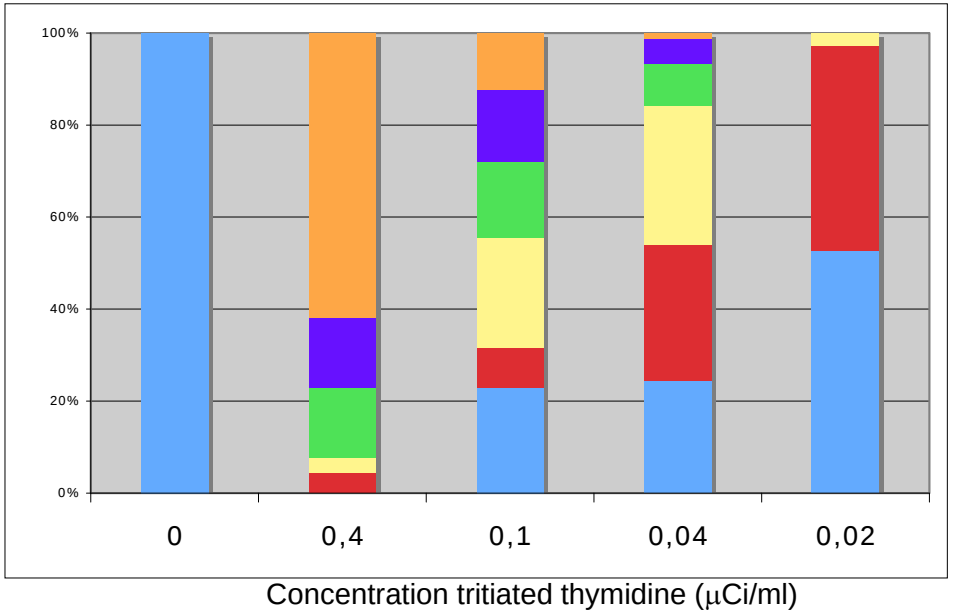


Good correlation between ^{3}H-Tdr concentration in the medium and the average number of ^{3}H-Tdr incorporated in individual cells

