A METHOD OF OBTAINING PREPARATIONS OF CHROMOSOMES OF HEMOPOIETIC CELLS IN DOGS AND THEIR POSSIBLE USE FOR IDENTIFICATION OF BONE-MARROW CHIMERAS

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The transplantation of bone marrow for the treatment of radiation sickness and of various hematological diseases is a problem of considerable practical and theoretical importance. The radiation chimeras of the bone marrow arising in these circumstances provide, at the same time, a convenient model for the solution of problems in immunology, hematology, and so on. Many methods have recently been proposed for the reliable identification of the hemopoietic elements of the donor in the body of the recipient, based on precise histological, histochemical, immunological, and physicochemical methods of investigation [1].

A reliable histochemical method is the karyological identification of the marrow cells, based on the individual features distinguishing the chromosomal complexes of donor and recipient, which can be detected during mitosis at the metaphase stage. Using this as the basis of their investigations, Ford and co-workers [3] identified marrow cells in homologous and heterologous chimeras of rats and mice, and also in homologous mouse chimeras using mice of line T_6 with a marker chromosome as donor.

In this paper a method of obtaining preparations of chromosomes of dogs' bone marrow cells is described, which consists of a combination of several methods [2, 4, 5]. This method was used for identification of allogenic marrow cells by sex chromosomes (XX and XY) in radiation chimeras (female donors, male recipients). Bone marrow obtained by puncture of the sternum or femur, in a volume of 1-2 ml, was added to 20 ml of glucose saline (600 mg NaCl and 700 mg glucose to 100 ml distilled water) with colchicine (in a dose of 2 ml of 0.04% colchicine solution to 20 ml nutrient medium). The suspension of marrow cells was incubated at 37° for 2-3 h, after which the original volume was diluted five times with a 0.44% solution of sodium citrate and left to stand at room tempera-

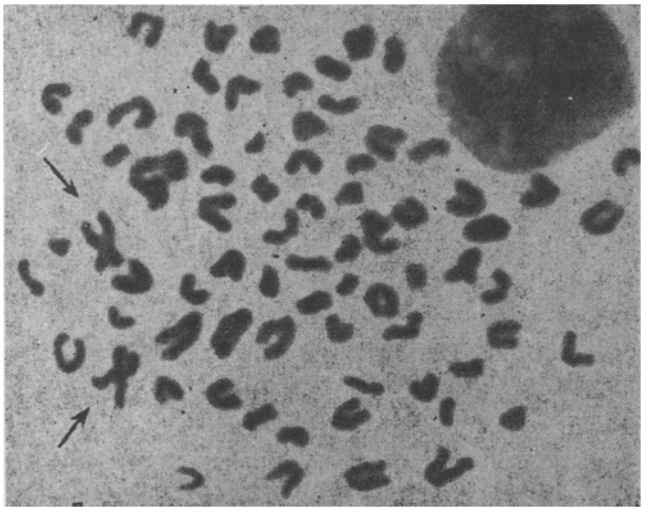

Fig. 1. Metaphase plate of a bone marrow cell from a female. The arrows point to large metacentric chromosomes.

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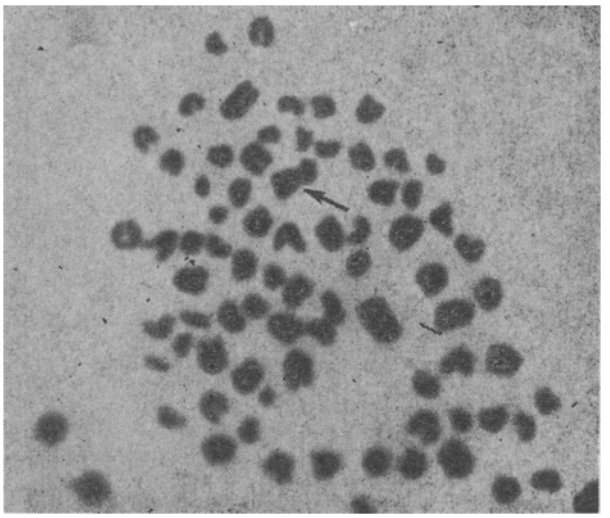

