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INTERMITTENT FEEDING AS A FACTOR ENHANCING HEMOPOIETIC STEM CELL PROLIFERATION AND SPLEEN COLONY FORMATION IN IRRADIATED MICE

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Abstract

The influence of metabolic stimulation induced by a 3 weeks' adaptation of the animals to intermittent food intake on hemopoietic stem cells was investigated in mice. The methods used included transplantation of bone marrow to lethally irradiated recipients, assay of CFUs number, seeding efficiency, and incorporation of ^{125}iododeoxyuridine into the DNA of spleen cells. A stimulatory effect of the metabolically influenced hemopoietic environment on the proliferative activity in stem cell compartments and on the recovery of hemopoietic organs was demonstrated. These stimulatory effects were most marked when the bone marrow of metabolically influenced donors was transplanted to similarly influenced recipients.

Our recent investigations have shown that adaptation of mice during 2 to 3 weeks to intermittent food intake enhances their resistance to gamma-irradiation, as manifested by increased post-irradiation survival and a more effective recovery of some hemopoietic indices (6–8). These effects are probably mediated by some adaptive metabolic consequences of the altered food intake regimen, including utilization of larger amounts of nutrients at a time, increased metabolic activity of tissues and enhanced formation of energy reserves (3). In order to understand better the hemopoietic conditioning of radioprotective effects of the altered metabolic state, the influence of intermittent feeding on the behaviour and/or properties of hemopoietic stem cells was studied. The exocolonizing test was used enabling estimation of the number of colony-forming cells and of the seeding efficiency of syngeneic transplanted bone marrow stem cells. Furthermore, the repopulating ability of bone marrow was measured in terms of splenic ^{125}iododeoxyuridine uptake after grafting. The repopulating capacity of the graft of the bone marrow from metabolically influenced donors was studied as well as the effects of metabolically influenced host environment on the graft.

Material and Methods

Animals and experimental regimen of intermittent feeding (IF). Male (CBA/JPh × C57BL/10ScSnPh)F_1 mice were kept under controlled lighting (12 h light : 12 h darkness; 6 a.m.—6 p.m.—6 a.m.) at a temperature of 22±2°C; 20 animals per cage. At the age of 12 months the mice were during 3 weeks subjected to alternating periods of 24 h fasting and subsequent 24 feeding (realimentation). The animals consumed standard pelleted laboratory diet with a carbohydrate content of about 50 cal%. Food was always given at the beginning of the light period, water was offered ad libitum. Control (C) animals were fed without interruption. The integral consumption of food in intermittently fed animals and the body weight at the end of experimental regimen did not differ from ad libitum fed

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Table 1

Numbers of spleen colonies (CFUs), spleen weight values, and seeding efficiency in controls (C) and intermittently fed (IF) mice after irradiation and transplantation of 1×10^5 nucleated femoral marrow cells from controls or intermittently fed donors

Donor	Controls		Intermittently fed mice	
Recipient	C	IF	C	IF
Colonies number	10.62±1.03	13.48±1.59	14.05±0.93*	14.96±0.95**
Spleen weight (mg)	34.91±1.97	43.34±2.77*	44.12±1.93**	45.99±3.65*
Seeding efficiency (%)	8.19±0.76	3.84±0.67**	7.82±0.48	4.32±0.37**

* $p<0.05$; ** $p<0.01$, compared with animals not subjected to intermittent feeding (C–C). For the determination of mean values 10 to 15 mice were used. Evaluation by Student's t-test.

animals. The experimental animals thus compensated for food deficiency during fasting days by a higher food intake in days of realimentation.

Irradiation. The mice were whole-body irradiated (^{60}Co gamma-ray source, dose rate 0.5 Gy/min) with a total dose of 8.2 Gy (experimental group always after 3 weeks of intermittent feeding and a 24 h period of realimentation).

Bone marrow cell preparation and transfusion. The bone marrow suspensions were made by flushing the femurs with ice-cold cultivation medium (M 199, USOL, Prague), pH 7.35, strained through a nylon gauze filter and resuspended to yield the appropriate concentration of cells. This was checked by counting the cells in a Coulter Counter. Suspensions of bone marrow cells, prepared from approximately 10 donors, were injected intravenously into mice irradiated 1 to 2 h previously. In all experiments 40 U heparin was injected intraperitoneally to recipient mice 10 min before cell injection.

