Fate of DNA-Labelled Bone-Marrow Cells from Different Mouse Strains Injected into Syngeneic Irradiated Recipients

Bone-marrow cells injected into irradiated recipients are known to repopulate myelopoietic and eventually lymphopoietic organs. The pathways by which this repopulation occurs have been studied using chromosome markers[1–4], but the early reactions of uptake of the injected cells are still poorly understood. Thus, it is disputed whether repopulation of the thymus occurs directly by cells taken up by this organ[4] or by cells first fixed in the bone-marrow[3]. As discussed in more detail below, chromosome markers are not entirely suitable for detecting the behaviour of the behaviour of the cells early after injection; we have, therefore, followed uptake and retention by spleen, thymus and bone-marrow of bone-marrow cells labelled in their DNA by injecting ^{3}H-thymidine to the donors.

Methods. The following inbred mouse strains raised in our laboratory were studied (AKR (LD 50/30 650 R), BALB/c (LD 50/30 575 R) and C 57 Bl (LD 50/30 725 R). In order to compare the results on the AKR strain with earlier cytogenetic data, marrow from AKR was injected into AKR/T 1 Ald. All animals were of 3 months age. The donors were given i.p. 150 μCi of ^{3}H-6-thymidine (specific activity 10 Ci/ml) divided into 2 equal doses 12 and 24 h respectively before sacrifice. These intervals were chosen in order to label as many as possible of the cells capable of entering into S phase. The recipients were whole-body X-irradiated with a dose of 650 R (sode rate 100 R/min, 250 KV, 20 mA, HVL 2 mm Cu, FSD 57 cm) and were injected immediately thereafter i.v. with 10^7 viable nucleated cells isolated from the femur of the donors. At different times after injection (1, 4 and 24 h, 3 and 6 days), recipients were sacrified, spleen, thymus and femoral marrow removed and homogenized in 0.5 ml of water. Perchloric acid was then added to yield a 0.6 *M* solution in order to precipitate proteins and nucleic acid. DNA was isolated from the precipitate by the Schneider method as recommended in[5], i.e. extracting DNA in 0.5 ml of 1.2 *N* hot perchloric acid. The activity of the extracted material was determined by liquid scintillation counting and its content of DNA by Giles[6] method.

Results. Data on weight and DNA content of spleen and thymus and on DNA content of the bone-marrow isolated from both femurs are shown in the Table. AKR mice have a larger thymus and a smaller spleen than the other strains (DNA content and weight 1 h after irradiation, i.e. the values corresponding to non-irradiated animals). The relative decrease in weight and in DNA content is, however, similar for all strains studied, but recovery of the spleen during the 6-day observation period is most marked for C 57 Bl and virtually absent for AKR mice.

Data on total activity of the 3 organs are presented in Figures 1–3 and corresponding data on specific activity are shown in Figures 4–6. Uptake of activity proceeds rapidly and is essentially complete after 4 h. Highest activities are found in the spleen, i.e. about 8% of the dose injected. Thymus takes about 0.5–1.2% and bone-marrow of the 2 femurs about 0.6–0.8% of the injected cells. Differences in uptake between strains relate mainly to uptake by thymus which is high for AKR and lower for the other strains. If one considers the specific activity of the DNA, the differences between the strains largely disappear. During the first day after irradiation, that is during the time when weight and DNA content of the organs diminish mainly as a result of interphase death of cells, most of the injected radioactivity is conserved. This results in an increase in specific activity of DNA isolated. Activity is lost slowly during the whole post-irradiation period, most rapidly from spleen and more slowly from thymus and bone-marrow. The decline in specific activity is also most pronounced in spleen and only slight or absent in thymus.

Discussion. Studies using chromosome markers of bone-marrow cells have provided important information

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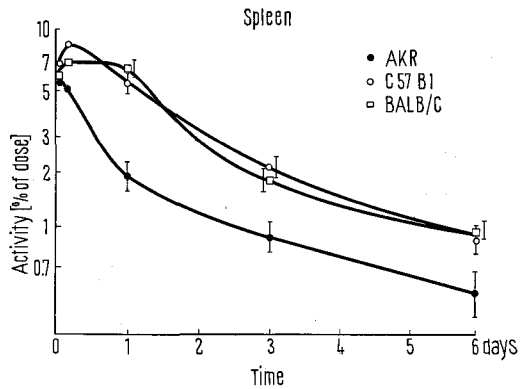

Fig. 1. Activity (in % of dose injected) as function of post-irradiation time (days) in spleen of different strains of mice (AKR ●; C57 Bl ○; BALB/c □) injected with 1×10^7 DNA labelled bone-marrow cells ($\approx 45 \times 10^3$ dpm) immediately after whole-body irradiation with 650 R. Values given represent means and standard deviation of the mean. For the 1 and 4 h period, standard deviation is not shown in order not to render the design confusing. (It is in the order of ≈7%.)