Fig. 2. Metaphase plate of a marrow cell of a male. The arrow points to a large metacentric chromosome.

ture for 25-30 min until the cells had reached the required degree of hypotonia. The cell suspension was centrifuged for 20 min at 800-1000 rpm, the supernatant fluid was removed, and the marrow cells were fixed by the addition or a mixture of 3 parts of absolute alcohol and 1 part glacial acetic acid, drop by drop, followed by incubation of the cell suspension for 1 h at 4°. If the cells were to be stored for a long time the fixing agent was replaced by a 45% acetic acid solution; immediately before the preparations were made this was replaced by 60% acetic acid solution. A few drops of the marrow suspension prepared in this manner were placed on a glass slide, preliminarily cooled with dry ice (to ensure uniform spreading of the drop over the glass), and dried over a Bunsen flame and stained with aceto-orcein. For the detailed analysis, photomicrographs were made of the dividing marrow cells.

This method was used to study the chromosomal apparatus of the bone marrow cells of healthy dogs (10 males and 8 females), and 236 metaphase cells were analyzed. Diploid chromosome sets of the marrow cells of dogs (males and females), containing 2n (78) chromosomes, are shown in Figs. 1 and 2. As is clear from Fig. 1, in the females 76 chromosomes were acrocentric and evidently distinguished only by size, while 2 large chromosomes were metacentric. In males (see Fig. 2) only one large metacentric chromosome was found, and the other 77 were acrocentric. Hence, the bone marrow cells of dogs (males and females) differed in their specific sex chromosomes, which were characterized by a pair of large, metacentric chromosomes in the females and by only one metacentric chromosome in the males, thus confirming the findings of Japanese authors who investigated a culture of lung cells of healthy dogs [6]. In this way the marrow cells of donor and recipient can be identified in dogs which are allogenic marrow chimeras, even without a detailed analysis of the chromosomal apparatus of the cells in these animals.

The method described above was used to identify the donor's marrow cells in a recipient (using a radiation bone-marrow chimera in dogs as model). A male was irradiated with two doses totaling 1000 R, and on the day after irradiation $9 \cdot 10^9$ nucleated marrow cells from a female were transplanted. The specimen of marrow obtained from the recipient by puncture on the 7th day after irradiation contained $0.5 \cdot 10^6$ cells, and was used for karyological analysis. Fourteen metaphase plates were found to be suitable for chromosome analysis. All contained pairs of metacentric chromosomes, on which basis they were identified as the donor's marrow cells, functioning in the recipient.

SUMMARY

A method is described which is used for identifying the cells of the allogenous bone marrow of the donor in the recipient body in dogs. The method is based on the characteristic features of sex chromosomes in male and female dogs – the presence of two large metacentric chromosomes in females and of only one large metacentric chromosome in males – and makes it possible to identify the bone marrow cells of the donor and recipient in dogs – allogenous bone marrow chimeras – without a detailed analysis of the chromosome apparatus.

LITERATURE CITED

1. K. A. Lebedev, Pat. Fiziol., No. 1 (1964), p. 68.

2. T. P. Tsessarskaya, F. V. Osechenskaya, and S. A. Khrustalev, Probl. Gematol., No. 1 (1964), p. 3.
3. C. E. Ford, J. L. Hamerton, D. W. H. Barnes et al., Nature, Vol. 177 (1956), p. 452.
4. C. E. Ford, P. A. Jacobs, and L. G. Lajtha, Ibid., Vol. 181 (1958), p. 1565.
5. M. Fox and I. M. Zeiss, Ibid., Vol. 192 (1961), p. 1213.
6. S. Takayama and S. Makino, Z. Krebsforsch., Vol. 64 (1961), p. 253.