by the combined treatment was less than after X-radiation alone.

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Zusammenfassung. Nachweis, dass i.p.-Injektion von Urethan und Ganzkörper-Röntgenbestrahlungen bei Ratten eine potenzierte leukämogene Wirkung hat, während die Zahl der entstandenen Haut- und Mamma-Tumoren geringer war als bei der Einzelbehandlung.

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Survival and Endogenous Colony Formation in Irradiated Mice Grafted with Normal or Infectious Mononucleosis Bone Marrow

Previously we attempted[1,2] to induce growth of a human haemopoietic cell line in lethally irradiated mice. Since cytotoxic drugs were found to be unable to prevent graft versus host reaction[3], an antihuman antilymphocytic serum was given after the transfer of human bone marrow. In most of the recipient animals, haemopoietic spleen colonies were found. Chromosomal analysis of these spleen nodules revealed a mouse karyotype suggesting an enhanced endogenous stemcell proliferation.

The present investigations deal with the influence of two irradiation doses and try to evaluate the nature of the colony inducing factor in human bone marrow. An increased activity was noted in the bone marrow derived from three infectious mononucleosis patients.

Adult NMRI (Naval Medical Research Institute) mice were used and received a total body irradiation of 850 or 975 rad as described previously[1]. Human bone marrow or buffy coat cells werve given within 3 h after the irradiation. The surviving animals were killed at day 9 and the spleen nodules counted under a dissecting microscope. In some experiments the grafted recipient animals also received 0.10 ml of a horse derived antihuman antilymphocytic serum (ALS), lymphotoxicity titer 1/3000–1/8000.

Results. Heavily irradiated mice (975 r) grafted with human bone marrow showed an improved 9 day survival. This was further enhanced by an antihuman ALS given after the cell transfer (Table I). The highest survival was observed in mice grafted with infectious mononucleosis bone marrow, while none of the animals grafted with cells from different malignant blood diseases (acute leukemia – lymphosarcoma) survived 9 days. Most of the irradiated and ALS treated animals grafted with human bone marrow showed haemopoietic colonies at the spleen surface. ALS alone did not induce colony formation although it slightly increased the survival. Chromosomal analysis of these colonies revealed a pattern of 40 acrocentric chromosomes, typical for a mouse karyotype (Figure).

In contrast to the 975 r irradiated mice, grafting of human cells into 850 r irradiated animals markedly decreased the survival (Table II). When the grafted mice were also treated with ALS, the survival was equal to the irradiated ungrafted recipients, Grafting of infectious mononucleosis bone marrow resulted in a survival similar to the normal human bone marrow and ALS-treated mice. Like in the 975 r, grafting of human cells and ALS treatment resulted in haemopoietic spleen colony formation.

Peripheral blood buffy coat cells derived from infectious mononucleosis patients given to the 850 r irradiated and ALS treated mice gave a doubling of the 9 day survival, compared to normal buffy coat cells (32% versus 16.6%). In both groups spleen colonies were found.

As a control, the influence of different samples of cell-free human plasma (normal – infectious mononucleosis) was tested. Only a small increase in survival and a few colonies were observed. There was a slightly higher survival in the animals treated with normal plasma compared to the mice who received plasma from a infectious mononucleosis patient.

In a last experiment, the colony-inducing ability of in vitro irradiated human bone marrow (10,000 r) injected into 975 r and 850 r irradiated mice was tested. The

[1] A. C. LOUWAGIE and R. L. VERWILGHEN, Nature *225*, 383 (1970).
[2] A. C. LOUWAGIE, R. L. VERWILGHEN and J. MEEKERS, 13th Congress of the International Society of Haematology, Munich 1971, Abstract Volume, p. 267.
[3] E. KELEMAN, Budapest (Hungary), personal communication.

Table I. Endogenous colony formation in 975 r irradiated anti-human ALS treated and /or human bone marrow grafted mice

Grafted bone marrow	ALS	9 day survival %	Animals with splen colonies (%)	Mean number of colonies positive spleen		
				Mean	S.D.	Range
Nihil	–	3.33 (1/30)	0	–	–	–
Nihil	+	13.3 (2/15)	0	–	–	–
Normal	–	11.7 (4/34)	50 (2/4)	1	–	1.1[b]
Normal	+	25 (14/54)	78.5 (11/14)	3.2[a]	2.97	1–11
Infect mononucleosis	+	61.5 (8/13)	75 (6/8)	2.8[a]	3.03	1–8
Malignant blood disease	+	0 (0/90)	–	–	–	–

( ); number of animals. [a] One recipient mice, having spleen colonies too numerous to be counted, was not included in the calculation of the means. [b] Individual number.

