INFLUENCE OF TOTAL BODY IRRADIATION ON BONE MARROW STROMA

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There is clear evidence that hematopoietic stem cell regulation depends on a functioning marrow microenvironment e.g. marrow stroma cells provide niches for hematopoietic stem cells, influence proliferation , self-renewal and differentiation, and restrict early stem cells and progenitors to the marrow site, in order to retain the stem cell pool. Several heterogeneous stromal elements take part in that regulatory system, like fibroblasts, adipocytos, macrophagos, end endothelial cells, producing and presenting specific adhesion molecules, cytocines, and extracellular matrix proteins with stimulatory or inhibitory effects on hematopoietic cells in a juxtacrine manner.

Much informations have been gained over the last 30 years about these stroma/stem cool interactions by animal models and in vitro systems like the long-term marrow culture (LTMC) or cocultures with clonal stromal cell lines, nicely reviewed by several experts on this field (Lichtman, 1981; Trentin, 1970; Verfaillie, 1993; Muller-Sieburg, 1995; Gronthos and Simmons, 1996). When hematopoietic stem cell transplantation started years ago, the focus of interest was the radiosensitivity of hematopoietic stem cells, and the surrounding microenvironment was thought to be relatively radioresistant. Thirty years later and after some ten thousands of transplants world-wide, it is well known that early or late graft tailures as well as poor marrow functions occur, which can not be explained solely by graft rejection phenomena. It is also clear that modern technology provides an increasing number of soluble factors with potential therapeutic impact on accelerating hematopoietic reconstitution in the transplant situation, but it bas been difficult thus far to demonstrate that any of the currently available cytokines act on the truly totipotent stem cell. Thus, increasing attention has turned to stroma dependent hematopoietic regulators in order to describe disease on treatment related dysfunctions of this system or to manipulate such stroma factors in order to ensure engraftment or enhance hematopoietic reconstitution.

Therefore, also the widely employed irradiation procedures within conditioning regimens for hematopoietic stem cell transplants should be re-evaluated for the impact on the function of the marrow microenvironment, knowing that animal models might not reflect the human situation, that dose-rates and fractionations modify the damaging effect on the stroma compartment, and last but not least that the stroma is not only heterogeneous in respect to the cell types involved, but also the radiosensitivity of each cell line. In addition, transplant patients usually receive a combination treatment including irradiation (TBI and others) and chemotherapy, and it is well known that also these chemotherapeutic substances like alkylating agents contribute to damaging effects on the marrow microenvironment.

Nevertheless, several conclusions can be drawn regarding irradiation effects on marrow stroma cells, pioneered by authors like FRIEDEN-STEIN, TAVASSOLI, KNOSPE, GREENBERGER, and others (Friedenstein et al, 1982; Tavassoli, 1982; Knospe, 1988; Maloney et al, 1983; Creenberger, 1991).

Localised high dose (20-100 Gy) irradiation to the limp of a rat produces not only marrow aplasia, but also a long lasting defect of the marrow microenvirontment, probably by damaging stromal elements as well as the sinusoidal structure. This defect prohibits a resettlement of hematopoietic stem cells from unirradiated marrow sites, unless there is not at least some repair of the irradiated stroma or an influx of unirradiated stroma cells from other parts of the body or syngeneic donors, which could be demonstrated in highly irradiated limbs by introducing marker genes into such stroma cells. The stroma tolerance was clearly greater, when fractionated radiation was employed instead or single dose exposure. Much less clear is the impact of radiation doses used in a clinical setting (8-12 Gy) and conflicting results exist between in-vivo and in-vitro data.

For in-vivo experiments irradiated bone fragments have been transplanted to extramedullary sites of an syngeneic animal and checked for hematopoietic supportive function. These experiments provided a lot of evidence, that the damaging effect on the stroma compartment already starts with doses from 5 Gy on, but is usually followed by an almost complete but slow recovery, demonstrating at least in these animal models contrasting irradiation sensitivities of microenvironmental and hematopoietic cells. In-vitro data finally allow a more precise evaluation of single cell components both of the hematopoietic as well as the stroma compartment, but also the

radiosensitivity of immature progenitors compared to the mature cell fraction in both systems. We and others looked for the radiation effect on hematopoietic precursors (CFU-GM) compared to those of the marrow stroma (CFU-F) in humans and demonstrated that the radiosensitivity is very close to each other with a Do for CFU-GM or 115 cGy and for CFU-F of 130 cGy (Ebell and Castro-Malaspina, 1982; Laver et al, 1986). Clearly the survival curve of stroma precursors revealed a broader shoulder then hematopoietic progenitors indicating the different repair capacity. Nevertheless, doses used in total body irradiation are followed by a long lasting proliferative defect of the stroma progenitors with little knowledge about the turnover of this cell system and therefore the impact of these findings. Once the stroma cells are allowed to grow to confluence in culture flasks and are then irradiated with doses of up to 10Gy, the hematopoietic supportive function is not greatly impaired.

Then we looked for the stroma situation in children after total body irradiation and marrow transplants and found a very similar picture as demonstrated in the in-vitro system. The stroma progenitors are considerably diminished for several months with a proliferative defect of the CFU-F derived fibroblasts. This numerical deficiency of stroma precursors is highly correlated with the hematopoietic reconstitution pattern. The stroma compartment might have also functional defects, for example regarding the production of stem cell factor, which is different from the in-vitro assumption. The post-transplant stroma compartment was exclusively of host origin as shown by others as well (Simmons et al, 1987).

Despite their impairment stromal cells were responsive to well know regulators for the proliferation of these cells like PDGF, FGF, and others, and also to factors influencing the factor production like TNF and IL-1. That might be a key to overcome stroma dysfunctions in the future and influence the donor hematopoiesis indirectly via the microenvironment instead of giving unphysiological cytokine cocktails with direct impact on the hematpoietic cells.

In conclusion, stromal cells are impaired by radiation doses used in total body irradiation but have in-vivo a large capacity for repair. The repair process without intervention is prolonged and requires 6-12 months or longer. Clinical relevance can be demonstrated already with doses up to 10 Gy. The critical dose without repair, however, is for single exposure above 20 Gy and for fractionated doses above 50 Gy.

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