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Comparison of Local Cellular Reaction to Tumor Grafts in Mice Treated with Some Plant Polysaccharides

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SUMMARY

Local cellular reactions around s.c. grafts of Sarcoma 180 were examined in mice treated with several plant polysaccharides, some known to be antitumor active and others known to be inactive.

Extensive outpouring of lymphoid cells, mostly plasma cells and macrophages, in the immediate vicinity of the graft at an early period (1 week after implantation) and later invasion into the graft by connective tissue cells characterized the effect of polysaccharides which suppressed the tumor grafts, namely, wheat straw hemicellulose B, lichen polysaccharide GE-3, and lentinan. These characteristic cellular reactions were either absent or slight in untreated controls, negative controls treated with inactive polysaccharides (wheat straw hemicellulose A and sunflower stalk hemicellulose B), and autochthonous controls against which none of the polysaccharides showed any effect.

The findings with transplanted allogeneic tumors cannot be evaluated on an exact immunological basis, but they may be of interest in suggesting a possible role of the local lymphoid cell reaction in allogeneic tumor graft rejection.

INTRODUCTION

Injections of several polysaccharides isolated from various plant sources induce resistance to transplanted tumors in mice (1, 2, 4–9). Although these studies were based on long-term-transplanted allogeneic tumors, mostly Sarcoma 180, the grafts and recipients were uniformly compatible, permitting 100% takes and progressive growth of the tumors in untreated, control mice. The fact that some polysaccharides conferred resistance while others did not was probably related to differences in the chemical structures of the polysaccharides.

Since the early work of Da Fano (3), it has become axiomatic that lymphoid cell infiltration, including plasma cells, lymphocytes, and macrophages, and connective tissue activities constitute the histological sign of resistance to transplanted tumors. From the biological point of view, therefore, it was considered probable that different polysaccharides might show some difference as to the cellular reaction around the tumor grafts, depending on whether the substances were antitumor active or inactive. This study was undertaken to determine this point.

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MATERIALS AND METHODS

Three representatives of the polysaccharides known to be active in inhibiting Sarcoma 180 implanted in mice were used. These were wheat straw hemicellulose B (6), lichen polysaccharide GE-3 (partially acetylated β-1,6-glucan isolated from a species of lichen, *Gyrophora esculenta*) (8), and lentinan [β-(1→3) linear glucan isolated from an edible Japanese mushroom, *Lentinus edodes*] (1, 2). For "negative controls," wheat straw hemicellulose A and hemicellulose B isolated from sunflower stalks (6) were selected as examples of inactive polysaccharides.

Normal Swiss albino mice weighing approximately 20 g were divided into groups of 10 each. All the mice were given implants of Sarcoma 180 (ascites form) s.c. in the right groin, and each group was treated with i.p. injections of one of the polysaccharides in a water solution. The dosages were 100 mg/kg for wheat straw hemicellulose B, 200 mg/kg for lichen polysaccharide, 1 mg/kg for lentinan, and 200 mg/kg for both wheat straw hemicellulose A and sunflower stalk hemicellulose B (Table 1). Injections were started 24 hr after the tumor implantation and continued daily for 5 or 10 days. One group of mice was held without treatment as "blank controls."

For comparison, similar groups of mice were set up in an autochthonous tumor-host system of spontaneous mammary adenocarcinoma in random-bred Swiss mice. These autochthonous tumor grafts are not influenced by any of the polysaccharides (10), and they were included here as "autochthonous controls."

Mice of all the groups were killed in lots of 3 or 4 each at 1, 2, and 3 weeks after tumor implantation. Examination before the 1-week period was considered useless because of the possible confusion of histological pictures by nonspecific reactions to the trauma of implantation. Tumor grafts with the surrounding s.c. tissue were removed for histological examination. Tissues were fixed in formalin, and sections were stained with hematoxylin and eosin.

RESULTS

Reaction in Mice Treated with Antitumor-active Polysaccharides. All the polysaccharides mentioned above as active in suppressing the growth of implanted tumor grafts (wheat straw hemicellulose B, lichen polysaccharide, and lentinan) showed essentially the identical effects on the local cellular reactions around the Sarcoma 180 graft.

In the specimens taken 1 week after tumor implantation,

i.e., after 5 daily injections of 1 of the active polysaccharides, the bulk of the tumor graft was in a state of necrosis, but many large and small groups of intact tumor cells were present in the peripheral parts. In the immediate vicinity of these tumor cell masses, there was extensive outpouring of lymphoid cells (Fig. 1), with plasma cells and macrophages composing the main cell types (Fig. 2). Small lymphocytes were not prominent. This plasma cell-macrophage mobilization was by far the most striking and characteristic feature of the local cellular reaction. The proliferation of connective tissue cells with lymphocytic infiltration was present in the area slightly distal to the very edge of the graft. Lymphocytic infiltration was seen in and about muscular tissue and also perivascularly near the tumor graft.

In specimens removed 2 or 3 weeks after tumor implantation, the plasma cell-macrophage reaction was no longer present; instead, invasion by connective tissue cells into the graft was actively in progress (Fig. 3). Here tumor cells were isolated and encircled by connective tissue cells, showing the typical picture of the destruction of allogeneic tumor grafts.

Blank (Untreated) Controls. The reaction to Sarcoma 180 grafts in untreated mice conformed to the well-known picture of successful allogeneic tumor grafts in general. At the end of the 1st week, there was no plasma cell-macrophage outpouring, as was so prominently observed in mice treated with active polysaccharides (Fig. 4). The formation of connective tissue stroma with adequate vascularization was evident. Tumor cells were often loosely spreading out or infiltratively invading the surrounding normal tissue. There was sporadic occurrence of lymphocytic infiltration in association with a connective tissue reaction around the tumor grafts, as may be expected in any case of allogeneic tumor transplantation.

