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The Effect of Iron Bound Transferrin on Megakaryocytopoiesis in Bone Marrow Culture

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Abstract: Megakaryocytopoiesis is a complicated phenomenon which involves the proliferation of progenitor cells, formation of a polyploid nucleus, cytoplasmic changes and formation of thrombocytes. It has been suggested that megakaryocytopoiesis resembles erythropoiesis. It is well known that iron is a required element for the proliferation of erythrocytes, lymphocytes and cancer cells but no consensus has yet been reached about its role in thrombocytopoiesis. Although thrombocytosis is generally found in patients with iron deficiency anemia, thrombocytopenia is also observed. Cell surface receptors for transferrin were initially characterized on cells with high iron requirements including hemoglobin producing cells and placental cells. More recently, it was found that transferrin receptors also exist on the other cells. Therefore, we assumed that megakaryocytes may have transferrin receptors and iron may affect the growth of megakaryocytes. We examined the proliferation of rat megakaryocytic cells with the presence of 50, 150, 300 µg/ml iron-saturated transferrin in a plasma clot culture system. According to our preliminary results, iron might not be required for the proliferation of megakaryocytic cells in vitro.

Key Words: Iron, Transferrin, Megakaryocytopoiesis, Bone marrow.

Introduction

Megakaryocytopoiesis involves proliferation of progenitor cells, formation of a poliploid nucleus and production of platelets (1). At some stages after commitment to the megakaryocyte lineage, megakaryocyte precursors stop cell division but continue to replicate their DNA creating poliploid cells with up to 32 times (64N) the usual complement of DNA. Once endomitosis stops, a massive increase in cytoplasm occurs and finally platelets are formed from megakaryocyte cytoplasm (2). Regulation of megakaryocytopoiesis and platelet production involve the interplay of soluble mediators as well as cell to cell interactions between megakaryocyte progenitors, mature megakaryocytes and bone marrow stromal cells (3). Since megakaryocytopoiesis is a complex phenomenon, which hormone and/or cellular factors affect the system, and where and how they affect are not clearly understood yet.

It has been suggested that megakaryocytopoiesis is similar to erythropoiesis, and 2N megakaryocyte precursors are equal to CFU-E by function and both cell types mature completely after 6-8 mitosis. The difference is that after maturation megakaryocytes become polyploid and their cytoplasms dissolve to give platelets (4). It is well known that iron is a required element for the proliferation of erythrocytes (5, 6). In those patients with iron deficiency anemia, thrombocytosis is usually seen, but thrombocytopenia is also observed (7). Therefore, no consensus has yet been reached about the role of iron in megakaryocytopoiesis.

Iron, which is transported to cells with transferrin (Tf) is obtained by receptor mediated endocytosis (8, 9). Cell surface receptors for Tf were initially characterized on cells with high iron requirements including hemoglobin producing cells and placental cells. It has been claimed that Tf receptors are present on the other cells also (10). We postulated that megakaryocytic cells might have Tf receptors and iron could affect mitosis and/or growth of these cells. We therefore studied the proliferation of megakaryocytes in vitro with the presence of 50, 150, 300 µg/ml iron bound Tf.

Materials and methods

Total of 20 Sprague Dawley rats weighing 150-200 g were used to obtain bone marrow cells. Experiments

included four groups: control with saline (n=6), 50 µg/ml iron bound Tf (Sigma) (n=6), 150 µg/ml iron bound Tf (n=6), 300 µg/ml iron bound Tf (n=6).

Under ether anesthesia, 20-30 mm length of femurs were cut and muscular tissues cleaned off. Bone marrow was flushed with Leibovitz (L)-15 (Sigma) from the femurs into a graduated and sterile plastic tube. The sample was homogenized, centrifuged at 4 °C at 2500 rpm for 5 minutes and the supernatant was discarded. The cells within the remaining 2 ml fluid was homogenized again and the nucleated cells were counted with a Coulter counter.

