Natural IgG antibody with anti- β -galactosyl specificity suppressed hepatoma cell invasion in culture

Yutaka Miura · Hiroshi Fujita ·
Fumihiko Sakai · Hiroyuki Tachikawa ·
Kazumi Yagasaki · Daisaburo Fujimoto

Received: 16 July 2012 / Accepted: 26 November 2012 / Published online: 19 December 2012
© Springer Science+Business Media Dordrecht 2012

Abstract The effect of natural IgG antibody recognizing β -galactosyl epitope on hepatoma cell invasion was investigated. Anti- β -galactosyl antibody dose-dependently suppressed hepatoma invasion underneath primarily cultured mesothelial cells monolayer without affecting the proliferation, to the same extent as natural IgG antibody with anti- α -galactosyl specificity, which had already been reported to have an anti-metastatic activity. The inhibitory effect of anti- β -galactosyl antibody was completely canceled by adding lactose (galactose- β -1, 4-glucose) to the medium, indicating that this antibody recognized some antigens with β -galactosyl epitope. Hepatoma cells pretreated with this antibody for 48 h showed reduced invasive activity, while the pretreatment of mesothelial cells with the antibody did not affect hepatoma cells invasion. Anti- β -

galactosyl antibody also suppressed hepatoma cells adhesion to mesothelial cells monolayer. These results suggest that natural antibody with anti- β -galactosyl specificity may recognize the β -galactosyl epitope in some adhesion-related molecules on hepatoma cells, thus suppressing adhesion and invasion to mesothelial cells monolayer. These results suggest possible therapeutic uses of this antibody in the treatment of metastatic tumors.

Keywords Natural antibody · Hepatoma · Invasion · β -galactosyl epitope

Introduction

Antibodies produced by an individual without a known history of sensitization to the relevant antigen are called natural antibodies. Many papers report the presence of several kinds of natural antibodies in human serum (Coutinho et al. 1995; Cramer 2000). Natural antibodies are known to have various physiological activities. Especially, natural antibody recognizing α -galactosyl epitope is known to be the major antibody which is involved in the rejection of transplanted xenografts (Sandrin and McKenzie 1994; Parker et al. 1999). Anti- α -galactosyl natural antibody is also reported to play an important role in the natural antitumor defense system (Catronovo et al. 1989;

Y. Miura (✉) · H. Fujita · F. Sakai · K. Yagasaki ·
D. Fujimoto
Department of Applied Biological Chemistry, Graduate
School of Agriculture, Tokyo University of Agriculture
and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-
8509, Japan
e-mail: eiyouym@cc.tuat.ac.jp

Present Address:

F. Sakai
Milk Science Research Institute, Megmilk Snow Brand
Co., Minamidai, Kawagoe, Saitama 350-1165, Japan

H. Tachikawa
Graduate School of Agriculture, The University of Tokyo,
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

Gollogly and Castronovo 1996; Larkin et al. 2006). Some patients with squamous intraepithelial lesions and early invasive cervical carcinoma are reported to contain high level of natural anti- α -galactosyl antibody in their sera (Tremont-Lukats et al. 1997). There are few studies describing the presence of natural antibody with β -galactosyl specificity as compared with anti- α -galactosyl antibody (Lucq et al. 2000). We have already found natural IgG antibody with β -galactosyl specificity in human sera and the serum concentration of this antibody is higher in some cancer patients with metastatic tumor than that in normal human or cancer patients without metastasis (Jung et al. 1992; Fujita et al. 1994). These results suggest that this antibody may also be involved in natural defense systems to tumor metastasis.

We have already developed an in vitro invasion assay system by co-culturing rat ascites hepatoma cell line of AH109A and rat mesentery-derived mesothelial cells (Miura et al. 1997a). Invasion is the most important and characteristic event in tumor metastasis (Liotta et al. 1988). Metastasis is a complex and sequential cascade of events that leads to the dissemination of tumor cells to distant tissues. In many cases, metastasis may be lethal in the course of clinical treatment of cancer. The inhibition of invasion is thought to lead to the inhibition of metastasis. We have already screened many substances in foods, which have anti-invasive activities using our assay system (Miura et al. 1997b; Kozuki et al. 2000, 2001).

