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Mitotic Features of Monocytes Occurring in the Peritoneal Fluid of Rats [1)]

By

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(With 13 Text-figures)

It has been generally accepted that there are in the normal peritoneal fluid of rats the following four cellular elements, the monocytes, lymphocytes, eosinophile leucocytes and mast cells, but little has been known about their mitotic events. For several years, the present author has engaged in observing the cytology of several ascites tumors of rats, and has had an opportunity to encounter mitotic figures of monocytes, particularly in the course of the transplantation experiment of a single tumor cell.[2)] Recently it has been found that the mitotic activity of monocytes is increased considerably by the use of some chemicals.

The knowledge on the somatic chromosome features of rats has been obtained from the studies in embryonal tissues and the regenerating adult liver (Tanaka 1953, Makino and Tanaka 1953). Quite recently, Makino and Hsu (1953), employing a new prefixation treatment of tissue cultures with a hypotonic saline, have demonstrated the exact location of the centromere in somatic chromosomes of the Norway rat.

Cytological investigations of several rat tumors carried out in the author's laboratory have made it clear that in tumor cells there exist certain pairs of clear V- and J-shaped chromosomes which vary in number with the kind of tumors (Makino 1952a, b, Makino and Kanô 1953). Since the tumor cells have lived together with the monocytes in the peritoneal cavity of rats throughout their development, it is interesting and important to learn the chromosomal features of monocytes. This interest prompted the present study.

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2) Details of this experiment will be published elsewhere.

Material and method

Young and full-grown albino rats (*Rattus norvegicus*), weighing 50 to 120 gm, were usually adopted as material, a few newborn and old rats being used in addition. It was found that the negative rats in the inoculation experiment of a single tumor cell proved as favourable material for this study, rather than the non-treated rats, because of the occurrence of high mitotic activity in the former. The rats which have received intraperitoneal injection with physiological saline or glucose solution at varouis concentrations also provided favourable material.

The following observations were made with smear preparations stained acetic dahlia (0.75 gm of dahlia dissolved in 100 cc of 30 % acetic acid) and acetic gentian violet (0.75 gm of gentian violet dissolved in 100 cc of 10 % acetic acid). Technique was the same as that described by Makino (1952b) and Tanaka and Kanô (1952).

Observations

Mitotic activity of monocytes: The mitosis of monocytes occurring in the normal peritoneal fluid showed no relationship to the age of rats. In the non-treated rats the mitotic activity was rather high in young rats having rich accumulation of body fluid. The mitotic figures of monocytes were found abundantly in the rats treated with reagents as mentioned above. Interestingly, in the body cavity of the negative rats after the transplantation experiment of a single tumor cell, the monocytes were increased in number with the increase of their mitotic figures. In some animals, mitotic activity of monocytes has remained constant during the period ranging from one to three weeks after inoculation. The mitotic rate was observed by counting the monocytes at mitotic stages in the total of 1000 cells of all kinds found in the peritoneal fluid. The mitotic rate of 0.9 percent was obtained when the mitotic figures are most abundant, though there were individual differences in mitotic activity.

General morphology of monocytes: The monocytes are considerably larger in size than any other cells occurring in the peritoneal fluid, with some variations. The nuclei of large monocytes are usually of kidney- or horseshoe-shape. Dividing figures were observed rather frequently in larger ones. The cells with two nuclei were rare in occurrence. The cytoplasm is relatively large in volume. It showed a slightly basophilic reaction by the technique employed. No visible granules occurred in the cytoplasm. On account of these morphological characteristics, the monocytes are clearly distinguishable from tumor cells in the peritoneal fluid.

Chromosomes of monocytes: The chromosomes of the monocytes tend to crowd and to stick together at metaphase, and therefore, good metaphase plates which allow a critical study of the chromosomes were of rather rare occurrence.

Forty metaphase plates showing distinct chromosomes were selected for study. The chromosome numbers observed in these cells are shown in Figure 1. The results indicate that the number of chromosomes varies within a range from 35 to 49. The cells showing the basic number (42) were highest in frequency (45 %), and the numbers showed a slight fluctuation around 42. There was a tendency indicating that cells having more than 42 chromosomes are more frequent than those having less chromosomes. Subtriploid cells (Fig. 12) and those of polyploid nature were very rare.

