CLIN. EXPL. METASTASIS, 1988, VOL. 6, NO. 2, 115–120

Inhibition of liver tumor cell colonization in two animal tumor models by lectin blocking with D-galactose or arabinogalactan

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(*Received 28 January 1987; accepted 22 April 1987*)

Repeated administration of the hepatic lectin blocking agents D-galactose or arabinogalactan completely prevented the settling of metastatic cells of sarcoma L-1 tumor in the liver of Balb/c mice and greatly reduced the colonization process of highly metastatic ESb lymphoma cells of the liver of DBA/2 mice. Therefore, when hepatic lectins were blocked with competitive glycoconjugates, tumor cell colonization of the liver could be prevented in two different model systems.

Introduction

In many tumor systems lung and liver are the organs most frequently involved in the formation of secondary tumor colonies. These organs are also the predominant sites of metastasis of chemically induced lymphomas (ESb) in DBA/2 mice [17, 18] and of sarcoma L-1 tumor in Balb/c mice [5, 16]. We previously described that organ-characteristic lectins (e.g. the hepatic lectin, discovered by Ashwell and Morell [1]) can apparently act as acceptors of neoplastic cells in metastatic 'homing' by interacting with cryptic carbohydrate receptors or precursor structures similar to the Thomsen–Friedenreich antigen [25] on the surface of metastatic tumor cells [3, 7, 13, 24, 25, 28]. This conclusion was supported by the following findings:

1. Spontaneous high-metastatic variants (ESb) of the mouse lymphoma Eb which show heavy liver involvement formed rosettes *in vitro* with isolated autologous hepatocytes whilst the low metastatic parental line Eb did not [18].
2. In two species (mouse and rat) galactosyl-specific receptors could be identified on liver cells which served as binding sites for tumor cells [7, 21].
3. More than ten different surface molecules of ESb tumor cells, and none of Eb-type tumor cells, served as carbohydrate ligands in the hepatocyte interaction [7].
4. ESb lymphoma cells express blood-group-related Thomsen–Friedenreich (T) antigen [24] with terminal Gal β 1-3-Gal NAc units; furthermore, soluble T antigen was a potent competitive inhibitor of ESb adhesion to liver cells [25].

In view of the galactosyl specificity of the hepatic lectin (HL) and the above *in vitro* findings we have suggested using competitive carbohydrate-bearing glycoconjugates *in vivo* to inhibit the metastatic spread of such tumor cells into the liver. The

experiments were designed in such a way that the liver receptors would be blocked before their first contact with circulating tumor cells. In accordance with Ashwell and Morell [1] blockade of the hepatic lectin (HL) could be demonstrated after intravenous administration of tritiated α_1-acid (asialo) glycoprotein which was rapidly cleared and taken up by the liver. However, pre-injection of D-galactose (arabinogalactan) caused a markedly delayed elimination of the asialoglycoprotein from the serum [5]. Recent experiments with neuraminidase-treated sarcoma L-1 cells, which colonize lung and liver of Balb/c mice, supported this hypothesis [29]. Pre-injection and repeated re-injection of D-galactose or arabinogalactan prevented tumor colonization of the liver but did not influence dissemination to the lung [5, 30]. Mannan and other galactose-free polysaccharides did not affect the initial pattern of organ colonization [5].

In the present paper we demonstrate similar *in vivo* inhibition results in the ESb model system. Liver colonization of this highly aggressive tumor line could be substantially reduced when lectin receptors were blocked with competitive sugars or with glycoconjugates.

Materials and methods

Animals

Inbred Balb/c mice (Central Institute for Experimental Animals, Hannover, FRG) and DBA/2 mice (Wiga Animal Breeding Comp., Sulzfeld, FRG) were used for the *in vivo* studies. The animals were kept in plastic cages and allowed free access to food and water.