In this paper, we have investigated the effect of natural β -galactosyl antibody on tumor cell invasion in vitro and clarified an anti-invasive ability of this natural antibody.

Materials and methods

Materials

Preparation of natural human antibodies with anti- α -galactosyl and anti- β -galactosyl specificities was described previously (Jung et al. 1992; Fujita et al. 1994). Male Donryu rats were purchased from NRC Haruna (Gunma, Japan). The animals were treated in accordance with guidelines established by the Animal Care and Use Committee at the Tokyo University of Agriculture and Technology. Other chemicals were best grade commercially available.

Cell culture

AH109A cells were obtained from the Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan) and maintained in the peritoneal cavity of Donryu rats as described previously (Miura et al. 1997a). AH109A cells cultured in MEM (Nissui Pharmaceutical Co., Tokyo, Japan) containing 10 % calf serum (JRH, Lenexa, KS, USA) (10 % CS/MEM) for at least 2 weeks after preparing from accumulated ascites were used for the following experiments.

Proliferation and invasion assays

The proliferative and invasive activities of AH109A cells were assessed as described previously (Miura et al. 1997a). Briefly, AH109A cells were seeded in the medium containing the antibody and/or the lactose and cultured for 20 h. Then MTT reagent was added to the culture medium and the absorbance of formed formazan was measured after further 4 h incubation. The proliferative activities were indicated as percent of control cells. As for the invasive activities, AH109A cells were co-cultured with primarily cultured mesentery-derived mesothelial cells (M-cells). After 24 h culture, the number of invaded cells and colonies were counted under phase-contrast microscope and the invasive activities were indicated as number of invaded cells and colonies/cm². In all experiments, normal human IgG antibodies with no specificities were added to the medium as control groups.

To assess the effects of pretreatment with antibody, AH109A cells or M-cells were treated with anti- β -galactosyl antibody for 48 h and treated cells were used for the invasion assays after washing twice with culture medium.

Binding assay

The binding activities of AH109A cells to M-cells were measured by modifying the above-mentioned invasion assay. That is, cells were co-cultured only for 2 h and washed gently and extensively with phosphate buffered saline. After 2 h co-culture, AH109A cells only adhered to M-cell monolayer and they had not invaded yet. The number of bound cells was counted under phase-contrast microscope and the binding activities were indicated as number of adhered cells/mm².

Statistical analyses

All data were shown as mean $\pm$ SEM. Data were statistically analyzed by one-way analysis of variance followed by Tukey–Kramer multiple comparison test. P value <0.05 is considered as significant.

Results and discussions

Anti- α -galactosyl antibody was reported to have anti-metastatic activities by inhibiting attachment of tumor cells to human endothelial cells or isolated extracellular matrices (Castronovo et al. 1987, 1989). We first compared the anti-invasive activities of anti- α -galactosyl antibody and anti- β -galactosyl antibody in our invasion assay system. Anti- β -galactosyl antibody showed the similar inhibitory effect on AH109A cell invasion as anti- α -galactosyl antibody as shown in Fig. 1a. This result indicated that natural antibody with β -galactosyl epitope might be also one of natural anti-tumor defense systems.

To investigate detailed effects of anti- β -galactosyl antibody on AH109A cell invasion, we checked dose-dependence of this anti-invasive effect. Figure 1B showed that anti- β -galactosyl antibody dose-dependently suppressed invasion of AH109A cells up to the concentration of 160 $\mu\text{g/ml}$. In our previous report, the serum concentration of anti- β -galactosyl antibody in normal humans was 10–280 $\mu\text{g/ml}$ (Fujita et al. 1994), further supporting the possibility that natural antibody with β -galactosyl epitope was one of the natural tumor defense systems.

