The Nature of Mammalian Lymphosarcoma Transmission by Isolated Chromatin Fractions

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In a recent publication, Stasney, Paschkis, and Cantarow (6) reported the successful transmission of the Murphy rat lymphosarcoma by inoculation of chromatin fractions isolated from the neoplasm according to the procedure of Claude and Potter (1). Although they could not exclude contamination of these fractions with intact living cells, they considered such a contamination to be highly improbable and supposed that the tumors appearing after subcutaneous and intramedullary injections of the chromatin fractions represented a neoplastic transformation of lymphocytes in the recipient animals, induced by the inoculated fraction or by some of its components.

Whether tumors developing after the inoculation of tumor preparations that are presumably free of viable cells represent a proliferation arising de novo in the injected host or are due to contamination of the material with viable tumor cells can be decided by the use of tumor material that is homologous to an animal strain of known genetic composition, according to the procedure of Law (4).

It seemed of interest to repeat the experiments of Stasney et al. with tumors homologous to highly inbred mouse strains. The procedure of Law was then employed to detect whether the tumors produced by the injection of isolated chromatin fractions are of the same genetic constitution as the original tumor or whether they have the genetic characteristics of the host in which they developed.

MATERIALS AND METHODS

Two strains of transplantable mouse lymphomas were used, one homologous to the DBA strain, described and studied by Goldie and Felix (2), and the 6C3H-ED lymphoma of the C3H strain (5). Both tumors were kept in the strains of origin, where the percentage of takes was nearly 100 per cent. The DBA lymphoma failed to take in 50 C3H and 20 C57 black mice, or regressed completely after a short initial growth period. The 6C3H-ED lymphoma failed to take or regressed in 30 Strong A and twenty C57 black mice. Both lymphomas were carried by intraperitoneal passages of ascitic fluid. When marked abdominal swelling was present, the animals were sacrificed, and their ascitic fluids and solid intra-abdominal tumors were collected. The solid tissue was minced and mixed with the ascitic fluid, until a dense pulp was obtained. From this point on, the procedure of Stasney et al. (6) for breaking the cells with sand and, subsequently, the method of Claude and Potter (1) for preparing the chromatin fraction were meticulously followed; the latter is the same as that employed by Stasney et al. The chromatin fraction was suspended in saline and injected subcutaneously and intraperitoneally as follows: (a) the DBA lymphoma into DBA X C3H F₁ hybrids and DBA X C57 black F₁ hybrids; (b) the 6C3H-ED lymphoma into A X C3H F₁ hybrids and into C3H X C57 black F₁ hybrids (Tables 1 and 2). From an aliquot of each chromatin fraction fresh smears were prepared, fixed with alcohol-ether (1:1) and stained by the Papanicolaou and the Feulgen methods. The animals were observed for tumor or ascites development, and the appearing tumors were re-injected subcutaneously and intraperitoneally into similar F₁ hybrids and into both parent strains of the particular F₁ hybrid in which the tumor arose. Histologic examinations have been routinely made of all tumors, while ascitic fluids were examined by Papanicolaou smears.

RESULTS

The results obtained are shown in Table 1 (DBA lymphoma) and Table 2 (6C3H-ED lymphoma).

Although the chromatin fractions were examined on stained smears as mentioned above, no evidence was found microscopically for the presence of whole cells or whole nuclei in the fields examined. The microscopic appearance of the fractions was very similar to that of Murphy lymphosarcoma chromatin fraction, as shown by the microphotograph of Stasney et al. (6).

Inoculation of the chromatin fractions of either lymphoma led to the development of solid tumors in a number of animals, as seen in Tables 1 and 2. Ascites was not produced after intraperitoneal inoculation, with the exception of one case. The tumors from the DBA lymphoma appeared 13–27 days after inoculation, and in the case of the 6C3H-ED lymphoma after 10–23 days. They grew progressively and led to the death of the host in all cases. Survival times ranged from 31 to 58 days and from 23 to 55 days, respectively, for the two tumors. No differences could be detected between

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the morphological structure of the original tumors and that of those tumors developing after injection of the chromatin fraction.