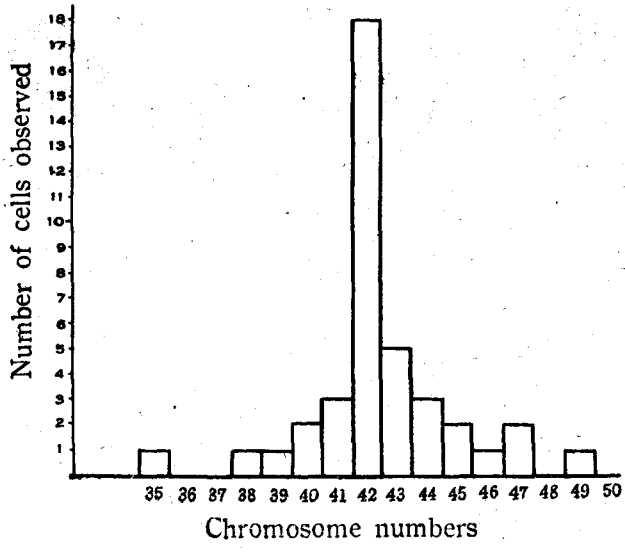


Fig. 1. Diagram showing the variation of chromsome numbers of monocytes and their distribution.

The behavior of chromosomes in mitosis : The mitotic behavior of chromosomes was found to be regular in most cases under study and there was no evidence of aberration, except for the occurrence of a few monocytes which showed such abnormalities as non-disjunction, lagging of chromosomes and the formation of the binucleate cell (Fig. 13).

The chromosomes of moncoytes arrange in the equatorial plate in a regular radial manner. Each chromosome shows generally a clear split separating it into two chromatids (Figs. 2–6, 9–12). Each chromatid seems to contain a pair of chromonemata.

Superficially, most of the chromosomes appeared to be rod-type, tapering at their inner ends and varying in shape and size. Mingled with these rod-shaped elements, there were a certain number of elements which indicate non-terminal

centromeres (Figs. 2–6, 8–12).

Recently Makino and Hsu (1953), working with tissue culture material, have shown that in the somatic complement of the Norway rat eight autosomal pairs and the X-element are characterized by metacentric structure having two dissimilar arms. According to them, the chromosomes in the descendent order, namely those numbered 1, 4, 8, 11, 12, 15, 16 and 19, are composed of the V- or J-shaped elements. In the present material it was in practice difficult to determine

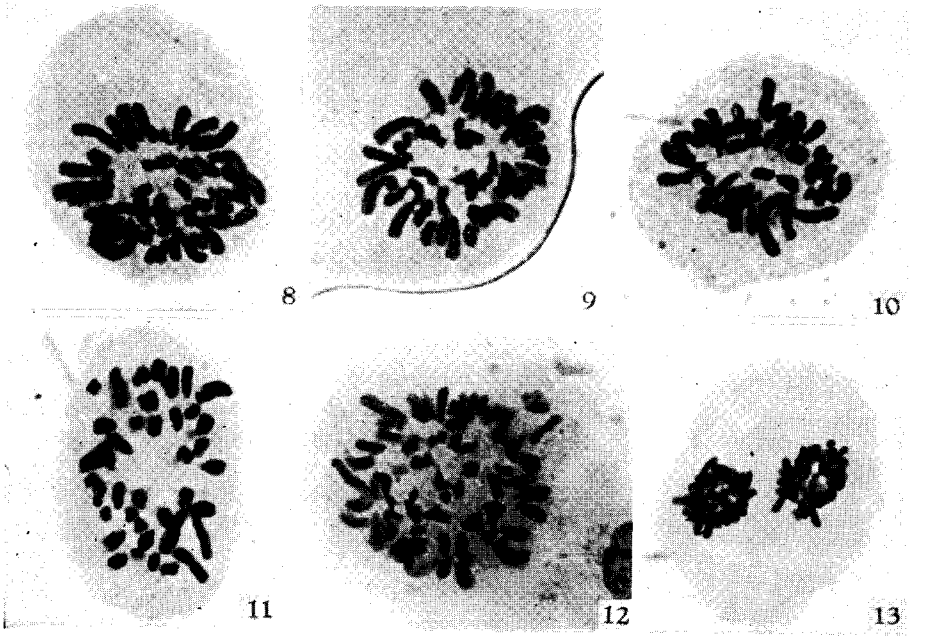
Figs. 2 to 7. Camera-lucida drawings, ca. 2200×. Figs. 2–6. Metaphase plates of monocytes having 42 chromosomes. (2–3, from the normal peritoneal fluid, 4–6, from the treated peritoneal fluid). Fig. 7. A metaphase plate of 3V-type cell of the Hirosaki sarcoma, having 38 chromosomes. Prominent three V's are indicated with asterisks.

the exact number of metacentric chromosomes, due probably to the technical difficulty. But it can be stated that there is a close similarity between the findings of Makino and Hsu (1953) and those of the present study. Noticeable and important are the following two facts : first, that the number of metacentric chromosomes having V- or J-shape is apparently dissimilar between the monocytes of rats and the tumor cells of the ascites sarcomas ; second, that the large, prominent V-shaped chromosomes which constantly occur in the tumor cells, varying in number from one to five according to the kind of the ascites tumors (Makino 1952b, Makino and Kanô 1953), are completely absent in the monocytes of rats.