Although anti- β -galactosyl antibody showed no or a little effect on the proliferation of AH109A cells (Fig. 1c), the inhibitory effect of anti- β -galactosyl antibody on the invasion was cancelled with the simultaneous addition of lactose (galactose- β -1, 4-glucose) (Fig. 1d). This result clearly indicated that this antibody showed its inhibitory effect on the hepatoma invasion by recognizing some epitopes with β -galactosyl structure on hepatoma cells and/or M-cells. In Fig. 1c, the proliferative activity of AH109A cells was slightly, but significantly suppressed by the addition of

Fig. 1 Effect of anti- β -galactosyl natural antibody on the invasion and proliferation of rat AH109A hepatoma cells. **a** The effect of anti- β -galactosyl antibody was compared with that of anti- α -galactosyl antibody. Both antibodies were added to the medium at a concentration of 200 $\mu\text{g/ml}$. **b** Dose-dependent effect of anti- β -galactosyl antibody on hepatoma cell invasion. **c** Effects of anti- β -galactosyl antibody (200 $\mu\text{g/ml}$) and/or lactose (1 mM) on the proliferation of hepatoma cells. **d** Effects of anti- β -galactosyl antibody (200 $\mu\text{g/ml}$) and/or lactose (1 mM) on the invasion of hepatoma cells. Values not sharing a common letter are significantly different by Tukey–Kramer multiple comparison test at $P < 0.05$

lactose alone and the addition of both anti- β -galactosyl antibody and lactose did not show further suppression in the proliferative activity. The reason of this suppression is not clear at present. This slight suppression was also seen in Fig. 1d (the invasive activities of cells in lactose-containing medium were slightly lower than for the control, but no significance was observed). To clarify whether the molecular target for anti- β -galactosyl antibody was present on AH109A cells or M-cells, both cells respectively pretreated with the antibody for 48 h and the change of invasive activities were assessed after the pretreatment. As shown in Fig. 2a, pretreatment of AH109A cells with anti- β -galactosyl antibody showed a similar suppressive activity of invasion with that of AH109A cells simultaneously treated with the antibody. However the pretreatment with the antibody of M-cells showed partial and weak suppression of invasion. This result suggests that anti- β -galactosyl antibody recognize some antigen(s) on AH109A cells rather than on M-cells.

The attachment of tumor cells to normal cell layer is known to be the first step for the invasion (Liotta et al. 1988). We finally investigated the effect of anti- β -galactosyl antibody on the adhesion of AH109A cells to M-cell monolayer (Fig. 2b). After 2 h co-culture, anti- β -galactosyl antibody slightly inhibited the adhesion of AH109A cells to M-cell monolayer and the pretreatment of AH109A cells with the antibody for 48 h resulted in a strong and significant suppression of

adhesion, although the pretreatment of M-cell monolayer did not show any effect on the adhesive activity of AH109A cells. In this binding assay, co-culture was done only for 2 h, so the effect of simultaneously-added anti- β -galactosyl antibody was weak compared with that in the pretreatment experiment for 48 h, but this result clearly indicate anti- β -galactosyl antibody inhibited invasion of hepatoma cells by inhibiting the adhesion of hepatoma cells to M-cell monolayer.

We had attempted to identify the molecular target of anti- β -galactosyl antibody, which was supposed to be present on AH109A cells, by western blot analysis of the membrane proteins of AH109A cells with the antibody. However, we could only detect some faint bands (data not shown) and could not identify the precise antigen. Our previous paper indicated that anti- β -galactosyl antibody did not recognize previously-reported tumor cell antigen, such as CPH, CTH or Forssman antigen (Jung et al. 1992; Fujita et al. 1994). Although some gangliosides, such as GM1a, GD1a, GD1b, or GT1b, were known to be involved in tumor cell motility or invasion and they have β -galactosyl bond in their structure, this antibody did not react with any gangliosides with β -galactosyl structure (Fujita et al. 1994). At present, the precise molecular target for the inhibitory effect of this antibody on hepatoma cell invasion was not identified. Further analyses seem to be needed for the identification of the antigen.

In conclusion, we have demonstrated that anti- β -galactosyl antibody could suppress hepatoma cell

Fig. 2 Effect of the pretreatment with anti- β -galactosyl natural antibody on the invasion and attachment of AH109A cells. AH109A cells or M-cells were pretreated with anti- β -galactosyl antibody for 48 h at the concentration of 200 μ g/ml. After washing cells, the invasive activity (a) and adhesive activity (b) were assessed as described in “Materials and methods”. Values not sharing a common letter are significantly different by Tukey–Kramer multiple comparison test at $P < 0.05$.

invasion in culture, suggesting that this natural antibody was one of the natural antitumor defense systems. These results suggest possible therapeutic uses of this antibody in the treatment of metastatic tumors.

