DIRECT AND SERIAL TRANSPLANTATION OF HUMAN ACUTE MYELOID LEUKAEMIA INTO NUDE MICE

N. NARA* and T. MIYAMOTO†

From the *1st Department of Internal Medicine, Tokyo Medical and Dental University, Tokyo, and the †Division of Hospital, National Institute of Radiological Sciences, Chiba, Japan

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ATHYMIC NUDE MICE have been used successfully for the hetero-transplantation of various human tumours. Haemopoietic malignancies, however, are very difficult to transplant (Sordat et al., 1977; Sharkey et al., 1978). For myeloid leukaemia, there have been only two reports of successful transplantation of the chronic form (Lozzio et al., 1976; Ueyama et al., 1977). The growth of acute myeloid leukaemia (AML) has not yet been reported. The present paper reports on the direct and serial transplantation of leukaemic cells from human AML into nude mice.

Case report.—A 40-year-old female had been well until October 1979, when she complained of fatigue, fever and cervical lymphadenopathy. Peripheral blood counts showed anaemia, thrombocytopenia and leucocytosis (RBC: 288 x 10^4/μl; haematocrit: 25%; Hb: 8·5 g/dl; reticulocytes: 0·5%; platelets: 1·8 x 10^4/μl; WBC: 32,700/μl; myeloblasts: 82%; band neutrophils: 1%; lymphocytes: 16%; monocytes: 1%). An aspirated specimen of sternal marrow was hypercellular (nucleated cells: 74·9 x 10^4/μl); 93·2% of nucleated cells were leukaemic, having large nuclei with a few nucleoli and fine granular chromatin network, and scant basophilic cytoplasm containing some fine granules (Fig. 1a). Leukaemic cells were strongly positive for peroxidase, positive for α-naphthyl acetate esterase, weakly positive for naphthol AS-D chloroacetate esterase, and negative for PAS stain. Serum lysozyme was 6·9 μg/ml (normal: 5·0–10·2) and urine lysozyme 1·4 μg/ml (normal: < 2). Cytogenetic study of the leukaemic cells revealed the normal karyotype (46, XX).

AML was diagnosed and combination chemotherapy with daunorubicin, cytosine arabinoside and vincristine induced complete remission within one month. Leukaemia, however, relapsed with nodular formation in skin and lymph nodes in June 1980. Remission induction chemotherapy was re-started, but she died on 14 April 1981.

Mice.—6-week-old male BALB/c nu/nu mice, bred in the animal colony of the National Institute of Radiological Sciences and fed under specific-pathogen-free (SPF) conditions, were used as recipients.

Leukaemic cells.—5 ml of marrow aspirate from the patient was on diagnosis added to an equal volume of modified McCoy's 5A medium (GIBCO, Grand Island, NY) supplemented with 20% foetal calf serum (Flow Lab., Rockville, MD), centrifuged at 150 g for 10 min, washed twice in the medium, and buffy coats collected.

Heterotransplantation.—Leukaemic cells were inoculated s.c. with a disposable sterile syringe with 26G needle into the backs of 4 nude mice, and wheals were made. The mice were administered with 5Gy γ-irradiation from a 137Cs source...
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immediately before transplantation. After inoculation, mice were kept under SPF conditions, and kept under observation.

The wheals on the backs of the mice disappeared gradually. After ~80 days, nodules began to appear at the implanted sites. They developed to about 18 × 17 × 8 mm-sized masses in 2 out of 4 recipients after 6 months. The tumour was greenish, resembling a "chloroma". Touched imprints of the tumour revealed clusters of blastic cells by Wright stain (Fig. 1b). Large round cells had round, ovoid or indented nuclei composed of a fine chromatin network with 1–2 large nucleoli. They were strongly positive for the peroxidase reaction. Histological sections of the tumour were occupied by uniform large undifferentiated blastic cells with large nuclei containing prominent nucleoli and scant cytoplasm. Several cells showed mitotic figures. Electron-microscopic examination of the tumour revealed that the cells had leukaemic characteristics (Fig. 2), viz., round nuclei, irregular in shape or indented, and with prominent nucleoli. The cytoplasm contained few granules, rough endoplasmic reticulum and abundant polysomes. No virus particles were found. Chromosomal analysis of the tumour cells showed a normal human karyotype (46, XX), indicating human origin of the tumour cells.

