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The growth capacity of bone marrow CD34 positive cells in culture is drastically reduced in a murine model of Down syndrome

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Abstract

Human trisomy 21, Down syndrome (DS), is characterized by mental retardation. In addition, high risks of developing hematological and immune disorders, as well as cardiac, skeletal and other abnormalities are life-long concerns. Recent data suggested that bone marrow contains progenitors, hematopoietic or stromal cells, which may have the potential of generating non hematopoietic tissue such as neural cells, cardiac cells or osteoblasts. Therefore we have used a model of Down syndrome, Ts65Dn mice, to investigate their bone marrow. We have found that the vast majority of $CD34^+$ cells in the bone marrow of adult Ts65Dn mice, but not of the $CD34^-$ cells, exhibit a drastic reduction in their in vitro growth capacity. In addition to neural antigens, cultured $CD34^+$ cells from trisomic and diploid mice also expressed mast cell markers. ***To cite this article: B. Jablonska et al., C. R. Biologies 329 (2006).***

Résumé

Réduction drastique de la prolifération des cellules $CD34^+$ cultivées à partir de la moelle osseuse de souris modèle du syndrome de Down. La trisomie du chromosome 21 chez l'homme, ou syndrome de Down, est essentiellement caractérisée par un retard mental. Il existe en outre un risque élevé de désordres hématologiques et immunitaires ainsi que des anomalies diverses affectant entre autres le cœur, les os et les muscles tout au long de la vie. Des études récentes suggèrent que la moelle osseuse pourrait contenir des progéniteurs, cellules hématopoïétiques ou stromales qui pourraient éventuellement être à l'origine de tissus non hématopoïétiques tels que les cellules neurales, ostéoblastiques ou cardiaques. Nous avons donc étudié la moelle osseuse de la souris Ts65Dn, qui constitue un modèle de la trisomie 21. La très grande majorité des cellules $CD34^+$, mais pas les cellules $CD34^-$, sont apoptotiques, ce qui a pour résultat une diminution très importante de leur capacité de multiplication in vitro. En plus d'antigènes neuraux, les cellules $CD34^+$ des souris trisomiques et diploïdes expriment également des marqueurs de mastocytes. ***Pour citer cet article : B. Jablonska et al., C. R. Biologies 329 (2006).***

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Mots-clés : Syndrome de Down ; Souris trisomiques ; Apoptose ; $CD34^+$; Moelle osseuse

1. Introduction

Human trisomy 21, Down syndrome (DS), is essentially characterized by mental retardation. In addition, the risk of developing hematological and immunological disorders is drastically increased in these patients [1, 2]. A mouse model of human trisomy 21, Ts65Dn, has been produced with partial trisomy of chromosome 16, since its distal third is syntenic to the distal end of chromosome 21 and comprises 104 trisomic genes. Ts65Dn mice survive to adulthood and exhibit phenotypic abnormalities that resemble those of DS individuals [3].

We have recently reported that bone marrow cells from adult mice cultured with IL3, IL6 and SCF can be grown in culture over 30 generations, so that over a 4 month period of culture 1×10^{15} cells were generated from 1×10^9 cells. These cells were $CD34^+$, expressed the hematopoietic stem cell markers: Sca1, AA4.1, and cKit, as well as neural antigens [4].

It has been recently reported that the bone marrow contains progenitors – either hematopoietic or stromal cells – that have the potential of generating a variety of non-hematopoietic tissues such as neural cells, cardiac cells or osteoblasts [5–7]. These data suggested that it might be of interest to explore the properties of bone marrow cells of Ts65Dn mice. Therefore, these cells were put into culture in conditions identical to those previously used for normal mice.

The main result is that the in vitro growth capacity of bone marrow $CD34^+$ cells from Ts65Dn mice is drastically reduced, yet they express a phenotype comparable to that of diploid cells.

2. Materials and methods

2.1. Mouse strains

The DS model mice – Ts(17^{16})65Dn (The Jackson Laboratory, Bar Harbor, ME) – were used in in vitro and ex vivo experiments. Diploid mice were the littermates of the Ts(17^{16})65Dn mice. These studies were conducted with the National Institutes of Health guidelines and the Institutional Animal Care Committee at University of Maryland (Baltimore, MD).

2.2. Preparation and experimental conditions of bone marrow cells

2.2.1. Preparation of ex vivo bone marrow cells

Bone marrow cells were obtained and prepared in the same way as reported previously [4]. Briefly, cells were collected aseptically from the femurs of Ts65Dn and diploid adult mice, fixed immediately in 4% paraformaldehyde, washed 2 times in 0.01M PBS (pH7.4) and deposited onto microscope slides by cytospin and stored at −80 °C until use.

2.2.2. Preparation of in vitro bone marrow cells

The sample of cells from one adult mouse femur was suspended in 10 ml of DMEM (GIBCO Invitrogen, Carlsbad, CA) containing 10% FBS with the following supplements: 5 ng ml^{-1} interleukin-3 (IL3), mouse 10 ng ml^{-1} interleukin-6 (IL6), 10 ng ml^{-1} mouse stem-cell factor (SCF) (R&D Systems, Minneapolis, MN), and a 1:1000 dilution of 10 µl 2-mercaptoethanol in 2.9 ml of H_2O. No matrix, substrate, or feeder cells were added to the liquid medium in the tissue-culture flasks. The medium was distributed into two T75 tissue-culture flasks to grow the cells at 37 °C in humidified 10% CO_2 in air. Passaging and feeding the cells with addition of 5 ml of fresh medium to each flask were usually done two times a week. Only the floating cells were passaged, leaving behind all the attached cells such as bone marrow stromal cells, endothelial cells, and macrophages, etc. Floating cells were subcultured in 50% conditioned medium from the previous culture and 50% fresh medium at 2×10^6 cells per 10 ml. After 3–4 weeks, the cultures contained only dividing floating cells, and the cells no longer differentiated into macrophages and other cells that attached to the flask. After 10 weeks of culture cells were fixed in 4% paraformaldehyde, washed two times in 0.01M PBS (pH7.4) and deposited onto microscope slides by cytospin and stored at −80 °C until use.

2.3. Immunocytochemistry

Immunocytochemical studies were performed on ex vivo and in vitro bone marrow cells. Paraformaldehyde-fixed cells on microscope slides were treated with 0.25% Tween 20 for 3 min at 21°C, washed three times in PBS, and analyzed by standard immunocytochem-