Anastomotic Sarcoma of Irradiated Parabiont Rats

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SUMMARY

In a series of 1003 pairs of parabiosed rats, in which the right partner of each pair received 1000 rads total-body irradiation, the incidence of soft-part sarcomas at the anastomotic site was 8.7%. The incidence in other parts of the irradiated partner was 5.8%, and in the nonirradiated partner it was 0.3%.

A combination of whole-body irradiation plus local factors of continuing trauma and scar formation is thought to account for the higher incidence of sarcomas in the anastomotic region. Systemic factors such as the endocrines may also play a secondary role.

INTRODUCTION

An excess incidence of sarcomas appeared in the connective tissue and muscle of the anastomotic region of long-term parabiosed rats, in which 1 partner had received supralethal total-body radiation (1000 rads) (Fig. 1). Sarcomas of these tissues were less frequent in unirradiated parabiont pairs and even less so in single animals. In addition to describing these sarcomas, we will discuss factors involved in their pathogenesis and localization within and outside the anastomotic region of the irradiated and nonirradiated rats. While osteogenic sarcomas and fibrosarcomas sometimes arose at the scapular union, these have been excluded because of the differences in tissue environment.

MATERIALS AND METHODS

The anastomotic sarcomas in this report occurred among 1003 parabiont pairs, with radiation of the right partner, and among 128 unirradiated pairs.

Excluded from the present series were 44 sarcomas, mostly osteogenic, occurring around the wire at the scapular anastomosis of the experimental 1003 pairs, and 9 similarly located sarcomas of the 128 nonirradiated control pairs. Although many of the same etiological factors were no doubt present here, the foreign body reaction to the wire, as well as chemical factors, may have introduced changes not present elsewhere in the anastomoses. The incidence of mesenchymal sarcomas in 362 single, unirradiated rats of similar age is also presented (Fig. 2). All of the rats were of the NEDH Slonaker strain, either inbred or penbred. In each series, the proportion of male and female rats was the same. Only animals living over 230 days were included, none of the mesenchymal tumors having appeared before that time.

Minor modifications in the methods of Bunster and Meyer (1) and of Sauerbruck and Heyde (15) were used to perform the parabioses. Our procedure has been to anesthetize, with Nembutal, 1-month-old littermate pairs of rats of the same sex. The operational fields were shaved, and aqueous Zephran was applied. A cutaneous incision was made along adjacent lateral body walls of each rat from the iliac crest to the base of the ear, curving slightly ventrally. The ventral margins were sutured with continuousatraumatic 4-0 chromic catgut. The abdominal muscles and peritoneum were then incised from the inguinal region to the lowest rib of each animal. A large peritoneal fistula was made along adjacent lateral body walls of each rat from the iliac crest to the base of the ear, curving slightly ventrally. The ventral margins were sutured with continuousatraumatic 4-0 chromic catgut. The dorsal skin margins were then sutured. Each animal received about 10,000 units of penicillin i.p. The great majority of the parabioses were perfomed by 1 operator and, hence, were technically uniform. However, minor variations in technique may be important since, in a group parabiosed by another operator, numerous sarcomas developed along the anastomosis.

Sixty to 90 days after operation, the right partners of healthy, healed pairs received 1000 rads of 250 kV X-rays at 15 ma with only intrinsic filtration. The scatter to the immediately adjacent tissue of the left, lead-shielded partner at the anastomosis was about 10% of the full dose. For the purposes of this study, the anastomotic region is defined as approximately 2 cm either side of the suture line in adult animals.

To gain some idea of the amount of soft tissue in the anastomotic region compared with the amount elsewhere in parabiosed rats, we dissected out and weighed the skin, s.c. tissue, and muscles for 2 cm on each side of the anastomotic scar. The remaining soft tissue of the pair was likewise dissected and weighed.

In addition to the parabiosed rats, 362 single, comparable, control rats were maintained until their natural death.