Spleen colonies. Nine days after irradiation and bone marrow cell injection the recipient animals were killed, their spleens excised, weighed and fixed in Bouin's solution. Surface colonies with a diameter of 0.25 mm and more were scored.

Seeding efficiency. The fraction of injected colony-forming cells seeding the spleen of irradiated recipients was determined by the double-transplant method according to LAHIRI & VAN PUTTEN (10) at 24 h following the injection of 1×10^5 marrow cells. The number of CFUs appearing in the recipient spleen (cell suspensions from about 10 spleens were prepared and injected similarly as bone marrow suspensions, after mincing the spleens with fine scissors) at this time interval was compared with the total number of CFUs in the original marrow inoculum as determined by assay in a separate group of irradiated recipients. The number of splenic CFUs was then expressed in relation to the total number of CFUs injected.

Assay of cell proliferation (4) consisted in ^{125}iododeoxyuridine (^{125}IUdR) incorporation into the spleen 3 and 5 days after transfusion of the bone marrow cells. ^{125}IUdR, a thymidine analogue, is specifically incorporated into DNA and is used as a label for proliferating cells. The mice were given intraperitoneally 3.7×10^4 Bq ^{125}IUdR (Amersham International, Amersham, England) in 0.4 ml saline. In order to inhibit endogenous thymidilate synthesis, 10^{-7} mol of 5-fluoro-2′-deoxyuridine (SERVA, Feinbiochemica, Heidelberg, FRG), was injected intraperitoneally 30 min before ^{125}IUdR (5, 14). Six hours after the isotope injection the mice were killed, the spleens were excised, weighed and placed in 10% buffered formalin for 48 h to remove radioactive iodine not incorporated into DNA (2). The activity of spleens was measured using the Nuclear Chicago Automatic Well Counting System and expressed as per cent of the total activity administered.

Statistics. The values given in the table and the figures represent the mean±SE. Statistical significance of the results was evaluated using the Student's t-test and two-factor analysis of variance.

Results

The experimental protocol included controls, metabolically influenced donors and bone marrow recipients. These variants enabled us to discriminate the roles of intrinsic (preselection of CFUs) and extrinsic (environmental changes) factors. The effects of the altered regimen of food intake on colony formation and seeding efficiency of CFUs are sum-

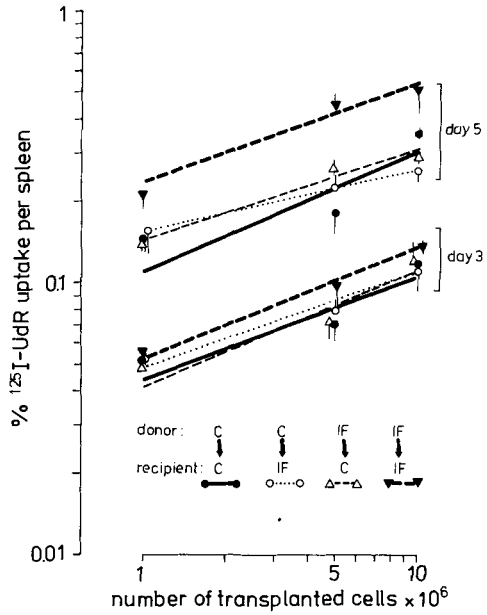

Fig. 1. Incorporation of ^{125}IUdR into DNA of splenic cells of mice (control or fed intermittently) subjected to different metabolic treatments on days 3 and 5 after irradiation and transplantation, in relation to concentration of injected femoral marrow cells from controls (C) or intermittently fed (IF) donors. Each point represents 5 to 7 animals.

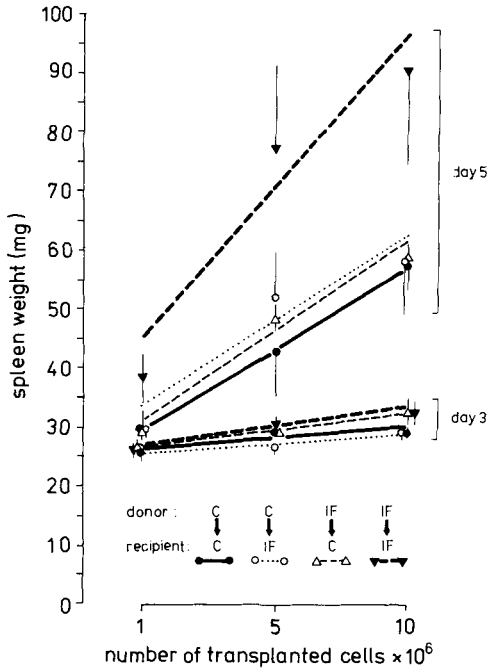

Fig. 2. Spleen weight values of mice on days 3 and 5 after irradiation and transplantation, found in the same experiment as summarized in Fig. 1. The arrangement and symbols used are identical.

