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Tumoricidal activation of murine resident peritoneal macrophages on pancreaticcarcinoma by interleukin-2 and monoclonal antibodies

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INTRODUCTION

Macrophages play an important role in tumor lysis and growth inhibition. They can be activated to a tumoricidal state by a variety of agents such as IFNr, TNFα or IL2. The killing machanisms of activated macrophages have been extensively investigated[1,2]. Recently, it has been proved that antibody dependent cellular cytotoxicity (ADCC) is one of the potent arms to lyse tumor cells resistant to cytotoxic macrophages, and that the antitumorous effect of a macrophage activator is significantly augmented by the combined use of mAbs capable of inducing ADCC to tumor cells[3].

The present study was undertaken to investigate these possibilities using Capan-2 human pancreatic carcinoma cell line. IL2 and YPC3 mAb inducing ADCC of murine splenic lymphocytes served as activators. The anti-pancreatic carcinoma efficacy of activated macrophages derived from murine peritoneal effusion cells (PEC) was observed *in vitro*.

MATERIALS AND METHODS

Media and reagents

The medium used for all experiments and cell maintenance was RPMI 1640 (Gibco, USA). ABC kit and IL2 were purchased from Vactastain and Ellite Co. Ltd, USA respectively. $Na_2{}^{51}CrO_4$ was supplied by the Isotope Institute of Chinese Academy of Medical Sciences.

Animals and cell lines

Capan-2 cell line was obtained from the University of California, USA, and Balb/c mice from the Experimental Animal Center of Sun Yat-Sen University of Medical Sciences.

Monoclonal antibodies

YPC3 mAb was extracted from acites pretreated with YPC3 hybridoma cells in Balb/c mice and purified as described previously[4,5]. 1-F/7 mAb against Denque fever virus was provided by the Department of Microbiology of Sun Yat-Sen University of Medical Sciences. Both mAbs were dissolved in pH 7. 4, 0.01M PBS (0.5g/L). Immunoreactivity was assessed by enzyme-linked immunosorbent assay (ELISA) on Capan-2 cells and avidin-biotin-peroxidase co mplex (ABC) immunohistochemistry of Capan-2 xenograft samples according to the methods described previously.

Effector cells (E)

One mL of incomplete RPMI 1640 medium was injected into the peritoneal cavity of Balb/c mice for 3 days in order to increase the number of PEC in mice. PEC was harvested by lavage of the peritoneal cavity of mice, washed, centrifuged twice and suspended in RPMI 1640 medium with 15% FBS at a concentration of $3\text{-}5\times10^6$/mL. PEC was further fractionated into non-adherent and adherent cells by 2 h incubation in glass dish. Non-adherent cells were removed by washing three times with warm medium. Adherent cells (more than 85% of macrophages) were resuspended to a final density of 10^6 cells/mL. PHA and IL2 (1000 U/mL) were added to adherent cell culture flasks. The flasks were incubated at 37 ℃ for 3 days at a humidified 5% CO_2 atmosphere. The cells were then collected, was hed and resuspended in complete medium. Adherent PEC without r-IL2 served as control cells. Lymphocytes generated from murine splenic cells were used for experimental comparison.

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Cytotoxicity test[6,7]

Four hours ^{51}Cr-release assay was used to evaluate the cytotoxicity of peritoneal macrophages and lymphocytes activated by r-IL2. ^{51}Cr labeled Capan-2 cells acted as target cells (T). E/T ratio was 25/1. The percentage of specific cytolysis in triplicate cultures was calculated by the formula:

$$\% \text{ specific cytolysis} = \frac{\text{Experimental cpm-spontaneous cpm}}{\text{Maximum cpm-spontaneous cpm}} \times 100$$

Maximum cpm was measured after incubation with 0.1N HCl. The spontaneous cpm, measured by incubation of the target cells alone, should be less than 30% of maximum cpm. To test ADCC, 25 mg/L of mAbs was incubated with the target cells for 30 min before addition of the effector cells. The percentage of specific cytolysis for ADCC was determined as described above.

RESULTS

mAb immunoreactivity assessment

Measured by ELISA on Capan-2 cells, the titer of YPC3 mAb (0.5 g/L) was more than 1:1000. YPC3 mAb was reacted with most of the pancreatic carcino ma cells determined by ABC immunohistochemistry on Capan-2 xenograft samples. No immunoreaction was found with control 1-F/7 mAb in ELISA and ABC immunohistochemistry. The results showed that YPC3 mAb might combine selectivel y with Capan-2 human pancreatic carcinoma cells.

