Granulocytosis induced in vivo by a mouse marrow stromal cell line, BMA1, which produces colony stimulating factor

K. Fujita, Y. Shimomura, J. Fujita and K.J. Mori

Department of Orthopedic Surgery, National Defence Medical College, Saitama 359 (Japan), and Department of Microbiology, Faculty of Medicine, Kyoto University, Kyoto 606 (Japan), 20 March 1984

Summary. Nude mice were inoculated with BMA1 cells. These are cells which produce granulocyte-macrophage colony stimulating factor (GM-CSF); They are derived from mouse bone marrow stromal cells transfected with adenovirus 5 DNA. Progressive neutrophilia developed as the tumor grew, but disappeared quickly after local tumor excision. Media conditioned with tumor cells had GM-CSF but neither erythropoietin nor burst-promoting activity. In all the tumors which developed, focal areas of bone formation were found among fibrosarcomatous tissues.
Key words. BMA1 cell line; colony stimulating factor; bone marrow stromal cell; bone formation.

It has been suggested that the bone marrow is of critical importance in sustaining and regulating the proliferation and differentiation of hemopoietic populations (reviewed by Dexter[1]). In long-term bone marrow cultures, cells in the adherent layer seem to play a central role in stem cell maintenance and differentiation through cell-to-cell interactions and/or local production of humoral regulators[2]. Among various regulators, GM-CSF has been most intensively studied, and it has been suggested that it plays a role in granulopoiesis both in vitro and in vivo[3,4]. In order to facilitate the biochemical and physiological understanding of stromal-hemopoietic cell interaction, we established a GM-CSF producing cell line BMA1 from DDY mouse bone marrow stromal cells transfected with adenovirus 5 DNA in vitro[5]. In the present study, we inoculated BMA1 cells into nude mice and assessed their tumorigenicity and effects on granulopoiesis.

Materials and methods. BMA1 cells were maintained in Fisher's medium as previously described[5]. $1–3 \times 10^6$ cells were inoculated s.c. in the dorsum of a nude mouse (6–8 weeks old) and the size of the tumor was estimated in three dimensions using vernier calipers. Mice were sacrificed by cervical dislocation and the tumors developed were fixed in formalin and examined histologically. In certain mice, tumors were excised locally under ether anesthesia. At the same time sham operations were done on other tumor-bearing mice as a control. Peripheral blood was obtained by retroorbital puncture and used for determinations of the leukocyte and differential counts. GM-CSF assay was similar to that described previously[5]. DDY mouse bone marrow cells were incubated in 0.3% agar with a test substance at 37 °C for seven days. Colonies consisting of 50 or more cells were counted with the use of an inverted microscope.

Results and discussion. After inoculation of BMA1 cells, 10 out of 10 mice developed tumors. Progressive rises in peripheral leukocyte count were observed as the tumors grew (fig. 1). The rise in total leukocyte count was due almost entirely to the rise in total neutrophil count. No effect was observed on the peripheral erythrocyte or platelet count.

Histologically all tumors had the appearance designated as fibrosarcoma-like. The tumors consisted of atypical spindle-shaped cells and giant cells were sometimes seen. Focal areas of bone formation were found in tumor tissues and this bone had some lamellar structure but did not have hemopoietic features (fig. 2).

One of the tumors developed (BMA1/N5) was further transplanted subcutaneously into six nude mice. 19 days later, three of them underwent local excision of the tumor. As shown in figure 1B, the peripheral leukocyte counts decreased rapidly after removal of the tumor, whereas they increased in the control group. Sera from BMA1-tumor bearing mice as well as media conditioned with tumors developed after inoculation of BMA1 cells showed GM-CSF but no erythropoietin or burst-promoting activity in vitro (data not shown). These facts strongly suggest that the observed leukocytosis was caused by GM-CSF produced by BMA1 tumor cells.

Various transplantable tumors, tumor cell lines and bone marrow cell lines have been reported to produce GM-CSF[6–9]. As previously reported[5], the BMA1 cell line was established from adenovirus DNA-transfected mouse tibial marrow cells which consisted of macrophages, fibroblastoid cells, endothelial cells

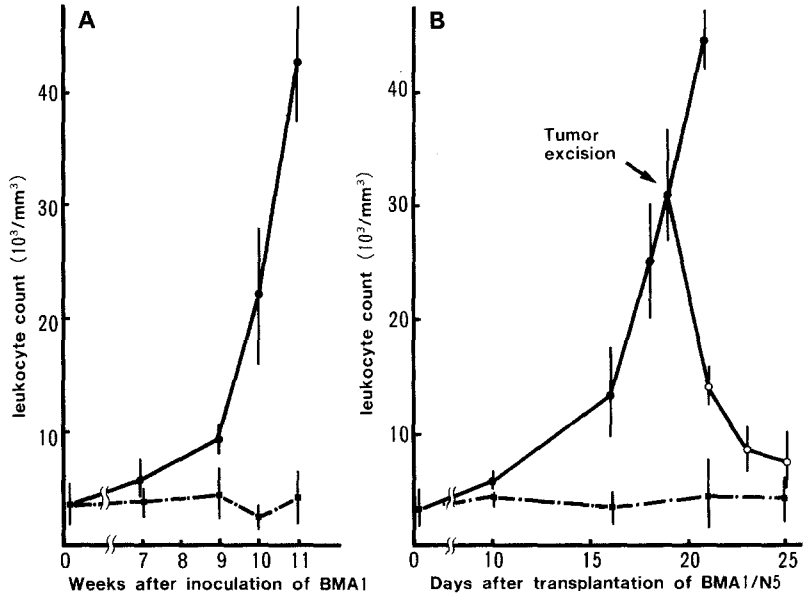

Figure 1. Peripheral leukocyte counts after inoculation into nude mice with *A* BMA1 cells ($1–3 \times 10^6$ cells/mouse) or *B* fragments of the tumor induced in a nude mouse with BMA1 cells (BMA1/N5), on day 0. (●–●), Tumor-bearing mice; (■–·–·–■), control; (○–○), tumor-excised mice. Each point represents the mean of (A)10 or (B) 3–6 nude mice.