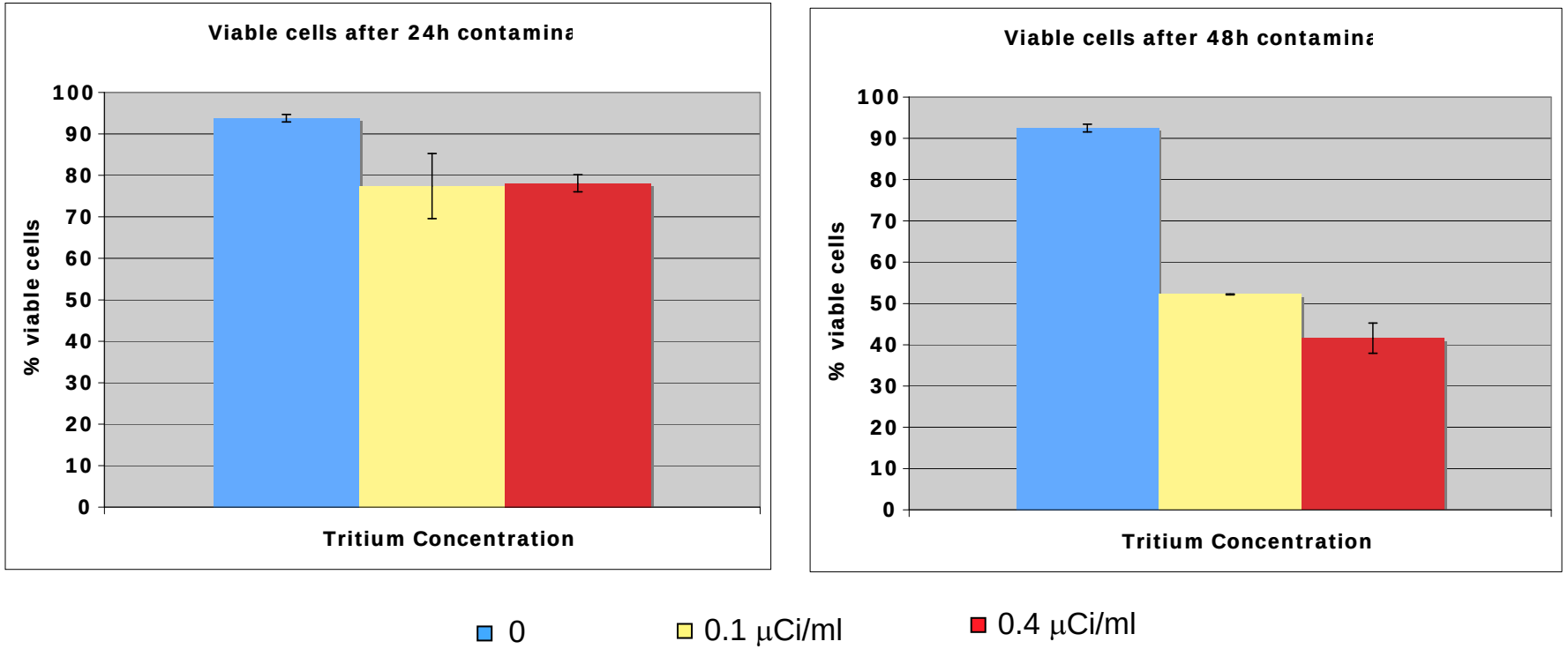
Effects of ^{3}H-Tdr on HSCs

3. Distribution of ^{3}H-Tdr incorporation per cell





^{3}H-Tdr incorporation in HSCs and cell death



48h incubation of ^{3}H-Tdr leads to a decreasing proportion of viable cells but cell death is not dramatic after a 24h incubation of ^{3}H-Tdr

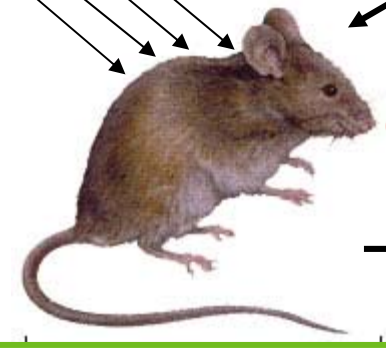
In vivo effects of ^{3}H-Tdr contamination of HSCs

Primary transplantation

Purification of HSCs

24h ^{3}H-Tdr incorporation

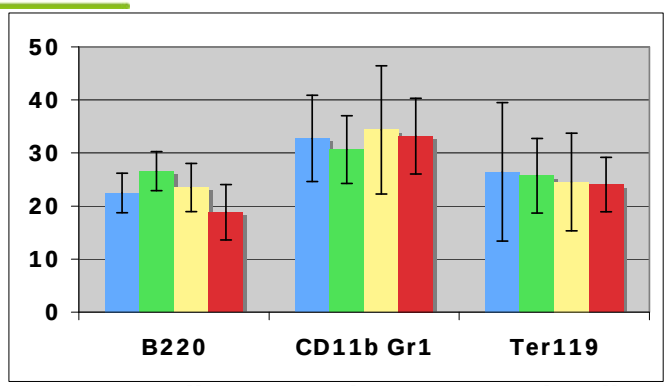
Lethal Irradiation (10Gy)