marized in the Table. A significantly higher spleen colony yield, and a spleen weight increase (9th day after irradiation) were found after injection of bone marrow cells from metabolically influenced donors, irrespective of the host conditions (IF–C, IF–IF). In a system using control bone marrow transplantate and metabolically influenced recipients (C–IF) the mean production of spleen colonies was also higher, but not statistically significant. Nevertheless the significant spleen weight increase suggests enhanced repopulation processes in these conditions. The given data of spleen colony production denote the CFUs numbers per 10^5 of injected cells. As no differences in femoral cellularity were found between control and metabolically influenced donors, the data illustrate relative differences in the content of CFUs in whole femurs. The mean background colony counts found in simultaneously irradiated non-transfused mice were smaller than 1, did not differ in controls and metabolically influenced animals, and were thus not taken into account. The seeding efficiency of injected colony-forming cells is also shown in the Table. A significant reduction in splenic seeding (about 50%) was found when metabolically influenced mice were used as recipients. The stem cells from controls and metabolically influenced donors injected to control recipients were characterized by similar seeding efficiencies.

The early proliferative capacity of the settled hemopoietic cells of the graft is illustrated in Fig. 1. The proliferative activity was measured by incorporation of the specific DNA precursor into spleen cells. Varying numbers of bone marrow cells were injected to irradiated recipients and the tracer uptake per whole spleen was measured on days 3 and 5 after irradiation. In all experimental variants the log of IUdR incorporation into spleen increased linearly with the log of number of cells injected. Analysis of variance revealed a significant increase in proliferative activity on days 3 and 5 ($p<0.01$) in metabolically influenced donors and recipients of the bone marrow (IF–IF). The background level of the tracer uptake in the spleens of non-transfused mice on days 3 and 5 after irradiation amounted to 7 to 8 per cent of the smallest activity achieved when injecting 1×10^6 of control marrow cells, was not influenced by the metabolically modified milieu and could thus be neglected. Spleen weights were monitored under the same conditions as the indices of proliferative activity (Fig. 2). Again, analysis of variance showed as statistically significant the greater spleen weight in metabolically influenced mice injected with bone

marrow from similarly influenced donors (IF–IF), as compared with controls (C–C) on day 5 after irradiation ($p<0.01$).

Discussion

The results suggest that stem cell compartments of the bone marrow as well as the inductive hemopoietic environment of the spleen may be modified by the effects of intermittent feeding. The increased spleen colony formation in control mice injected with the influenced grafts indicate an increased number of pluripotent stem cells in the bone marrow of intermittently fed mice. Furthermore, retransplantation experiments have shown effects of intermittent feeding on host spleen environment. Surprisingly, in the metabolically influenced recipients a lower seeding efficiency was found in both control and experimental bone marrows, in spite of the greater yield of spleen colonies measured on day 9 after irradiation. Similarly BERAN & TRIBUKAIT (1) described a lowering of seeding efficiency of control bone marrow in the spleens of irradiated mice pretreated by prolonged hypoxia, which is known to increase the capacity of self-renewal and generation of descendent cells determined 10 to 14 days later. These observations agree with the suggestion of MONETTE & DEMELLO (12) suggesting that colony-forming cells in cycle exhibit reduced spleen seeding when compared with normal marrow. One can hypothesize that the decrease of seeding efficiency as measured by the double-transplant method at 24 h after the injection of marrow cells is conditioned by the switching of transplanted CFUs into cell cycle induced by the metabolically influenced host environment. This assumption is supported by our findings indicating an enhanced cell proliferation on days 3 and 5 in the spleens of metabolically influenced recipients of the similarly influenced grafts. No such effect was, however, evident when control grafts were transplanted to metabolically influenced hosts. Perhaps, the early manifestation of the supposed proliferative stimulus needs some cooperation of the host environment and a preselection of CFUs endowed with a greater capacity for proliferation. Transplantable stem cells are not homogeneous with respect to potential for proliferation and differentiation (9, 10). Metabolically influenced grafts may contain higher numbers of cells which exhibit, in a proper environment, a higher proliferation rate. However, this does not mean that a metabolically influenced environment could not increase the repopulating ability of control-uninfluenced grafts at later post-irradiation intervals, i.e. after a longer time, which enables the manifestation of the proliferative stimulus. Increased spleen weights in metabolically influenced and irradiated hosts injected with control grafts support this suggestion (see the Table).