RESULTS

Occasionally, parabiont pairs attained an age of over 800 days. The average age was 562 ± 129 days for all experimental pairs.
pairs, approximately the same as that for rats bearing anastomotic sarcomas. At times, the irradiated partner developed as many as 4 individual primary benign or malignant tumors in different organs. Many of these occurred in endocrine organs such as the pituitary (23), adrenals (22), ovaries (6), and pancreas (20). The first evidence of mesenchymal tumor, some 7 to 8 months after parabiosis, was the appearance of a small firm nodule in the anastomotic region. In some instances, the diffuse nature of the growth precluded exact definition of its point of origin in relation to the scar. Most of the anastomotic tumors grew rapidly, attaining a diameter of 5 to 6 cm within 4 to 6 weeks (Fig. 1). The virulence of the tumors was expressed by early and extensive infiltration leading to ulceration of the skin and envelopment of abdominal organs, and also by a 33% incidence of metastasis, which was high, based on our experience with rat sarcomas. Metastases appeared most frequently in lungs and lymph nodes of either partner. Metastasis could have occurred by direct invasion of veins draining the tumor, since the tumor was supplied by vasculature derived from each animal as demonstrated by radiography after jugular injection. Metastasis could also have occurred from tumor tissue that had spread transperitoneally. Twenty-nine % of the extraanastomotic sarcomas in the irradiated partner also metastasized but, unlike the anastomotic sarcomas, they were restricted to the partner in which the tumor originated.

A number of anastomotic sarcomas have been transplanted successfully. The 87 anastomotic sarcomas were classified microscopically as follows: 64 fibrosarcomas, 10 undifferentiated sarcomas, 8 rhabdomyosarcomas, 3 extraosseous osteogenic sarcomas, 1 angiosarcoma, and 1 myxosarcoma. The fibrosarcomas were composed of fusiform cells interspersed through variable amounts of collagen (Fig. 3). The well-differentiated tumors contained occasional mitoses and much collagen, while the less differentiated ones exhibited many mitoses and scant collagen. Myxomatous areas were noted occasionally. Peripheral invasion was always demonstrable.

The cells of the rhabdomyosarcomas were larger and usually had an abundance of pink amorphous or fibrillar cytoplasm. Myofibrils and, more rarely, cross-striations could be identified in the cytoplasm. Large pleomorphic nuclei contained coarse dark chromatin, and mitoses were fairly numerous (Fig. 4).

The undifferentiated sarcomas were composed of plump, single or polygonal cells with large hyperchromatic nuclei, often in mitosis. Except where necrosis was present, the tumor cells were crowded into sheets with little intercellular substance (Fig. 5). Myxoid changes were seen occasionally.

Preliminary electron microscopic studies on a single fibrosarcoma by Dr. Micheline Federman have demonstrated the complexly folded cell membrane and abundant organelles often seen in tumor cells (Fig. 6). Secondary lysosomes containing laminated phospholipid bodies were frequently present, probably representing beginning autolysis of the cytoplasmic structures. These cells are now being studied in detail and will be reported upon later. Recognizable virus bodies have not been encountered. Survey immunological studies, for which we are indebted to the National Cancer Institute, demonstrated that the colony was free of polyoma virus. There was evidence of the occasional presence of the Toolan H1 or Kilham rat virus.

The distribution of soft-part sarcomas in 1003 irradiated parabionts was as follows: 8.7 ± 0.28% occurred in the anastomotic region, 5.8 ± 0.25% occurred elsewhere in the irradiated partner, and 0.3 ± 0.06% occurred in the nonirradiated partner, exclusive of the anastomotic region. In contrast to the 1003 experimental pairs, the 128 nonirradiated control pairs showed an incidence of 3.1 ± 0.17% in the anastomotic region (Fig. 2) and 2.3 ± 0.15% elsewhere in the soft tissues of each member of the control pairs. The soft-part tissues of the anastomotic region weighed less than one-fourth that of the soft parts of the pair outside the anastomosis.

The 362 nonirradiated single control rats had an incidence of 1.9% soft-part sarcomas.

DISCUSSION

At least 3 factors may account for the development and localization of mesenchymal sarcomas predominantly in the right partner and in the anastomotic regions, namely, hormonal environment, irradiation, and regional conditions at the anastomotic site which contributed to the density and extent of scar formation.

Evidence for the role of the endocrine factor present is coincidental rather than direct. An increase in circulating gonadotropins in parabiotic rodents, 1 partner of which had been castrated, has been reported (7, 9). In many of our irradiated parabiosed rats, the pituitary gland of one or the other partner showed hyperplasia or adenoma formation (23). This probably resulted from altered feedback from irradiated end organs such as gonads, adrenals, and pancreas, with subsequent increased stimulation of these organs by appropriate pituitary hormones. We have noted a modest preponderance of anastomotic tumors in males (11.4%) as compared with 6% in females. Some endocrine tumors occur predominantly in males, for example, adrenal medullary (22) and insular tumors (20). The marked deficiency of sarcomas in the shielded partners (0.3%) raised the question as to why their mesenchymal cells respond differently from those of the anastomotic site and of the irradiated partner. Certainly, hormonal imbalance is present in these animals, but whether this exerts a neoplastic effect on the mesenchymal cells of the anastomotic site or on the cells of the irradiated partner is hypothetical.