The above findings showed that the tumour consisted of the patient's leukaemic cells. The necropsy findings of the mice revealed no leukaemic-cell infiltration in marrow, peripheral blood, spleen or liver.

The tumour was implanted s.c. successively every 6 months into irradiated nude mice, and has been maintained to
date for 5 passages, with the preservation of histological and cyto-genetical characteristics. Fig. 3 shows the growth curves obtained from the primary to the 4th-passage tumours. They grew exponentially after the latent periods, the doubling time being \( \sim 20 \) days. The latent periods were \( \sim 80 \) days in the primary, 2nd and 3rd passages, and \( \sim 40 \) days in the 4th passage.

In the present study, we have demonstrated the direct and serial transplantation of human AML into whole-body irradiated nude mice. Why heterotransplantation of leukaemia is more difficult than for other solid tumours is not clear. The characteristic of leukaemic cells and/or the conditions of the host may not be suitable for the transplant.

Leukaemic cells are usually suspended in marrow or peripheral blood and seldom form tumours \textit{in vivo}. In our case, however, the leukaemic cells infiltrated the skin and lymph nodes to form nodules. This clinical finding suggests that the leukaemic cells of this patient easily clustered \textit{in vivo} and might therefore be suitable as implants. Haemopoietic cell lines with chromosomal abnormalities have been reported to show higher “take” rates than those free of abnormalities (Imamura et al., 1970). The leukaemic cells reported here, however, had no chromosomal abnormality.

In nude mice there are thymus-independent immune systems, including B cells, natural-killer cells and the monocyte-macrophage system (Campanile et al., 1977; Cudwitz, 1975; Herberman, 1978) and natural cytotoxic antitumour antibodies (Martin & Martin, 1974). The rich surface glycoprotein of haemopoietic cells is thought to provoke a thymus-independent immune system (Watanabe et al., 1980). In order to transplant the haemopoietic cells, the pretreatment eliminating
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Fig. 3.—Growth curves of implanted tumours. ⋄—⋄ the primary tumour (1 mouse); ▲—▲ tumour of 2nd passage (1 mouse); △—△ tumour of the 3rd passage (2 mice); ○—○ tumour of the 4th passage (3 mice; mean ± s.d.). Tumour volume = 1/3 π abc (a, b and c being the 3 dimensions of the tumour).

these immunities is considered necessary, e.g. whole-body irradiation (Watanabe et al., 1978), antilymphocyte serum injection (Ohsugi et al., 1980) or splenectomy (Watanabe et al., 1980). We implanted AML cells immediately after whole-body irradiation, when the immune system probably still had some function. The inoculated cells then gradually disappeared, macroscopically. After ~ 80 days, nodules began to appear and tumours were formed. These tumours must have been derived from residual leukaemic cells which had not been completely rejected by the immune system of the irradiated mice.

It is not known whether our present success in the transplantation of AML is due to the preconditioning of mice or is the specific characteristics of the leukaemic cells. Further transplantations of AML in the same condition should resolve this question.

Recently several in vitro human myeloid leukaemic cell lines have been established which would provide useful models for the study of the biology of myeloid leukaemia (Koeffler & Golde, 1980). However, these are not necessarily good models for studying the pathophysiology of AML in vivo or examining the effects of chemotherapy, radiation therapy and immunotherapy against AML in vivo. Of course, animal models such as murine myeloid leukaemia have limited applicability to the investigation of human AML. The successful serial transplantation of human AML into nude mice, reported here, should provide an important approach not only to studying the pathophysiology of AML in vivo, but also in examining therapeutic trials against AML in vivo.
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