The results obtained demonstrate a stimulatory effect of the environment of metabolically influenced animals on the proliferative activity of hemopoietic stem cells at the time of irradiation and at early intervals following irradiation. Thus they elucidate more closely the mechanism by which a faster repopulation of cells of the hemopoietic system is enabled in metabolically influenced animals in post-irradiation conditions (6, 8), and so contribute to a higher radiation resistance (6, 7). However, experiments of the type now reported do not allow deduction of more detailed mechanisms induced by the intermittent food intake, i.e. effects of the activated energy (8, 11, 13, 16) and particularly lipid metabolisms (8, 13, 15) on the proliferative activity of hemopoietic tissue cells in vivo. These mechanisms will therefore be the subject of further investigation.

REFERENCES

1. BERAN M. and TRIBUKAIT B.: Modification of the proliferative capacity of transplanted bone marrow colony forming units by changes in the host environment. J. Cell Physiol. 84 (1974), 57.
2. BÜRKI K., SCHAER J. C., GRIEDER A., SCHINDLER R. and COTTIER H.: Studies on liver regeneration. I. ^{131}Iododeoxyuridine as a precursor of DNA in normal and regenerating rat liver. Cell Tissue Kinet. 4 (1971), 519.
3. FÁBRY P.: Feeding pattern and nutritional adaptations. Academia, Prague 1969.
4. HÜBNER G. E., WANGENHEIM K.-H. and FEINENDEGEN L. E.: An assay for the measurement of residual damage of murine hematopoietic stem cells. Exp. Hematol. 9 (1981), 111.
5. HUGHES W. L., COMMERFORD S. L., GITLIN D. et coll.: Deoxyribonucleic acid metabolism in vivo. I. Cell proliferation and death as measured by incorporation and elimination of iododeoxyuridine. Fed. Proc. 23 (1964), 640.
6. KOZUBÍK A. and POSPÍŠIL M.: Adaptation to intermittent fasting as a factor modifying the radiation resistance of mice. Experientia 38 (1982), 958.
7. — — Protective effect of intermittent fasting on the mortality of gamma-irradiated mice. Strahlentherapie 158 (1982), 734.
8. — — and HOŠEK B.: Stimulatory effect of intermittent feeding on hemopoietic recovery in sublethally

gamma-irradiated mice. Acta radiol. Oncology 24 (1985), 199.
9. KRETCHMAR A.-L. and CONOVER W. R.: A difference between spleen-derived and bone marrow-derived colony-forming units in ability to protect lethally irradiated mice. Blood 36 (1970), 772.
10. LAHIRI S. K. and VAN PUTTEN L. M.: Distribution and multiplication of colony forming units from bone marrow and spleen after injection in irradiated mice. Cell Tissue Kinet. 2 (1969), 21.
11. LEVEILLE G. A. and CHAKRABARTY K.: Diurnal variations in tissue glycogen and liver weight of meal-fed rats. J. Nutr. 93 (1967), 546.
12. MONETTE F. C. and DEMELLO J. B.: The relationship between stem cell seeding efficiency and position in cell cycle. Cell Tissue Kinet. 12 (1979), 161.
13. PETRÁSEK R., POLEDNE R., VAVREČKA M. and SLABOCHOVÁ Z.: Dynamics of changes of fat formation and some other metabolic indicators after infrequent feeding. *In:* Proceedings of the Eighth International Congress, September 1969, p. 285. Edited by J. Mašek, K. Ošancová and D. P. Cuthbertson. Excerpta Medica, Amsterdam 1970.
14. TAKADA A., TAKADA Y., KIM U. and AMBRUS J. L.: Bone marrow, spleen, and thymus regeneration patterns in mice after whole-body irradiation. Radiat. Res. 45 (1971), 522.
15. TEPPERMAN H. M. and TEPPERMAN J. T.: Adaptive hyperlipogenesis. Fed. Proc. 23 (1964), 73.
16. — FÁBRY P. and TEPPERMAN J.: Effect of periodic fasting and refeeding on rat kidney cortex enzymes, gluconeogenesis and water intake pattern. J. Nutr. 100 (1970), 837.