The increased incidence of mesenchymal sarcomas in the irradiated partners of 1003 parabiosed rats seems to establish irradiation as an etiological factor. Excluding for a moment the anastomotic site, 5.8% of the mesenchymal sarcomas occurred in the irradiated partner as against 0.3% in the nonirradiated partner. This also exceeds the 2.3% in each control parabiont and the 1.9% in the single controls. These results are similar to those of Koletsky and Gustafson (10) who found a 7.3% incidence of fibrosarcomas in skin and s.c. tissue of single, Wistar male rats following whole-body X-radiation with 660 R.

The greatest number of sarcomas arose in the anastomotic...
region (8.7%). The right half of this region received the full dose of 1000 rads, and the left half received a diminishing amount of irradiation. In this region, additional factors were present, namely, chronic irritation, continuing stress from movement, and alterations in histocompatibility, all of which contribute to scar formation. The possible effect of local factors at the anastomotic site, uncomplicated by irradiation, were shown in the control series of 128 parabionts. Here, an incidence of 3.1% compares with 2.3% in each of the partner's soft tissues outside the anastomosis. The incidence of sarcomas in the anastomotic region of both the experimental and control parabionts becomes more impressive, since we have shown that the mass of soft tissue in the anastomotic region was less than one-fourth of that outside it. There is also some support in the literature for assigning etiological significance to factors at the anastomotic site, uncomplicated by irradiation, which may contribute to scar formation. The possible effect of local factors operative at the anastomosis site.

The general incidence of soft-part sarcomas in rats is low. In 1 series of Sprague-Dawley rats, the incidence was reported as 1.6% (18). In 2 series of Wistar rats (3, 14), in 1 of the Osborne-Mendel strain (16), and in other strains (4), it was reported as less than 1%.

Under appropriate chemical stimulation, the connective tissue of the rat has a considerable propensity to develop sarcomas (8). This is also true for mice (2) and guinea pigs (17, 21).

A more subtle etiology is apparently operative in the genesis of mesenchymal sarcomas arising in scar tissue around inserted plastic films. In 1941, Turner (19) produced fibrosarcomas in the scar tissue around Bakelite disks implanted in the s.c. tissue of rats. Seven years later, Oppenheimer et al. (13) reported the occurrence of fibrosarcomas around cellophane film, and followed this with reports of s.c. sarcomas occurring adjacent to such plastic films as Dacron, nylon, Saran, and polyethylene (12), as well as around metal foils (11) and glass coverslips (5).

Analysis of the scar tissue around cellophane has shown an increase in insoluble collagen around the films prior to the development of sarcoma (5).

The operation of some regional factor at the anastomotic site in our parabionts and related to scar formation was further suggested by a group of 172 rats not included in the present series. Here the incidence of anastomotic sarcomas in the hands of 1 operator reached 42% in contrast to the usual incidence of 7 to 9% in the hands of others. At present the reason for this unusual increase is not apparent, and further experiments are being conducted in attempts to produce denser and more extensive scars by wider incisions, vascular occlusion, and increased local trauma.

A review of the lineage of tumor-bearing rats showed no consistent pattern.

Subtle differences in the histocompatibility of the 2 partners might produce imperfect continuity of mesenchymally derived cells and cause the cells of 1 animal to act as an impenetrable film to the cells of the other animal, thus stimulating scar tissue reaction and ultimate neoplasia.

REFERENCES


Fig. 1. Characteristic location of anastomotic sarcomas.

Fig. 2. Diagrammatic representation of the location of mesenchymal sarcomas in experimental parabiont pairs, control parabiont pairs, and single control rats. The region considered to be anastomotic is stippled. The highest incidence of sarcomas is in the anastomotic region of those pairs in which the righthand animal received 1000 R. The irradiated member of the pair shows a greater incidence of sarcomas than does the shielded partner. The incidence in control parabiont pairs is somewhat larger than in control single animals.
Fig. 3. Well-differentiated fibrosarcoma; fairly abundant collagen; rare mitoses. H & E, × 500.

Fig. 4. Rhabdomyosarcoma with tumor giant cells, myofibrils, and cross-striations. Phosphotungstic acid-hematoxylin, × 600.

Fig. 5. Undifferentiated sarcoma with poorly defined cytoplasmic boundaries and numerous mitoses. H & E, × 600.

Fig. 6. Portion of typical tumor cell representing characteristic findings as seen in 1 sarcoma. Note the secondary lysosomes containing laminated phospholipid bodies, probably the result of autolytic digestion of mitochondria (Rat PRA 73). Electron photomicrograph, × 40,000. (Courtesy of Dr. Micheline Federman.)
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