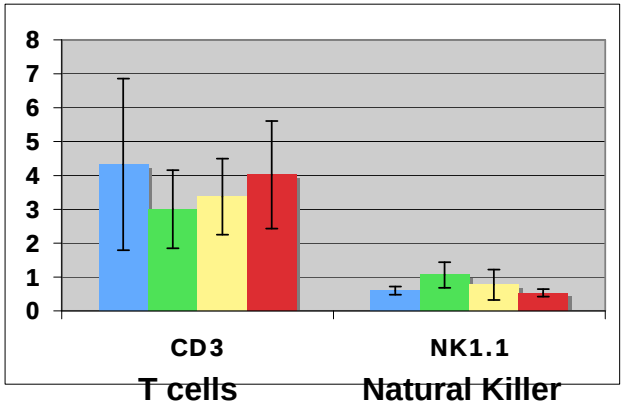


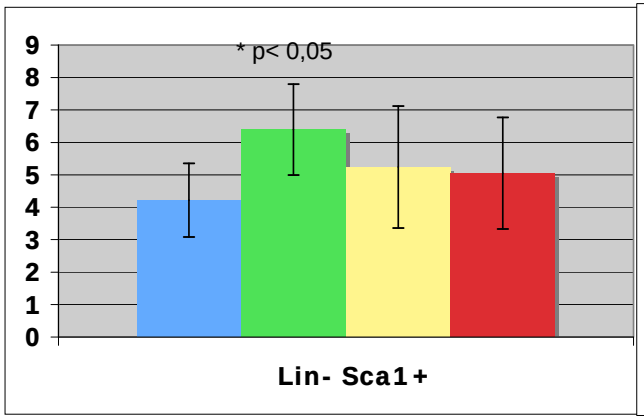
Transplantation of contaminated HSCs

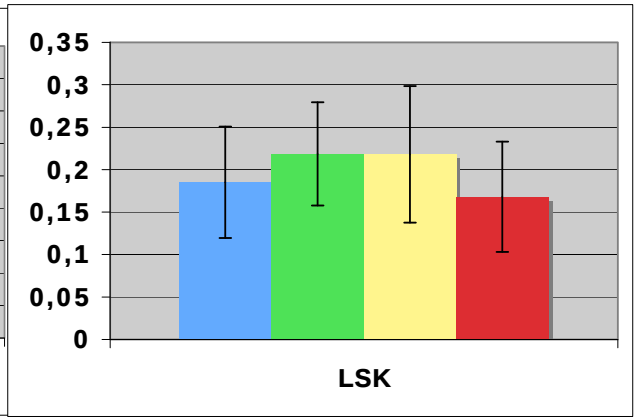
Analysis of the different hematopoietic populations four months after transplantation

Analysis of long term hematopoietic reconstitution by ^{3}H-Tdr contaminated HSCs









- 0
- 0.04 µCi/ml
- 0.1 µCi/ml
- 0.4 µCi/ml

Four months after transplantation of 3H-Tdr contaminated HSCs, only a slight but significative increase in hematopoietic progenitors ($Lin^{-}Sca^{+}$) at the lowest dose of contamination can be observed

In Vivo effects of ^{3}H-Tdr contamination of HSCs

Competition Transplantation

C57Bl6/45.1 mouse

Purification of HSC from bone marrow

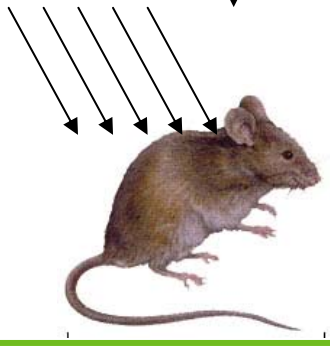
C57Bl6/45.2 mouse

Purification of HSC from bone marrow

24h ^{3}H-Tdr incorporation

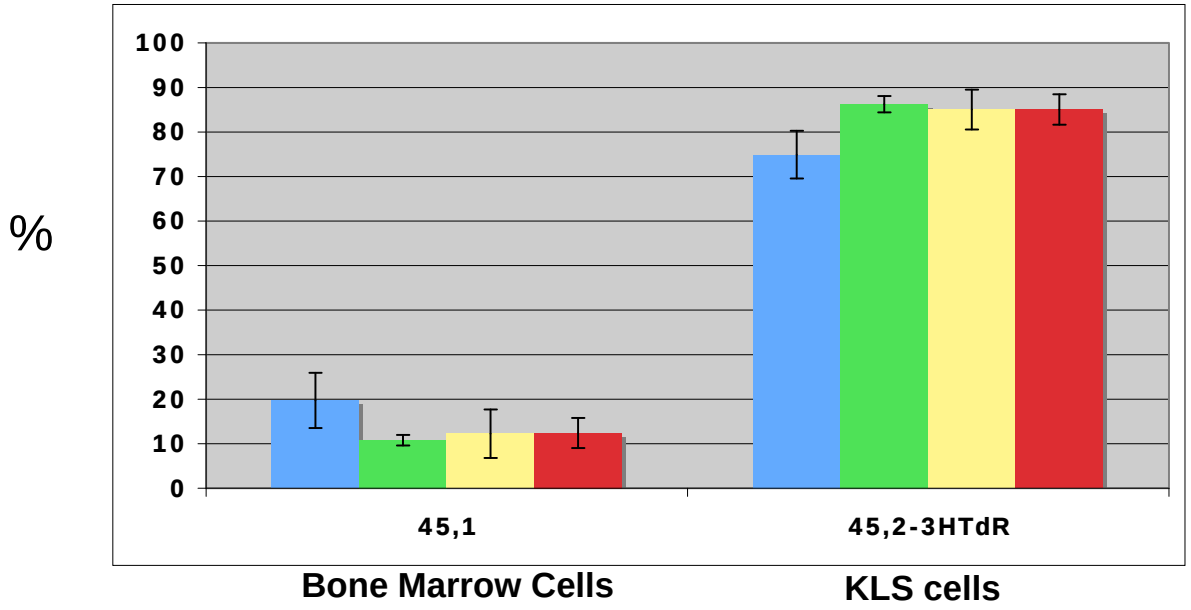
Co-Transplantation

Lethal Irradiation



Cell chimerism analysis four months after transplantation

Competition Transplantation



No difference in the repopulating capacity of KLS cells contaminated with different doses of ^{3}HTdr

