Effect of Experimental Ehrlich Ascites Tumors on Healing of Abdominal Wall Wounds in Mice

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Abstract: Many systemic factors may influence the healing process. The present study aimed to analyze histological modifications induced by the presence of Ehrlich ascites tumors on laparotomic surgical scars in BALB/c mice. A total of 52 mice were used. Half of the mice were injected with Ehrlich tumor cells, and 7 days later (day 7) all mice underwent laparotomy. On day 11, the scar was resected in 10 mice with the tumor and in the 10 control mice. The procedure was repeated on day 14 with the remaining animals. The scar tissue was histologically evaluated by means of semiquantitative analysis for acute inflammation, re-epithelization, formation of granulation tissue, chronic inflammation, fibroblast proliferation, and collagenization. Mice injected with tumor cells gained weight due to ascites growth. Histologic results showed that Ehrlich ascites tumor cells did not affect initial acute inflammation, re-epithelization, and formation of granulation tissue (P = ns). Chronic inflammation and fibroblast proliferation were, however, significantly decreased in mice with tumors, whereas collagenization had increased (P = 0.001). These results show that Ehrlich ascites tumors affect the healing process in mice. Despite a decrease in chronic inflammation and fibroblast activity, scars in these animals had more collagen, were more fibrous, and were better organized.

The Ehrlich tumor as described in 1906, originated from a murine breast carcinoma. It is one of the best characterized transplantable tumors as it may be maintained in solid or ascitic form, and is currently one of the most frequently used experimental models. The experimental Ehrlich ascites tumor establishes in 1 week and results in angiogenesis, cell proliferation, and invasion, which is similar to other neoplastic processes.

Healing is a process whereby injured tissue is replaced with scar tissue and is not dependent on the cause of the wound. It is now accepted that healing has 5 stages—an acute inflammatory process that proceeds through cell proliferation, formation of connective tissue, contraction, and finally wound remodeling. In both rats and mice, the inflammatory stage lasts 4 days, fibroplasia lasts 14 days, and maturation begins soon after.
Currently, surgical procedures are based on notions of surgical metabolism, dietetic and anesthesia variables, and accurate surgical techniques, which aim to shorten surgical time and reduce trauma to avoid any effects on the healing process. The process may also be influenced by systemic factors that are intrinsic to the patient such as circulatory conditions, nutritional status, diabetes, and immunosuppression. Local factors related to the wound such as infection, early movement, presence of foreign bodies, and location, size, and wound type are also involved.

A patient with cancer will have the presence of systemic factors that possibly affect healing mechanisms within the various stages of the disease.

The objective of this work was to analyze histological alterations of laparotomy scars in mice induced by experimental Ehrlich ascites tumors and to determine if the neoplastic condition influences the healing process.

**Materials and Methods**

Fifty-two 2-month-old female BALB/c mice weighing between 18 g and 22 g were used in this study. The animals were weaned at day 20 and were maintained in individual cages in standard temperature and humidity conditions with a 12-hour light cycle. Food and water were interrupted at 6 hours and 1 hour, respectively, before submitting the animals to the surgical procedure. All animals received humane care and the protocols were in compliance with the institution’s guidelines after receiving approval from the University of Taubaté Research Ethics Committee (no. 0202/04) and from the University of Taubaté (no. 362/2003).

The first step in preparing the Ehrlich tumor cells for introduction on half of the 52 mice abdomen was to collect ascites from a female with a tumor.

Thirty-mL of phosphate-saline-buffer (PBS) was added to 5-mL of ascites and was spun for 5 minutes at 1200 rpm. The supernatant was refused and filled with 30-mL of PBS; the procedure was repeated by spinning it four times.

The tumor cells viability was assessed using trypan blue stain 0.4% and was > 90%.

The cells were counted using an optical microscope at 40x magnification and the ordinate reading using a Newbauer chamber.

A group of 26 mice (group 1 [G1]) was injected in the left inferior quarter of the abdomen with 400-µL of PBS. Group 2 (G2), also composed of 26 mice, was injected with 400-µL of a suspension of Ehrlich tumor cells at 5 x 10^7 cells/mL.

Seven days later, the mice were weighed and anesthetized in doses in which the 20%-30% weight gain due to the tumor was not considered. Atropine sulphate was used for pre-anesthesia, and xylazine-ketamine hydrochloride was used for anesthesia via intramuscular injection.

Surgery was performed after antisepsis of the inferior third of the thorax and abdomen of the animals with 10% polyvinylpyrrolidone in an alcohol solution. A median incision was made below the xiphoid process that extended 2.5 cm and included all structures of the abdominal wall. The abdomen of all animals was examined, and any ascites observed was preserved. The abdominal wall was closed in two layers with mononylon 5-0 monofilament suture. The skin was closed with the same mononylon 5-0 suture.

Four days later (day 11), 10 G1 mice and 10 G2 mice were weighed and anesthetized as described above. The

**Figure 1.** Young scars with granulation tissue, inflammation, and a small amount of collagenization (H&E stain, 40x).

**Figure 2.** Whole epithelium, under which there is moderate inflammatory infiltrate and signs of collagenization (H&E stain, 100x).

**Figure 3.** Neoplastic infiltration with discontinuity of the epithelium lining (arrows) (H&E stain, 40x).
anterior wall of the abdomen was resected and the animals were sacrificed. The procedure was repeated 3 days later (day 14) with the remaining mice (13 G1 and 7 G2). In the research draft two groups were created (control and experimental) that were sacrificed in different days in an effort to study younger and more mature scars in mice with and without a tumor.