The cytotoxicity of peritoneal macrophages activated by r-IL2 and YPC3 mAb

Table 1 shows that the cytolysis of peritoneal macrophages was evidently enhanced after activated by r-IL2 and YPC3 mAb. The percentage of cytolysis of peritoneal macrophages+Y-IL2+YPC3 mAb was 70.0%, 67.1% and 39.4% hig her than the single peritoneal macrophages, peritoneal macrophages+Y-IL2 and peritoneal macrophages+ YPC3 mAb, respectively. 1-F/7 mAb had no significant effect on cytotoxicity of peritoneal macrophages against Capan-2 cells.

Table 1 Cytolysis of peritoneal macrophges with different agents

Groups	*n*	Cytolysis (%, $\bar{x}\pm s$)
Macrophages	3	27.72±1.52[a]
Macrophages+γ-IL2	3	22.47±4.32[a]
Macrophages+YPC3 mAb	3	26.67±1.58[a]
Macrophages+γ-IL2+1 F/7 mAb	3	27.02±2.78[a]
Macrophages+γ-IL2+YPC3 mAb	3	37.06±10.78

[a]$P<0.05$, *vs* macrophages+ Y-IL2+YPC3 mAb.

The comparison of ADCC between the effect of peritoneal macrophages an d splenic lymphocytes activated by γ-IL2

Y-IL2 is a multifunctional protein which influences numerous cell types and activates both macrophages and lymphocytes. Essentially similar cytotoxicity levels of activated macrophages and lymphocytes were obtained with 25 mg/L of YPC3 mAb (37.06±10.78 and 30.13±9.15 respectively, $P>0.05$). The results indicated that ADCC depended mainly on the reaction of antibodies with membrane antigens expressed on the target cells.

DISCUSSION

ADCC is a mechanism of immunologic lysis, in which cellular targets sensitized by specific antibodies are selectively lysed by FcR bearing nonsensitized effector cells[6]. Macrophages bearing Ig-FcR have ADCC effect on tumor cells mediated specific anti-tumor mAbs. This ADCC is one of the important killing machanisms of macrophages[3].

Murine YPC3 mAb, belonging to IgG1 isotype, was produced against the human pancreatic carcinoma cell line. It reacted with pancreatic carcinoma in 28/32 patients, did not react with 11 normal human pancreas tissues and only 9/78 non-pancreatic tumors showed weak cross-reation by ABC immunohistochemi stry[4]. Radioimmunol-ocalization of human pancreatic carcinoma by ^{99}m-Tc labeled YPC3 mAb was achieved in *Balb/c* nude mice[5]. YPC3 mAb increased the anti pancreatic carcinoma efficacy of splenic LAK cells *in vitro* and the simultaneous injection of LAK cells and YPC3 mAb completely inhibited the growth of Capan-2 cell line in nude mice[7]. In this experiment, YPC3 mAb showed good affinity with Capan-2 cells and good synergism with Y-IL2. When combined with r-IL2, YPC3 mAb enhanced the cytolysis of peritoneal macroph ages on Capan-2 cells, which was 70% more than peritoneal macrophages alone, and was higher than that of 1-F/7 mAb.

Some curative effects of lymphokine-activated killer cells and interleukin 2 alone on metastatic carcinoma of serosa cavity have been progressed. But the therapy needed a lot of effector cells and large doses of activators with obvious side-effect[8,9]. Kawase *et al*[3] reported that combined thera py of C3H/HeN mice bearing ascitic MH134 hepatoma with ip injection of γ-IL2 and tumor specific 11G2 mAb brought about potent suppression of the tumor growth, resulting in the significant increase in the number of tumor-free mice, whereas neither Y-IL2 nor the mAb could exhibit such a potent antitumor effect when used alone. The results provided possibility that antitumor mAbs capable of inducing ADCC could overcome a limitation of LAK therapy.

The prognosis of pancreatic carcinoma remains poor because of early peritoneal metastasis and lack of effective therapy in advanced patients[10,11]. The combination of γ-IL2 and YPC3 mAb can increase

the effect of anti-pancreatic carcinoma *in vitro*. Ip injection of γ-IL2 and YPC3 mAb may provide a new way for local treatment of pancreatic carcinoma. But the research in the host bearing pancreatic carcinoma will be continued.

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