Tumor

ESb is a spontaneous, highly metastatic variant of Eb, a Heidelberg subline of methylcholanthrene-induced DBA/2 mouse lymphoma L 5178 Y. These tumor cells were passaged in tissue culture using RPMI-1640 (Grand Island Biological Co., Gibco, Grand Island, N.Y., USA) with 10 per cent fetal calf serum (FCS) and 2×10^{-5} M 2-mercaptoethanol.

Sarcoma L-1 tumor (Institute of Oncology, Warsaw, Poland) arose spontaneously in the lung of a Balb/c mouse and was maintained in this strain [16]. Material was used from serial passages (110–116) of the tumor. 'Primary' tumors were dissected from donor mice, minced with scissors and rubbed through a steel sieve. The cells were washed, suspended in RPMI and counted. All cell suspensions injected into mice were > 95 per cent viable as assessed by trypan blue dye exclusion, and were examined microscopically for signs of aggregation. Suspensions exhibiting any obvious aggregates were discarded since the extent of tumor cell clumping can affect colonization capacity [14]. Preparation and administration of the cells were as previously described [5].

Chemicals

D-galactose (Gal) and arabinogalactan (a heterogeneous Gal residues-containing compound from *Larix Europaea* with an average mol wt of about 70,000 daltons) were obtained from Serva GmbH, Heidelberg, FRG; mannan from Sigma Chemicals Co., St. Louis, MO, USA; and phosphate buffered saline (PBS) from Flow Laboratories GmbH, Meckenheim, FRG.

Experiments

1×10^5 sarcoma L-1 cells (Balb/c mouse origin) and 1×10^4 ESb cells (DBA/2 mouse origin) were intravenously inoculated into the tail veins of syngeneic mice. D-galactose (2 mg/g body weight) or arabinogalactan (1 mg/g body weight), solubilized in 0·2 ml PBS, were intraperitoneally pre-injected (1 h before tumor cell inoculation) and regularly administered (12 h intervals) for 3 days or 7 days respectively (sarcoma L-1/Balb/c-model and ESb/DBA/2-model). From our previous investigations these doses of D-galactose/arabinogalactan, as well as the schedule of administration and the tumor cell dose, proved to be optimal [5]. Furthermore, no side-effects of this regimen were obvious in mice. Lung and liver surface tumor nodules were counted under a dissecting microscope by two independent observers 14 days after sarcoma L-1 cell inoculation and 7 days after administration of the metastatic ESb cells as previously described [5].

Results

Two model systems were investigated and compared: sarcoma L-1 in Balb/c mice and ESb lymphoma in DBA/2 mice. In both experimental tumor systems the hepatic lectin-blocking capacity of D-galactose and arabinogalactan were studied. Pre-injection (1 h before intravenous inoculation of tumor cells) and regular intraperitoneal re-injection of arabinogalactan completely prevented the settling of sarcoma L-1 tumor and greatly decreased the dissemination of highly metastatic ESb tumor cells into the livers of syngeneic mice (table 1; 78 per cent for arabinogalactan and 69 per cent for D-galactose). However, this treatment did not influence the homing of tumor cells to other organs e.g. lung as the number of tumor nodules (range 150–200) did not differ from the control group. The rapid metabolism and elimination of D-galactose prohibits complete lectin-blocking during a 12 h period. Compared to intravenous administration, intraperitoneal injection results in a prolonged presence in the circulation. The discontinuous application (same timing scheme as arabinogalactan), however, is responsible for the

Table 1. Mean number of liver colonies in mice injected with tumor cells and treated with hepatic lectin blocking agents.

	Mean number of liver colonies (±SD)*			
Intravenous inoculation	PBS	D-galactose	Arabinogalactan	Mannan
1×10^5 sarcoma L-1 cells into Balb/c mice	12·0 (±6)	0·2 (±1)*	0*	10·3 (±5)
1×10^4 ESb cells into DBA/2 mice	74·5 (±14)	22·8 (±11)**	16·3 (±9)**	n.t.

D-galactose 2 mg/g body weight; arabinogalactan or mannan 1 mg/g body weight. Ten to twenty mice per group were injected with indicated materials. In all mice the number of long colonies was high (range 150–200) and not precisely estimated.