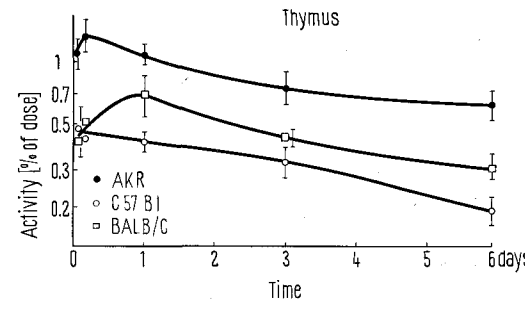

Fig. 2. Activity (in % of dose injected) as function of post-irradiation time (days) in thymus of different strains of mice (AKR ●; C57 Bl ○; BALB/c □) injected with 1×10^7 DNA labelled bone-marrow cells ($\approx 45 \times 10^3$ dpm) immediately after whole-body irradiation with 650 R. Values given represent means and standard deviation of the mean. For the 1 and 4 h period, standard deviation is not shown in order not to render the design confusing. (It is in the order of ≈7%.)

Weight and DNA content of thymus, spleen and bone-marrow (DNA content of femurs) in 3 mouse strains after irradiation

Time after exposure to 650 R		Thymus			Spleen			Bone-marrow (both femurs)		
		AKR	C57Bl	BALB/c	AKR	C57Bl	BALB/c	AKR	C57Bl	BALB/c
1 h	Weight (mg)	117 ± 3	59 ± 3	67 ± 6	89 ± 6	131 ± 6	107 ± 8			
	DNA content (μg)	1960 ± 100	950 ± 60	700 ± 40	1170 ± 25	1890 ± 30	1270 ± 80	152 ± 10	281 ± 30	215 ± 33
4 h	Weight (mg)	123 ± 9	54 ± 4	66 ± 7	72 ± 4	110 ± 8	93 ± 5			
	DNA content (μg)	2010 ± 120	1040 ± 60	770 ± 40	905 ± 40	1790 ± 100	1190 ± 70	138 ± 7	344 ± 35	225 ± 20
24 h	Weight (mg)	58 ± 3	47 ± 2	42 ± 4	45 ± 3	61 ± 3	52 ± 3			
	DNA content (μg)	410 ± 20	196 ± 20	190 ± 20	235 ± 35	330 ± 35	454 ± 30	71 ± 5	125 ± 12	110 ± 13
3 days	Weight (mg)	41 ± 6	45 ± 4	34 ± 7	39 ± 2	47 ± 3	44 ± 4			
	DNA content (μg)	210 ± 30	135 ± 10	97 ± 19	206 ± 15	270 ± 30	313 ± 11	40 ± 3	104 ± 25	84 ± 10
6 days	Weight (mg)	49 ± 4	57 ± 4	40 ± 7	54 ± 6	142 ± 4	95 ± 10			
	DNA content (μg)	175 ± 27	175 ± 20	115 ± 20	289 ± 27	1470 ± 35	890 ± 100	30 ± 1.7	68 ± 16	85 ± 4

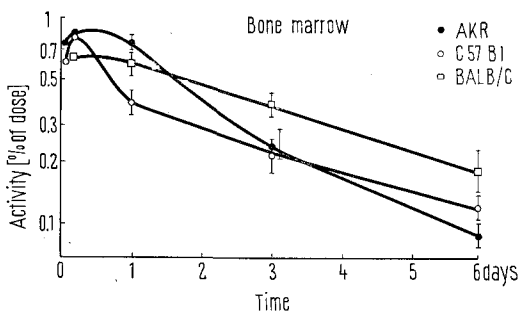

Fig. 3. Activity (in % of dose injected) as function of post-irradiation time (days) in bone-marrow of different strains of mice (AKR ●; C57Bl ○; BALB/c □) injected with 1×10^7 DNA labelled bone-marrow cells ($\approx 45 \times 10^3$ dpm) immediately after whole-body irradiation with 650 R. Values given represent means and standard deviation of the mean. For the 1 and 4 h period, standard deviation is not shown in order not to render the design confusing, (It is in the order of ~7 %.)