A modification of the method of Nakeff et al. was used to culture the bone marrow cells in 96 well plastic plates (11). Each 0.3 ml plasma clot culture contained the following percentages (v/v) of these ingredients: 50 % L-15, 20 % rat serum (Sigma), 10 % bone marrow cells (10^6 /ml), 10 % iron bound Tf or isotonic saline, 10 % rat plasma, respectively. All the cultures were incubated at 37 °C in an atmosphere of 5 % CO_2 in air and 95 % relative humidity for seven days. Each experiment was repeated at least three times.

Plasma clots were transferred daily to glass slides, fixed with 5 % glutaraldehyde and stained by the use of the "direct-coloring" thiocholin method for acethylcholinesterase activity (12). Following a one minute rinse in 0.1 M sodium phosphate, post-fixation in absolute methanol for 10 minutes, and 50 % methanol for 30 seconds, the cells were counterstained in Harris' hematoxylin for two minutes. The slides were then blued by dipping in a 2 % ammonium hydroxide solution. The final preparations containing acethylcholinesterase positive megakaryocytic cells were examined under a light microscope. For the determination of statistical significance two way analysis of variance was used. Results are presented as the MEAN ± SEMEAN.

Results

Figure 1 shows the bone marrow culture environment and acethylcholinesterase positive megakaryocytic cells.

About 140 % increase in the cell number of control group was calculated on the fifth day compared with day zero. When the numbers of megakaryocytic cells on day zero were compared with the numbers on day 5, there were rises of about 105 % in 50 µg/ml, 167 % in 150 µg/ml and 196 % in the 300 µg/ml iron-saturated Tf groups ($p<0.01$, respectively). These results may indicate a healthy culture system presently used.

On the fifth day alone, there was not enough increase in the cell number of 50 µg/ml Tf group when compared with its control ($p>0.05$). Almost no difference in the cell number of 150 µg/ml Tf group on day 5 was seen when compared with its control ($p>0.05$). On day 5, there was not a significant rise in the number of cells of 300 µg/ml iron-saturated Tf (Figure 2) when compared with its control group on the same day ($p>0.05$).

Discussion

The role of iron in thrombopoiesis has long been disputed (7). Both thrombocytosis and thrombocytopenia

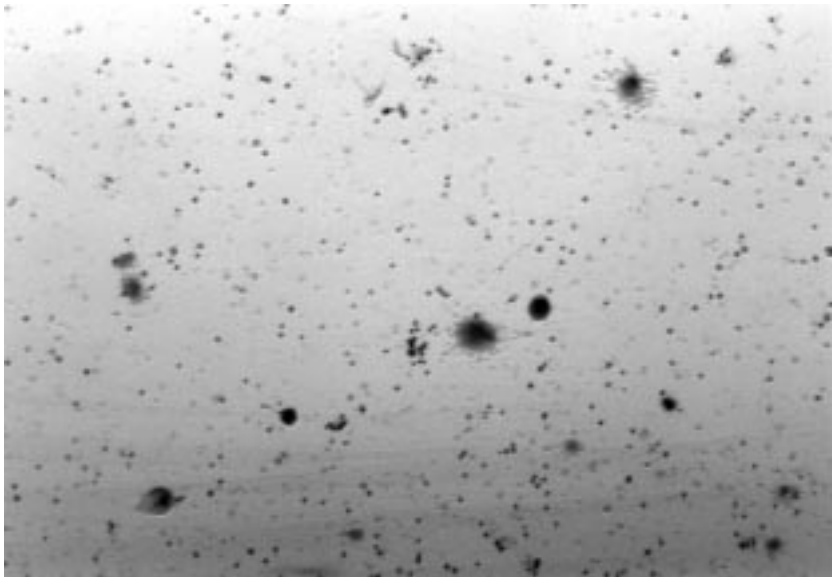

Figure 1. Seven-day old bone marrow culture environment and acetylcholinesterase positive megakaryocytic cells (x 200).