Re-inoculation of the solid tumors that developed in the susceptible F1 hybrids into both susceptible and resistant parent strains and into similar F1 hybrids gave unequivocal and consistent results for both tumors. All inoculations led to tumor development in members of the susceptible parent strain (strain of origin of the tumor) and in similar F1 hybrids, while no progressive growth was observed in members of the resistant parent strain.

DISCUSSION

After transplantation into genetically controlled material, the behavior of the tumors that arose in suitable F1 hybrids upon inoculation of the chromatin fractions is proof that these tumors had the same genetic constitution as the original tumor. Established fact (5) that low cell-doses of ascites-producing tumors do not lead to typical ascites tumors but only to solid tumor development.

SUMMARY

The experiment of Stasney et al. (6) on tumor production by the injection of chromatin fractions isolated from a rat lymphosarcoma was repeated, with two transplantable mouse lymphomas that originated and grew in genetically controlled animal strains. The development of lymphomas after subcutaneous inoculation of chromatin fractions has been confirmed, but evidence has been presented to show that the tumors which result do not possess the genetic constitution of the host in which they arise, but that of the original tumor. The most probable explanation of these findings is contamination of the chromatin fractions with surviving cells, although, as in the experiments of Stasney et al., intact cells or nuclei could not be detected microscopically in any of the fractions studied.

REFERENCES

Fig. 9.—Portion of gastric mucosa (Rat 416, untreated control) 4 months after implantation showing deep almost isolated cystic adenoma-like formation. Neighboring sections demonstrated the connection with the mucosa clearly. Hematoxylin and eosin. ×20.

Fig. 10.—Isolated group of gastric acini (Rat 394, normal control) in deepest layers of flap 6 months after transplantation. Connection with mucosa demonstrated in neighboring sections. Hematoxylin and eosin. ×35.

Fig. 11.—Metaplastic bone formation in mucosa of normal control rat (421) 3 months after transplantation. Periodic acid-Schiff stain. ×200.

Fig. 12.—General configuration of flap 11½ months after transplantation (220). Although this rat was treated by painting with MCA, the extensive ulceration and crusting of the mucosa in the central portion is similar to that seen in normal controls as is the degree of general growth indicated. The absence of any overgrowth of skin epithelium is also evident. Mucicarmine. ×5.
Fig. 1.—Gastric flap (Rat 899-R) immediately after implantation, showing skin at left with portion of whole stomach wall lying on the muscle of the anterior abdominal wall. Hematoxylin and eosin. X20.

Fig. 2.—Detail of mucosa of flap shown in Figure 1. Note the mixture of parietal and mucous cells of normal gastric mucosa. Hematoxylin and eosin. X225.

Fig. 3.—Gastric flap (Rat 433) 1 week after transplantation showing marked ulceration of the surface epithelium and almost complete replacement by acute inflammatory reaction. The submucosa is greatly thickened, and the muscle fibers of the wall of the stomach are separated by the exudate which also extends into the layer between the flap and the anterior abdominal wall. Hematoxylin and eosin. X18.

Fig. 4.—Higher magnification of the border between intact epithelium and the exudate shown in Figure 3, showing the polymorphonuclear character of the exudate. Hematoxylin and eosin. X79.

Fig. 5.—Gastric flap (Control rat 3-430) 2 weeks after transplantation, showing regenerating mucosa growing into the exudate and regenerated mucus type of epithelium. The exudate beneath the epithelium contains capillaries and lymphocytes and plasma cells in addition to polymorphonuclear leukocytes. Hematoxylin and eosin. X187.

Fig. 6.—Squamous epithelium of skin adjacent to flap (Control rat 3-430) 2 weeks after transplantation showing marked hyperplasia. Infiltration of inflammatory exudate may be seen beneath it and around its junction with the flap at the extreme right. Hematoxylin and eosin. X81.

Fig. 7.—Gastric flap (Control rat 3-440) 3 weeks after implantation, showing the cystic dilatation of the regenerating epithelium. Deep islands of gastric mucosa may be seen at the lower portions of the figure. Periodic acid-Schiff stain. X30.

Fig. 8.—Detail of Figure 7, showing the mucus nature of the regenerated epithelium and the submucosa composed of newly formed fibrous tissue and capillaries infiltrated with lymphocytes and plasma cells. Periodic acid-Schiff stain. X210.
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