At the end of the 2nd and 3rd weeks, grafts were generally established as solid tumor masses with some central necrosis, and connective tissue bordering the growing margin of the tumor was devoid of any remarkable cellular reaction.

Negative Controls. The histological picture of Sarcoma 180 grafts in mice treated with wheat straw hemicellulose A and with sunflower stalk hemicellulose B, both already shown to be inactive (6), was not different from the above description of untreated controls. There was but a slight local cell reaction around the graft at the 1-week period, and plasma cells and macrophages were not in evidence in this reaction. The tissues surrounding the graft gradually became quiescent, and in the 2- and 3-week specimens sarcoma tissue was generally actively growing, with adequate support of stromal connective tissue and copious vascular supply. There was no sign of invasion into the tumor masses on the part of connective tissue cells.

Autochthonous Controls. The autochthonous grafts of spontaneous mammary adenocarcinoma always grew in the usual acinus form. With or without polysaccharide treatment, there was no plasma cell and macrophage reaction, which characterized the inhibition and regression of allogeneic tumor grafts. The stromal connective tissue proliferation and vascularization were very active, and lymphocytic infiltration, if any, was only very slight.

DISCUSSION

In the study of plant polysaccharide effects, there are many points yet to be elucidated, for example, possible different effects of different polysaccharides on peritoneal cell exudation after i.p. injections, on peripheral white cell counts, on reticuloendothelial function, etc. Also, I am aware that the transplanted allogeneic tumor used does not permit well-controlled immunological investigation. In justification, however, the allogeneic tumor graft-recipient system I used ensured 100% takes of the grafts and their progressive growth leading to the death of the hosts.

In this study, I have examined Sarcoma 180 grafts and surrounding tissues and found an extensive lymphoid cell outpouring followed by connective tissue invasion into the graft in mice treated with wheat straw hemicellulose B, lichen polysaccharide, and lentinan. These polysaccharides are known to induce resistance, with eventual complete regression of

Table 1

Effect on Sarcoma 180 of several plant polysaccharides (compiled from published data)

Polysaccharide and dose	No. of mice	Complete regression	Tumor weight (g)	Reference
Wheat straw hemicellulose B, 100 mg/kg	10	8/10	0.04[a] (0–0.2)[b]	Nakahara *et al.* (6)
Control	10	0/10	7.2 (2.7–11.5)	
Lichen polysaccharide GE-3, 200 mg/kg	10	8/10	0.06 (0–0.5)	Shibata *et al.* (8)
Control	10	0/10	7.4 (3.5–12.0)	
Lentinan, 1 mg/kg	7	7/7	0	Chihara *et al.* (2)
Control	8	0/8	9.5 (4.0–11.4)	
Wheat straw hemicellulose A, 200 mg/kg	8	0/8	9.9 (6.0–14.1)	Nakahara *et al.* (6)
Control	8	0/8	10.5 (8.1–13.0)	
Sunflower stalk hemicellulose B, 200 mg/kg	9	0/9	5.6 (2.0–11.3)	Nakahara *et al.* (6)
Control	10	0/10	7.9 (5.1–10.9)	

[a] Mean.
[b] Range.

many of the tumors. In mice treated with wheat straw hemicellulose A, as well as with sunflower stalk hemicellulose B, both known to be inactive, the lymphoid cell reaction was insignificant or absent, and there was no invasion of the graft by connective tissue cells. A similar lack of cellular reaction was noted in untreated controls.

The tumor transplantation data relevant to the difference in cellular reaction described above are summarized in Table 1.

The absence of plasma cell and macrophage mobilization against autochthonous grafts was in accordance with previous experiments with spontaneous mammary tumors in mice (10), which demonstrated that the plant polysaccharides known to be potent in suppressing the growth of allogeneic tumor grafts entirely failed to inhibit local recurrence, including the growth of autografts, after surgical removal of the primary tumors, to prolong the postoperative survival period, to reduce the rate of new tumors developing at sites away from the primary growth, and to reduce the rate of lung metastasis formation found at autopsy.

The plant polysaccharides may be of no real value for improving tumor immunity, since they fail to influence autochthonous tumors. When used under appropriate experimental conditions, however, these materials may serve as convenient tools in the study of the mechanism of allogeneic tumor graft rejection. Also, the antitumor effect, as determinable by tests with allogeneic tumor transplantation, may offer useful bioassay methods in the elucidation of structure-activity relations in the field of polysaccharide chemistry.

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Fig. 1. A portion of a Sarcoma 180 graft, closely attended by a profuse outpouring of lymphoid cells, 1 week after implantation in a mouse treated with wheat straw hemicellulose B. H & E, × 100.

Fig. 2. An area of the lymphoid cell reaction in the above figure. High-power view showing the characters of cell types (macrophages and plasma cells) participating in the reaction. H & E, × 400.

Fig. 3. Invasion of connective tissue cells into Sarcoma 180 graft, showing a few isolated sarcoma cells encircled by the proliferating connective tissue with some lymphocytic infiltration. Two weeks after tumor implantation in mouse treated with wheat straw hemicellulose B. H & E, × 100.

Fig. 4. A portion of a Sarcoma 180 graft in an untreated mouse, showing no plasma cell-macrophage reaction between the border of tumor growth and the necrotic area. Compare with Fig. 1, which shows an extensive cellular reaction at the corresponding location. One week after tumor implantation. H & E, × 100.