References

- Castronovo V, Foidart JM, Li Vecchi M, Foidart JB, Bracke M, Mareel M, Mahieu P (1987) Human anti- α -galactosyl IgG reduces the lung colonization by murine MO4 cells. *Invasion Metastasis* 7:325–345
- Castronovo V, Colin C, Parent B, Foidart JM, Lambotte R, Mahieu P (1989) Possible role of human natural anti-Gal antibodies in the natural antitumor defense system. *J Natl Cancer Inst* 81:212–216
- Coutinho A, Kazatchkine MD, Avrameas S (1995) Natural autoantibodies. *Curr Opin Immunol* 7:812–818
- Cramer DV (2000) Natural antibodies and the host immune responses to xenografts. *Xenotransplantation* 7:83–92
- Fujita H, Jung SK, Mukainaka Y, Tachikawa H, Fujimoto D, Kosuge T, Shirai T, Takeiri A, Suzuki Y (1994) Characterization of natural IgG antibody with anti- β -galactosyl specificity. *Biomed Res* 15:17–25
- Gollogly L, Castronovo V (1996) A possible role for the α 1–3 galactosyl epitope and the natural anti-gal antibody in oncogenesis. *Neoplasia* 43:285–289
- Jung SK, Sakano Y, Fujimoto D (1992) Natural human IgG antibody with anti- β -galactosyl specificity. *Biomed Res* 13:317–320
- Kozuki Y, Miura Y, Yagasaki K (2000) Inhibitory effects of carotenoids on the invasion of rat ascites hepatoma cells in culture. *Cancer Lett* 151:111–115
- Kozuki Y, Miura Y, Yagasaki K (2001) Resveratrol suppresses hepatoma cell invasion independently of its anti-proliferative action. *Cancer Lett* 167:151–156
- Larkin JM, Norsworthy PJ, A'Hern RP, Eisen TG, Gore ME, Porter CD (2006) Anti- α Gal-dependent complement-mediated cytotoxicity in metastatic melanoma. *Melanoma Res* 16:157–163
- Liotta LA, Wewer U, Rao NC, Schiffmann E, Stracke M, Guirguis R, Thorgeirsson U, Muschel R, Sobel M (1988) Biochemical mechanism of tumor invasion and metastasis. *Prog Clin Biol Res* 256:3–16
- Lucq J, Tixier D, Guinault AM, Greffard A, Loisançe D, Pilatte Y (2000) The target antigens of naturally occurring human anti- β -galactose IgG are cryptic on porcine aortic endothelial cells. *Xenotransplantation* 7:3–13
- Miura Y, Shiomi H, Sakai F, Yagasaki K (1997a) Assay systems for screening food components that have anti-proliferative and anti-invasive activity to rat ascites hepatoma cells: in vitro and ex vivo effects of green tea extract. *Cytotechnology* 23:127–132
- Miura Y, Furuse T, Yagasaki K (1997b) Inhibitory effect of serum from rats administered with coffee on the proliferation and invasion of rat ascites hepatoma cells. *Cytotechnology* 25:221–225
- Parker W, Lin SS, Yu PB, Sood A, Nakamura YC, Song A, Everett ML, Platt JL (1999) Naturally occurring anti- α -galactosyl antibodies: relationship to xenoreactive anti- α -galactosyl antibodies. *Glycobiology* 9:865–873
- Sandrin MS, McKenzie IF (1994) Gal α (1, 3)Gal, the major zenoantigen(s) recognized in pigs by human natural antibodies. *Immunol Rev* 141:169–190
- Tremont-Lukats IW, Avila JL, Hernández D, Vásquez J, Teixeira GM, Rojas M (1997) Antibody levels against α -galactosyl epitopes in sera of patients with squamous intraepithelial lesions and early invasive cervical carcinoma. *Gynecol Oncol* 64:207–212