Paraffin sections were prepared and stained with hematoxylin-eosin (H&E) and picrosirius. Eight fields from each section were examined with 10x eyepiece and 4, 10, and 40x objectives. Sections were examined for acute inflammation, re-epithelization, formation of granulation tissue, chronic inflammation, fibroblast proliferation, and collagenization. Parameters were scored as (+), (++), or (+++) for mild, moderate, or intense alterations, respectively, in semi-quantitative analysis (Figures 1–3).

**Results**

Twelve animals died during the experiment. Six control group animals died after anesthesia and surgery. The 6 animals from experiment group died as a result of the tumor.

The groups were compared for weight gain by the Student’s t-test for non-related samples.

The descriptive analysis showed that mice from G2 had a tendency for greater weight than G1 mice (Figure 4).

For histologic results, control and experimental mice were compared with a log linear data model $P < 0.05$ was considered significant.

Comparison of groups 1 and 2 showed that acute inflammation, re-epithelization, formation of granulation tissue and angiogenesis were not affected by the treatment, with similar results found in animals with or without ascites tumor. Twelve mice died during the course of the experiments, thus the results from 23 mice (G1) and 17 (G2) are shown (Table 1).

Conversely, statistically significant differences between G1 and G2 were observed for chronic inflammation, fibroblast proliferation, and collagenization (Table 1).

In the G1 mice without a tumor, higher chronic inflammation and fibroblast proliferation was observed. However, the collagenization was lower than that of G2, and even with lower chronic inflammation and fibroblasts formation, had scars with more collagen.

**Discussion**

In this study, injection of Ehrlich tumor cells in mice ultimately resulted in the development of the tumor and ascites, as shown by the increased weight of treated animals. Clinical alterations were observed similar to those induced by malignant neoplasias in moderate or advanced stages. These disturbances involved respiratory, nutritional, hydro-electrolytic, and acid-base balance parameters.
Soluble and cellular mechanisms induced by the tumor may influence the healing process, as previously shown for other nutritional and metabolic parameters\(^{11,12}\).

The rapid tumor growth, and the need for surgical interventions, and sacrifice of the experimental animals prevented the study of later stages of the healing process, which would be interesting in view of the differences in healing stages between humans and mice.

The surgical procedure included suture in two layers with separate stitches, which presents better results than continuous suturing in one layer\(^{13}\). The pathologic investigations were designed for early wound healing in which independent and comparable histologic elements were analyzed\(^{14}\).

Acute inflammation was similar in mice from both groups, showing that the soluble and cellular elements that induce chemotaxy were not influenced by the presence of the tumor\(^{5}\). On the fourth postoperative day, acute inflammation was already diminished and the cell proliferation had begun. Macrophages participate more intensely of this stage, and some modification of local or systemic elements related was expected.

Chronic inflammation was significantly more intense in mice from G1, indicating that the tumor interferes with this process. Similar results were observed for fibroblast proliferation, which is related to chronic inflammation and later stages of the healing process, decreased in mice with a tumor. Since macrophages are most likely involved with the proliferation of fibroblasts in the surgical wound, \(^{15}\) the statistically higher proliferation rate of fibroblasts in mice from G1 is perhaps due to a logical sequence of the chronic inflammatory process, and the presence of macrophages and their possible activity\(^{16}\).

The extension of angiogenesis depends on the presence of stimulating and inhibitory factors; in normal tissues and surgical wounds, inhibitory factors are more frequent contrary to what happens in tumor tissues\(^{16,17}\). Granulation tissue and vascular neoformation were not modified by the presence of Ehrlich tumor cells in the present study. Peritoneal tumor blocks present tumor angiogenesis. An influence on inflammatory, healing, and physiological angiogenesis in the surgical wound was not seen. Tumor cells in some of the mice invaded the wound. It is possible that the angiogenic process was different in the surgical wound and scar as compared to the tumor with the predominant activity of inhibitory and stimulating factors, respectively. The surgical wound presented physiological progression.

The process of collagenization, contrary to the other parameters, was significantly more intense in mice with Ehrlich ascites tumors. Fibroblasts showed greater capacity to produce collagen in animals with tumors despite having less stimulation by macrophages, which were possibly less involved with the processes of chronic inflammation and fibroblast proliferation, as observed in the scars of G2 mice. It is possible that fibroblasts in these animals were stimulated by the more intense production of soluble factors, which explains the greater collagen production despite the smaller number of producing cells\(^{19}\). Tissue oxygenation and angiogenic factors may also have been involved\(^{18}\).

Scars in mice from G2 showed a more organized structure with more collagen, a superiorly structured fibrosis and remodeling, and probably faster healing than control mice. Tumor cells invaded the wound, but they did not have the capacity or perhaps enough time to invade tissues and reach blood or lymph vessels. Histological examination of the sections did not show vascular invasion of tumor cells, as previously reported\(^{19}\). Invasion of tumor cells interfered with, but did not prevent, the healing process in mice from G2.

The local influence of ascites and the pressure resulting from the process could explain the histological aspects more frequently observed in mice from G2. These results, however, were not significantly different when data from day 11 and day 14 were compared, although weight and ascites contents of the animals were different. Pressure and distention due to ascites progressively increased during the 7 days following the first surgery, but did not influence the establishment of better organized fibrosis and the presence of collagen.

**Conclusion**

Future perspectives of this investigation include the modification of oxygen supply, which might provide important information. Hyperoxia, observed during anesthesia in intensive care units, and hypoxia might interfere with healing and tumor growth processes\(^{20}\). The relationship of hyperoxia and hypoxia with physiological angiogenesis, wound healing, and tumor angiogenesis is an important point to be considered\(^{21}\).

**References**

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