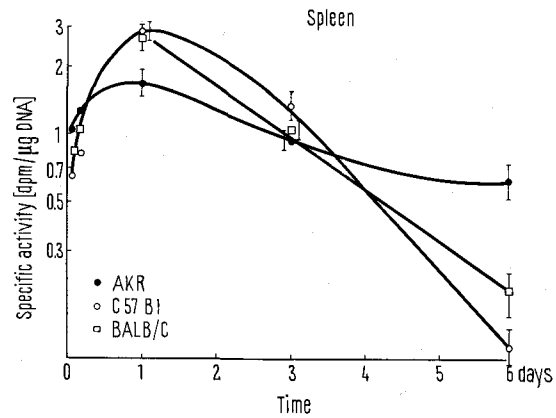

Fig. 4. Specific activity (dpm/μg DNA) as function of post-irradiation time (days) in spleen of different strains of mice (AKR ●; C57 Bl ○; BALB/c □) injected with 1×10^7 DNA labelled bone-marrow cells ($\approx 45 \times 10^3$ dpm) immediately after whole-body irradiation with 650 R. Values given represent means and standard deviation of the mean. For the 1 and 4 h period, standard deviation is not shown in order not to render the design confusing. (It is in the order of ≈7%.)

concerning the fate of these cells in irradiated[1–4] and non-irradiated[7] recipients and on the origin of the cells repopulating myelopoietic and lymphopoietic organs in irradiated animals. Nevertheless, the cytogenetic technique has certain shortcomings: it can only be applied in strains where suitable markers are available; it takes in account only cells which are dividing and thus does not provide truly quantitative information concerning the ratio of different cell populations; and finally it demands a considerable effort in time.

Determination of the fate of radioactivity from cells labelled by injection of ^{3}H-thymidine to the donor is easily carried out in all strains, yields information already during the time when the injected cells are not yet dividing, and also allows us to determine the relationships of different cell population independently of their rate of cell division. On the other hand, it remains uncertain whether the cells responsible for repopulating the lympho- and myelopoietic organs are labelled at all under these conditions and whether the different species of labelled bone-marrow cells all behave in the same way. Moreover, one must consider reutilization of label by other cells and the possibility of radiation damage to the injected cells by the incorporated activity.

Reutilization of label from cells broken down is probably negligible during the early time after irradiation, since cell division is stopped. Radiation damage to injected cells seems unimportant in view of the small amount of activity injected (about 20 μCi) and of the efficient repopulation of spleen by the injected cells. An unambigous interpretation of the data in terms of distribution of colony forming cells will, however, be possible only on the basis of a comparison of data on radioactivity (including autoradiographic ones) and those on cytogenetics. Nevertheless, the results presented allow, in our opinion, not only a comparison of uptake of cells between different strains and different organs but also certain conclusions concerning possible transfer between organs.

The following factors must be considered in interpreting the curves shown. A change in total activity represents uptake or loss (migration or breakdown) of labelled cells. An increase in specific activity as seen early after irradiation reflects preferential destruction of radiation-damaged non-labelled cells of the recipient. The decrease in specific activity at a later time, so far as it is not a result of changes in total activity and therefore of cell loss, follows DNA replication and therefore regeneration.

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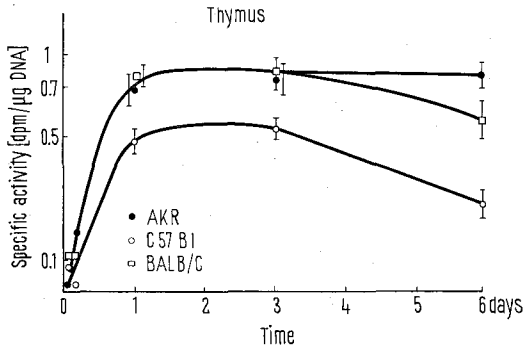

Fig. 5. Specific activity (dpm/µg DNA) as function of post-irradiation time (days) in thymus of different strains of mice (AKR ●; C 57 Bl ○; BALB/c □) injected with 1×10^7 DNA labelled bone-marrow cells (≈ 25–30×10^8 dpm) immediately after whole-body irradiation with 650 R. Values given represent means and standard deviation of the mean. For the 1 and 4 h period, standard deviation is not shown in order not to render the design confusing. (It is in the order of $\approx$ 7%.)