* $P < 0{\cdot}05$; ** $P < 0{\cdot}005$, both are statistically significantly different from controls, but there is no difference between D-galactose and arabinogalactan treatment results within the same model.

n.t. = Not tested; PBS = phosphate buffered saline; SD = standard deviation.

negligible number of liver tumor nodules in D-galactose treated Balb/c mice. In comparison with D-galactose, arabinogalactan has a much higher molecular weight which is responsible for its longer half-life in the circulation of mice (unpublished data), leading to a more effective hepatic lectin blockade. Consequently, the discontinuous application of arabinogalactan requires lower amounts to inhibit the tumor spread into the liver than of D-galactose. Mannan (administered as a control polysaccharide without Gal; the same timing scheme as Gal) altered neither the pattern or organ colonization nor the number of tumor nodules in Balb/c mice.

Discussion

Recent *in vitro* [4, 6] and *in vivo* experiments [5, 29] are in accordance with our hypothesis and show that the 'homing' of tumor cells into the liver can be prevented or decreased either by non-immunogenic galactans (e.g. arabinogalactan) or by D-galactose. This lectin-mediated recognition process is highly specific [29] and obviously not related to the number of D-galactose residues, but more to their steric arrangement [2, 5] fitting into the combining area of the lectin. Previous fine specificity analysis of ESb-hepatocyte rosette inhibition by various mono- or disaccharides revealed that Gal β 1-3 Gal NAc was the best inhibitor [24]. At higher concentrations, such as 0·1 M, Gal and Gal NAc caused about 50 per cent inhibition, while glucose and ribose were negative [7]. Most appropriate for *in vivo* application should be such carbohydrate-bearing molecules which are non-toxic, have a long half-life, express the right carbohydrates in the correct steric arrangement and at a density sufficient for multiple ligand interactions with the corresponding cell surface lectins of organ parenchymal cells. It is clear that the molecules used in this study are not optimal with respect to all of these criteria, and that more research into such ligands is required.

The basic mechanisms of adhesion of tumor cells [4, 8] and adhesion of bacteria [6, 22] appear to have much in common, since lectins can be involved in both. This may also be true for macrophages [10] which also have several different lectins on their surface [23]. Even tumor cells themselves have been reported to express carbohydrate-binding lectin-like molecules [11, 15]. Furthermore, when highly metastatic tumor lines were compared with their low metastatic counterparts, differences were noted quite regularly in the expression of endogenous sugar-binding proteins [12]. Because of these findings we suggest that lectin–carbohydrate interactions may have some significance for the metastatic process. Accordingly, it might be possible to inhibit the spreading of certain tumor cells into the liver (e.g. during the surgical treatment of malignant diseases) by blocking organ-characteristic lectins with monosaccharides or appropriate neoglycoconjugates. However, in other tumor systems metastatic to liver, adhesion molecules not possessing D-galactose recognition may be involved [8]. The macrophage lectins may also be blocked under such conditions, which should be taken into consideration.

In order to evaluate immune responses during intermittent prophylactic D-galactose treatment, we examined various representative parameters of the immune system (IgM production; delayed type hypersensitivity to oxazolone; monocyte, granulocyte and complement activation) and found no differences compared with control groups. Furthermore, no abnormal effects of high and prolonged doses of D-galactose (160 g Gal/24 h, administered orally for 5 days) were seen in human volunteers (unpublished data).

In conclusion, the data from two different experimental tumor systems (ESb cells in DBA/2 mice and sarcoma L-1 cells in Balb/c mice) suggest that the metastatic dissemination of these malignant tumors into the liver can be inhibited by tolerable concentrations of D-galactose or arabinogalactan.

Acknowledgments

This study was supported by the Deutsche Forschungsgemeinschaft (DFG). The authors are very grateful to Mrs D. Hauck, Mrs R. Pfeiffer and Mrs C. Böttinger for their excellent technical assistance.

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