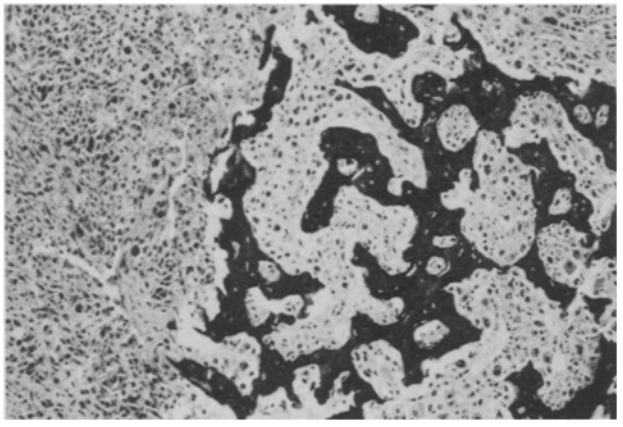

Figure 2. Histology of the tumor obtained by inoculating BMA1 cells into nude mice (six weeks after inoculation). Note the bone formation among tumor tissues (hematoxylin and eosin × 160).

and others, and it contains cells expressing adenoviral early antigens, which are known to immortalize and transform the cultured cells[10]. In the present study tumorigenicity of this cell line was established. Adenovirus DNA was demonstrated in DNA of the developed tumor (data not shown). Chondro-osteogenic gene activation is induced at the onset of the morphogenetic phase of bone development and is regulated by a combination of extra-and intracellular factors as well as intrinsic genetic and epigenetic factors[11]. The bone marrow stromal cells are considered to be more sensitive to bone morphogenic protein than almost any other known mesenchymal cell population in the body[12]. Although BMA1 cells have been considered to be derived from marrow stromal cells which have the capacity to produce GM-CSF[5], they also contain cells which may form bones or produce bone inducing substance(s). The identity of these two kinds of cells and the possible gene activation by adenovirus transformation in them are currently under investigation using cloned cells.

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Diploidy and triploidy in the hybrid minnow, *Phoxinus eos* × *Phoxinus neogaeus* (Pisces: Cyprinidae)[1]

G.R. Joswiak, R.H. Stasiak and B.F. Koop

Office of Computer Services, Oakland University, Rochester (Michigan 48063, USA), Department of Biology, University of Nebraska at Omaha, Omaha (Nebraska 68182, USA), Department of Anatomy, Wayne State University, Detroit (Michigan 48202, USA), 5 April 1984

Summary. Two presumptive hybrid populations are examined. Nebraskan hybrids, all having allozymic F1 electromorphs, exist as both diploid (2n = 50) and triploid (3n = 75) forms. This supports the hypothesis of parthenogenesis as the mode of hybrid reproduction. The maximum number of nucleoli per cell is suggested as an indicator of ploidy level. In contrast, electrophoretic analysis of a postulated Mendelian hybrid population in Quebec failed to detect any allozymic heterozygotes or recombinants. A previous conclusion of introgressive hybridization in this population is not supported.
Key words. Cyprinid; triploidy; polyploidy; parthenogenesis; nucleoli.

The hybridization between the minnows *Phoxinus eos* and *Phoxinus neogaeus* is geographically widespread, although the occurrence and frequency of hybrids vary greatly among localities[2–4]. The question of hybrid fertility and mode of reproduction has been unanswered in previous studies. Two morphometric studies have led to varying conclusions, one suggest that the hybrids may be a all-female parthenogenetic species, the other suggesting introgressive hybridization. Based on samples from the United States, one researcher found that the hybrids were essentially all females, deviated from intermediacy, and where sometimes taken in the absence of one of the parental species[2]. Parthenogenesis was postulated as a method of maintaining all-female hybrids in the absence of one parent. Supporting this hypothesis are the facts that hybrids have been reported from Colorado and Montana where pure *P. neogaeus* has never been taken[5,6]. In contrast, an investigation based on discriminant analyses concluded that hybrids of both sexes exist in some Quebec lakes, with the hybrids forming a Mendelian population[7].

In an earlier allozymic investigation of two *Phoxinus* populations in Nebraska, we found data strongly supporting the parthenogenetic hypothesis[8]. We assayed specimens for three enzyme marker loci where fixed allelic differences occur between the species[8]. These loci encoded the following enzymes; phosphoglucomutase (E.C. 2.7.5.1), malate dehydrogenase (E.C. 1.1.1.37) and superoxide dismutase (E.C. 1.15.1.1). Hybrids, all females, comprised most of the *Phoxinus* at those localities, and were coexisting with only one parental species, *P. eos*. All the hybrids had patterns typical of F1 hybrids at each of the three marker loci: no recombinants classes were found. Despite their singular allozymic phenotype, the hybrids exhibited some morphological variability, with some resembling one parental species more then the other. These characteristics are strongly suggestive of hybrid parthenogenesis, possible of a diploid-triploid complex. Diploid-triploid unisexual complexes of hybrid origin are known to exist in a number of fish, amphibian and lizard species[9].

In this paper we present new data on both the Nebraskan and