The evidence may be well understood by reference to Figure 7 by way of comparison with Figures 2 to 6.

Discussion

Previously, the origin and nature of monocytes has been discussed by many haematologists. Most authors have emphasized that the monocytes bear a close interrelationship to the lymphocytes, plasma cells, fibroblasts, reticular cells, histiocytes and endothelial cells. In contrast to them, Amano (1943, 1948) put



Figs. 8 to 13. Photomicrographs showing the mitotic figures of monocytes, ca. 1200×. 8, a metaphase plate showing 45 chromosomes. 9–11, the same showing 42 chromosomes. 12, the same showing subtriploid chromosomes. 13, showing the formation of a binucleate cell.

forth a new theory on "Monozytenreihe" based on his extensive investigations. It was constructed by establishing an independent monocytic series throughout the course of their development.

Because of the rarity of cell division in the mature monocytes both in the blood and in the peritoneal fluid, knowledge about the mitosis of monocytes in mammals has remained meagre up to the present, with only fragmental descriptions (Sabin et al. 1925, Amano 1943, 1948, Yoshida 1949). Earlier workers have

claimed that the method of division in monocytes is amitotic (cf. Schafer 1934). Evidence of amitosis has never been observed in the present study. From the observations of active proliferation of monocytes in the chicken blood culture, Amano (1943, 1948) has assumed that monocytes alone are capable of mitosis in the peripheral blood of mice and men.

It has been generally recognized in haematology that the monocytes usually increase following the neutrophile reaction caused by inflammation occurring in the peritoneal cavity. As described above, a considerable increase in monocytes took place after inoculation of a single tumor cell, or after treatment with some chemicals. This suggests itself as being the intraperitoneal cellular reaction. According to Amano (1943, 1948), the monocytes are formed originally in the bone-marrow and spleen, but secondarily arise in the *omentum majus* of the abdominal cavity. His view is in agreement with the fact obtained in this study that dividing figures of monocytes appear abundantly in the peritoneal fluid following various stimulations.

Cytological investigations of blood cells of mammals have been carried out in the bone-marrow (La Cour 1944, Polli 1946, 1950) and lymph nodes (Maximow 1928), with special attention to the mitotic cycle. The chromosome study was made with megaloblastic elements of human pernicious anaemias by Polli (1946), finding the diploid (48), triploid (72) and hypoploid cells. Recently, Tanaka (1953) has made a detailed study of the chromosomes of somatic cells from various organs including bone-marrow of embryo rats. The chromosome number observed in the bone-marrow was found to be mostly 42, the basic number, while a few showed a variation ranging from 32 to 49. Interestingly, the characters of chromosomes in the bone-marrow cells are closely similar in nature to those of monocytes. However, the occurrence of the basic number of chromosomes is a little higher in embryonic blood cells than in adult monocytes. Such a tendency was found to occur in the liver (Tanaka 1953, Makino and Tanaka 1953).

Makino (1952a, b), and Makino and Kanô (1953) have established that the chromosome complexes of the ascites tumors of rats consist of two distinct groups, namely, a group of rod-shaped chromosomes and a group of V- and J-shaped ones which are characteristic according to the kind of the tumor. As already described, the exact number of the metacentric chromosomes in the monocytes could not be determined in this study, but the metacentric chromosomes occurring in the monocytes are apparently dissimilar in number from those in the tumor cells. Further the prominent V- shaped chromosomes of large size which occur constantly in the tumor cells, varying in number from one to five according to the kind of the tumor, are completely absent in the monocytes. Due to these differences, the tumor cells are clearly distinguishable from the monocytes in the peritoneal fluid of rats.

Summary

This paper reports studies of mitotic activity and the chromosome features of monocytes occurring in the peritoneal fluid of albino rats.

It was found that the negative rats after inoculation with a single tumor cell, and the animals which have received intraperitoneal injection with normal saline or glucose solution showed a high mitotic activity of the monocytes in the peritoneal fluid. The number of chromosomes in the monocyte varies from 35 to 49, showing a gradual fluctuation around 42. The cells having the basic number (42) are highest in occurrence. Most of the chromosomes are of rod-type, but there are certain pairs of metacentric two-armed elements. It was found that the metacentric chromosomes occurring in the monocytes are dissimilar in number and in feature from those found in the tumor cells of ascites sarcomas.

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