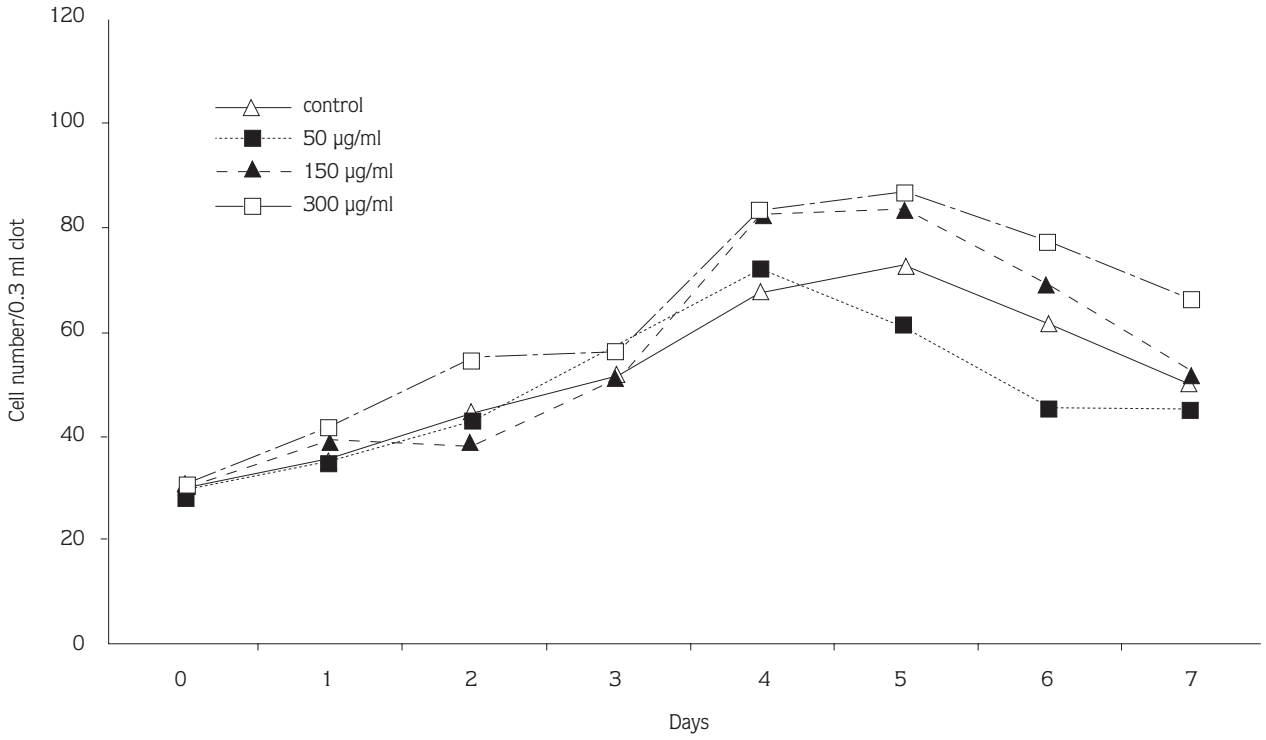

Figure 2. The number of megakaryocytic cells in plasma clot culture system containing 50, 150, 300 µg/ml iron bound Tf and of control group ($p>0.05$, $X_{mean} \pm SEM$).

have been reported in patients with iron deficiency anemia. Although it has been suggested that these apparently contradictory observations can be resolved if iron plays both a synthetic and a regulatory role in thrombopoiesis, the mechanisms involved are not well understood (13).

Acute blood loss in guinea pigs resulted in a 1.2 fold increase in platelet count while concomitant iron administration produced even higher increase in the platelet number (14). Although it has been reported that iron-containing proteins, hemin and Tf, are important components in both megakaryocyte and platelet production (15), and that iron is essential for megakaryocytopoiesis and thrombopoiesis (7), our results did not support the idea that iron is a required element for the megakaryocyte proliferation. We suggest that megakaryocytes might not have a Tf receptor.

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