Table II. Endogenous colony formation in 850 r irradiated anti-human ALS treated and/or human bone marrow grafted mice

Graft or treatment	Number of cells	ALS	9 day survival (%)	Animals with spleen colonies (%)	Mean number of colonies positive spleen		
					Mean	S.D.	Range
Nihil		—	40 (12/30)	0	—	—	—
Bone marrow							
Normal	5×10^6	—	17.4 (6/35)	33.3 (2/6)	1	—	[a], 1[b]
Normal	5×10^6	+	40 (40/100)	75 (30/40)	1.76[a]	1.03	1–4
Infect. Mononucleosis	1.2×10^6	—	37.5 (6/16)	83.3 (5/6)	3.2	1.30	2–5

( ) Number of animals. [a] One recipient mice, having spleen colonies too numerous to be counted, was not included in the calculation of the means. [b] Individual numbers.

irradiated bone marrow had the same activity as the non-irradiated marrow derived from the same donor, showing that the viability of the graft was not directly involved.

Discussion. Although there is no proof of a take[1,2], a xenograft of human bone marrow increases the survival of the 975 r irradiated mice. A similar protective influence has been observed by LORENZ and CONGDON[4]. The graft, however, was harmful to the 850 r irradiated recipients. Their decreased survival might be due to a host versus graft reaction as a result of a less complete destruction of the host's immunological reactivity[5]. We wonder if the protective effect of the anti-human ALS treatment is due either to an inhibition of the graft versus host reaction or to an aspecific immunosuppressive effect on the host.

Looking at the origin of the graft, a higher survival was found when the graft was derived from infectious mononucleosis patients. In contrast, mice grafted with bone marrow derived from different malignant blood diseases did not survive. Administration of cell-free human plasma to 850 r irradiated mice gave a slightly improved survival. There was only a minimal difference between the mice treated with plasma from normal and infectious mononucleosis patients.

Although a human bone marrow graft in 975 r and 850 r irradiated mice resulted in an opposite influence on the survival, in both groups endogenous spleen colonies were induced. A similar endocolonization has already been described by PONS and PETRAKIS[6] and FISHBARG et al.[7] after injection of human bone marrow and lymphocytes. Our results suggest that the endocolonization might be due to a cell-bound factor, since it was practically absent in the plasma treated mice. The viability of the cells, however, does not seem to be involved since in vitro irradiated bone marrow (10.000 r) was equally active. A similar observation was made by DELMONTE et al.[8], who induced endocolonisation in baboon marrow grafted mice and found lysed baboon cells equally effective.

The higher protective effect of bone marrow and buffy coat cells derived from infectious mononucleosis patients is a remarkable but unexplained phenomenon. As plasma from a mononucleosis patient is rather less effective compared with normal plasma, the effect seems to be cell-bound.

Résumé. L'administration intraveineuse de cellules médullaires ou de «buffy coat» humain induit la formation de colonies hématopoiétiques chez les souris irradiées. L'analyse caryotypique en a démontré l'origine murine. Les phénomènes décrits sont plus prononcés chez les souris traitées avec des cellules provenant de malades atteints de mononucléose infectieuse, mais sont indépendant de la viabilité des cellules.

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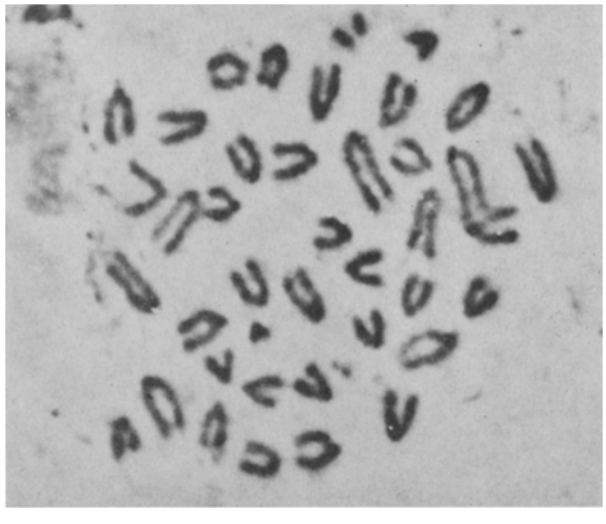

Karyotype in a spleen colony of a human bone marrow grafted and ALS treated 975r irradiated NMRI mice.

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[9] We thank Dr. C. VERMYLEN, director of the Red Cross Blood Transfusion Centre, Leuven, for providing the anti-human ALS. We are grateful to Miss SUZY NELEMANS, Miss MONIQUE RENSON and Mrs. MIA LESAGE-OTTEVAERE for their skilled technical assistance.