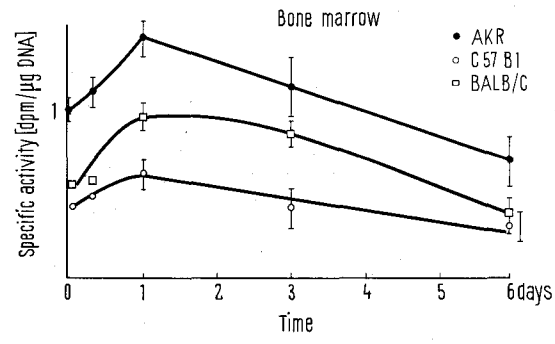

Fig. 6. Specific activity (dpm/µg DNA) as function of post-irradiation time (days) in bone-marrow of different strains of mice (AKR ●; C 57 Bl ○; BALB/c □) injected with 1×10^7 DNA labelled bone-marrow cells ($\approx 45 \times 10^8$ dpm) immediately after whole-body irradiation with 650 R. Values given represent means and standard deviation of the mean. For the 1 and 4 h period, standard deviation is not shown in order not to render the design confusing. (It is in the order of $\approx$ 7%.)

The data presented indicate, therefore, that bone-marrow cells are capable of entering spleen, bone-marrow and thymus, the organs being enumerated in order of importance of uptake. During the experimental period of 6 days a certain percentage of the cells is lost (70–90%) in spleen and bone-marrow and 50% in thymus but it is evident that cells lost by the bone-marrow and 50% in thymus but it is evident that cells lost by the bone-marrow cannot account for the labelled cells in thymus. Regeneration during the period of observation commences in spleen and is most marked for C 57 Bl and BALB/c mice[8].

Zusammenfassung. Knochenmarkszellen, durch Injektion von ^{3}H-Thymidin in syngenischen Spendern markiert, wurden in bestrahlte Empfänger von AKR-, C 57 Bl- oder BALB/c-Mäuse injiziert, wobei grösste Aktivität in der Milz, dann im Knochenmark und im Thymus gefunden wurde. Aktivitätsverlust war während der 6tägigen Beobachtungsperiode in Milz und Knochenmark am stärksten. Auch die Regeneration und die Veränderungen der spezifischen Aktivität der DNA dieser Organe waren bei C 57 Bl- und BALB/c- Mäusen am ausgeprägtesten. Die markierten Spenderzellen im Thymus scheinen nicht von Zellen zu stammen, die über das Knochenmark dorthin transportiert wurden, sondern wurden unmittelbar vom Thymus aufgenommen.

G.B. Gerber, A. Decleve, J.R. Maisin, A. Léonard and Gh. Mattelin

Euratom and Radiobiology Department, C.E.N./S.C.K., B-2400 MOL (Belgium), 26 July 1971.

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The Effect of Protein Biosynthesis Inhibitors upon the Development of Cellular Membranes Hyperpolarization

The purpose of the present work was to study the effect of injuries at various steps of protein biosynthesis process upon the development of a cellular membrane hyperpolarization.

Experiments were conducted on various kinds of cells, such as single muscle fibres and neurons of the sensomotor cortex region. The inhibition of protein biosynthesis was reached by administration of actinomycin D (inhibiting the synthesis of messenger RNA on structural genes); of puromycin (blocking the protein synthesis in ribosomes); of ribonuclease (destroying the molecules of RNA)[1].

Methods. Experiments were performed on 280 albino rats aged 8–10 months. The membrane potential of muscle fibres of the m. gastrocnemius and of neurons of the sensomotor cortical region was measured by microelectrodes in situ, according to the universally adopted methods. The hyperpolarization of m. gastrocnemius fibers was effected by i.p. administered insulin (0.16 U/100 g of body wt.), deoxycorticosterone acetate – DOCA (500 µg/100 g), estradioldipropionate – EDDP (100 µg/100 g) and by the denervation of this muscle (cross-section of the n. ischiadicus). Actinomycin D (ACM-D) was administered i.p. at a dose of 1–10 µg/100 g; puromycin at a dose of 50–100 µg/100 g; RNA-ase – 300 µg/100 g. In the experiments with reproduction of hyperpolarization of the sensomotor cortex cells, the protein biosynthesis inhibitors were applied to the investigated area with ACM-D (0.5×10^{-5}), RNA-ase (1×10^{-4}). For inhibition of various links of energy exchange we used sodium fluoride (0.01 *M*/100 g), monoiodacetate (0.005 *M*/100 g), 2, 4-dinitrophenol (0.002 *M*/100 g).

Results and discussion. Administration of insulin, estradioldipropionate, DOCA as well as denervation cause a distinct hyperpolarization of the muscular fibres (Table I). Administered doses of ACM-D, RNA-ase and

[1] These experiments were conducted in cooperation with L. A. Gromov, V. G. Korotonozhkin and O.A. Martynenko.