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Review

Delivery and processing of exogenous double-stranded DNA in mouse CD34 + hematopoietic progenitor cells and their cell cycle changes upon combined treatment with cyclophosphamide and double-stranded DNA

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ABSTRACT

We previously reported that fragments of exogenous double-stranded DNA can be internalized by mouse bone marrow cells without any transfection. Our present analysis shows that only 2% of bone marrow cells take up the fragments of extracellular exogenous DNA. Of these, ~45% of the cells correspond to CD34+ hematopoietic stem cells. Taking into account that CD34+ stem cells constituted 2.5% of the total cell population in the bone marrow samples analyzed, these data indicate that as much as 40% of CD34+ cells readily internalize fragments of extracellular exogenous DNA. This suggests that internalization of fragmented dsDNA is a general feature of poorly differentiated cells, in particular CD34+ bone marrow cells.

When linearized plasmid DNA was used as a source of exogenous DNA, we observed that exonucleolytic processing and ligation of double-stranded DNA termini occurred in the bone marrow cells that had this DNA internalized. We also recovered "hybrid" plasmids that encompass kanamycin-resistance gene from the exogenous plasmid DNA and the fragments of plasmids from host enterobacteria, which is suggestive of recombination events taking place upon DNA internalization.

CD34+ cells make up the distinctive bone marrow cell population that internalizes extracellular DNA. Cell cycle analysis of CD34+ cells treated with cyclophosphamide only or in combination with dsDNA, suggests that these cells have distinct biologic responses to these treatments. Namely, whereas upon cyclophosphamide treatment bone marrow stem cells become arrested at S–G2 phases, combined cyclophosphamide + dsDNA treatment leads to cell cycle progression without any delay. This indicates that when the genome is undergoing repair of interstrand crosslinks, injection of fragmented exogenous dsDNA results in immediate reconstitution of genome integrity. We observe that cyclophosphamide-only or a combined cyclophosphamide + dsDNA treatment of cells lead to two distinct waves of apoptosis in CD34+ progenitors. We also show that cyclophosphamide and cyclophosphamide + dsDNA injections promote division of CD34+ cells at distinct time periods.

Contents

Abbreviations: BMCs, bone marrow cells; CP, cyclophosphamide; DSB, double-stranded break; dsDNA, double-stranded DNA; HSCs, hematopoietic stem cells; ICL, interstrand cross-link.

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1. Introduction

Cyclophosphamide (CP) is known to induce apoptosis in bone marrow stem cells, which is accompanied with myelosuppression and subsequent abortive increase in hematopoietic progenitor cell counts. This phenomenon has been described in both humans and lab animals (mice in particular) (Dolgova et al., 2009; Likhacheva et al., 2007). It has also been established that injections of double-stranded DNA (dsDNA) preparations into CP-treated mice result in rapid recovery of leukocyte population (leukostimulatory effect) (Dolgova et al., 2009).

Recently, we demonstrated that injections of exogenous DNA 18–30 h after CP treatment (during the so-called "death window" period) lead to specific symptoms and death of experimental animals (Dolgova et al., 2012). We showed that animals succumbed to multiple organ failure resulting from severe immunodeficiency due to accidental involution of lymphoid organs. This total depletion of immunocompetent organs was caused by massive inflammation and activation of all components of immunity. Concomitantly, administered dsDNA specifically targets CD34+ bone marrow cells (BMCs) as they recover from CP treatment and repair interstrand cross-links (ICLs), thereby hematopoietic stem cells (HSCs) temporarily lose their ability to differentiate into lymphoid lineage. Thus, the organism fails to maintain active anti-infective immune status; opportunistic infections develop and in most cases such animals die. We also showed that BMCs, CD34+ cells in particular, internalize dsDNA fragments both in vivo and upon co-incubation ex vivo, without any transfection reagents. Our analysis suggests that the most critical step in this cascade of molecular, cellular and organ failures is when dsDNA participates in the final steps of ICL repair and DNA integrity is restored.

The present paper continues to describe and analyze the experimental series outlined above. Here, we quantify the BMC population that takes up the fragments of exogenous DNA. We show that the property to internalize the fragments of dsDNA is characteristic of poorly differentiated cells, CD34+ bone marrow cells in particular. To trace the internalization events, we used a fluorescently labeled (TAMRA) DNA probe homologous to human *Alu* repeat. We also analyze the structural changes in exogenous DNA that eventually reached the internal compartments of BMCs. We report that internalization of dsDNA may occur during the cell repair, namely, we observed that the linearized plasmid DNA was subject to exonucleolytic and ligase activities in the cells. The exogenous dsDNA that became internalized by the cells may also participate in recombination events, which was demonstrated using ex vivo co-incubation of BMCs with plasmid DNA.

When dsDNA is administered to CP-pretreated animals during the "death window" period, the ability of CD34+ HSCs to differentiate into lymphoid lineage is greatly compromised, which results in severe damage to the organisms of treated mice. This prompted us to perform comparative analysis of these cell populations several days after CP or CP+ dsDNA treatments, in more detail. We observed that injection of dsDNA into CP-treated mice leads to rapid, and apparently, abortive completion of the repair process in CD34+ BMCs. This results in that HSCs fail to give rise to certain cell populations, such as lymphocytes (Dolgova et al., 2012).

2. Materials and methods

2.1. DNA sources and probe delivery

Three distinct exogenous dsDNA probes were used in our experiments. Mouse BMCs were incubated with either human *Alu* repeat DNA (500 bp) or linearized plasmid pEGFP-N1 (4.7 kb). To study cell cycle effects in mouse CD34+ HSCs, human dsDNA was used (Likhacheva et al., 2007). CP and exogenous DNA were injected into mice intraperitoneally.

2.2. TAMRA labeling of human Alu repeat DNA

DNA was labeled using PCR (Saifitdinova, 2008). PCR template was human *Alu* repeat sequence cloned in pUC19; this repeat encompassed the start and the end of the tandemly repeated *AluJ* and *AluY* (NCBI: AC002400.1, 53494–53767). Standard M13 primers were used for amplification. Purification of PCR products was done by standard phenol–chloroform extraction followed by ethanol precipitation, as previously described (Saifitdinova, 2008). DNA concentration and incorporation of dUTP-TAMRA label were measured using Nanodrop (Eppendorf, USA) and calculated by comparing the signal before and after PCR re-precipitation.

2.3. Analysis of TAMRA-labeled exogenous DNA internalization by CD34 + and CD34 − cells

BMCs were perfused from tubular bones of intact mice using RPMI-1640 medium, pelleted at 400 *g* for 5 min and washed once in 1 ml of the same medium. Red blood cells were lysed in the lysis buffer (0.15 M NH_4Cl, 10 mM Tris–HCl pH 7.5, 0.5 mM EDTA), and 10–11 million cells were incubated with 0.8–1 μg TAMRA-labeled human *Alu* DNA in 0.5 ml of culture medium for 3 h. Cells were washed 3–4 times with cell culture medium as described above and resuspended in PBS + 0.1% NaN_3 and 1% FBS. Samples were then incubated for 40 min at +4 °C with FITC rat anti-mouse CD34 conjugates (BD Pharmingen) or isotype controls (FITC rat IgG2a, κ Isotype Control, BD Pharmingen), 3 μg per 3 million cells/ml. Then the cells were FACS-sorted into CD34+ and CD34− populations on BD FACSAria (Becton Dickinson, USA). Positive and negative cells were defined based on the isotype control levels.