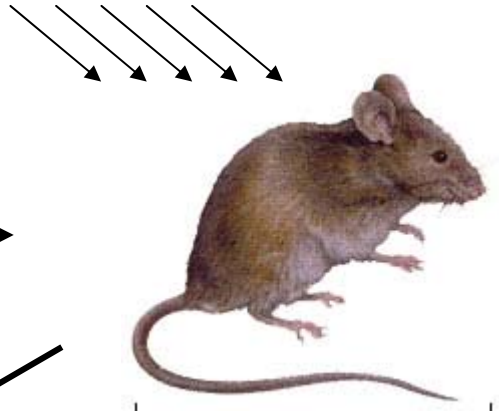
In vivo effects of ^{3}H-Tdr contamination of HSCs

Secondary transplantation

Mouse transplanted with
^{3}H-Tdr HSCs

Bone marrow
cells

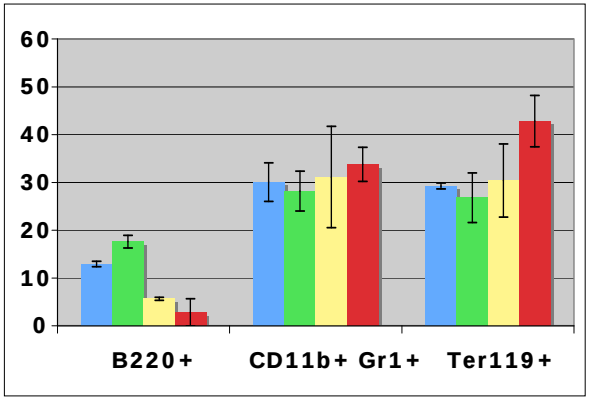
Lethal Irradiation
(10Gy)

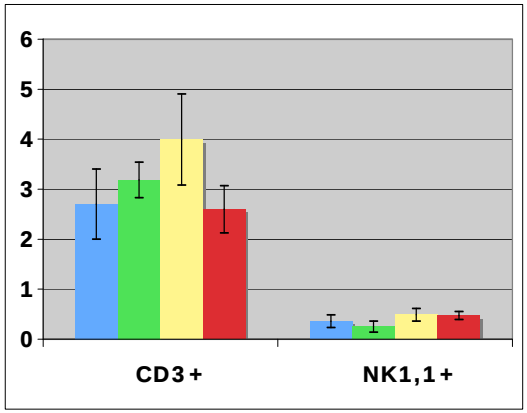


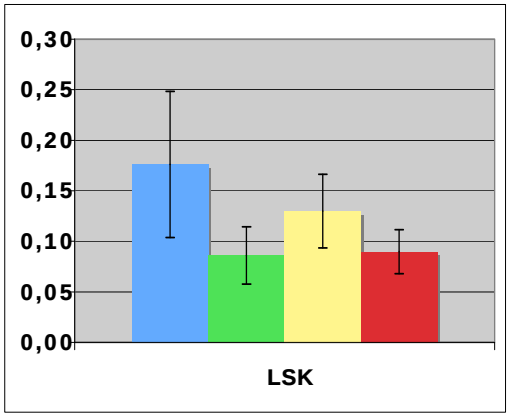
Transplantation

Analysis of the different hematopoietic
populations 4 months after transplantation

Defect in B lymphopoiesis in secondary transplantation







B cells **Granulocytes** **Red Cells** **T cells** **Natural Killer** **Hematopoietic Stem Cells**

0 0.04 μCi/ml 0.1 μCi/ml 0.4 μCi/ml

4 months after a secondary transplantation all the mice are alive but analysis of different hematopoietic populations shows a dose dependent decrease of B Lymphocytes in the bone marrow

Conclusions

The ^{3}HTdR incorporation increases the percentage of mortality of HSCs only after 4 hours of incubation

The contaminated HSCs

1. Didn't display any *in vivo* impaired potential as studied hematopoietic reconstitution except hematopoietic progenitors (lin-sca+).

2. Competition experiments indicated a similar hematopoietic potential before and after ^{3}HTdR incorporation

3. Secondary transplantation indicated a decreased number of B lymphocytes in the bone marrow

Aknowledgements

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