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Injection of physiological saline facilitates recovery of ascitic fluids for monoclonal antibody production

Julio M. Coll

Research Department, INVESGEN, Madrid, Spain

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The intraperitoneal injection of physiological saline into anti-C-reactive protein (anti-CRP) hybridoma tumor-bearing mice facilitates extraction of diluted ascitic fluids. The dilution with physiological saline before withdrawal of ascitic fluid reduces the processing time in large scale operations, facilitates further manipulations, increases the number of extractions per animal and permits the recovery of ascitic fluid containing monoclonal antibodies from solid tumor-bearing mice.

Key words: Ascitic fluid; Monoclonal antibody; Hybridoma

Introduction

Methods for the in vivo production of monoclonal antibodies in mice are used to obtain small volumes of highly concentrated antibody even when there is a demand for larger quantities, since in vitro methods still yield concentrations that are several magnitudes lower than those produced in vivo (Reuveny et al., 1986a; Velez et al., 1986). Some methods designed to increase ascitic fluid production in mice have been reported. For example, large amounts of polyclonal antibody were obtained by inducing ascitic fluid with a combination of initial pristane, Freund's complete adjuvant, immunogen and non-producer myeloma cells (Lacy and Voss, 1986). Moreover, priming with incomplete Freund's adjuvant instead of pristane permitted production of large amounts of monoclonal antibody (5–7 ml of ascitic fluid per mouse) in a short time (2 weeks of priming) and using low numbers of hybridomas (Mueller et al., 1986). In another recent study crosses between BALB/c males and SW/HPB females yielded mice producing four times more ascitic fluid than the BALB/c parent (Brodeur and Tsang, 1986). There are, however, some production problems either when large amounts of antibody are required or when hybridomas tend to grow in solid rather than ascites tumors. For example, the maintenance of a large colony of animals for the purpose of collecting and processing milliliter quantities of ascitic fluids from each animal lacks industrial efficiency because this is a time consuming effort.

We report here the use of a simple method to facilitate the yield and decrease the time involved in obtaining ascitic fluids from hybridoma tumor-bearing mice (either ascites or solid tumor). A 5 ml injection of sterile physiological saline, followed by extraction of diluted ascitic fluid facilitates and speeds up further processing of ascitic fluids such as centrifugation. The procedure is especially useful for the recovery of ascitic fluids from solid tumor-bearing mice.

Correspondence to: J.M. Coll, Research Department, INVESGEN, Madrid, Spain.

Materials and methods

Hybridoma cell lines

21 anti-human C-reactive protein (CRP) mouse hybridoma cell lines were raised in our laboratory in several fusions of the non-secreting mouse myeloma cell line X63/Ag 8.653 with spleen cells from BALB/c mice immunized with human CRP. All hybridoma lines and its monoclonal antibodies have been described (Iturralde and Coll, 1984; unpublished observations).

Production of diluted ascitic fluid

Female BALB/c mice (5–10 weeks) were primed with an intraperitoneal injection of 0.5 ml of pristane (Aldrich, Chemical Co, Milwaukee, WI). 3–10 days later groups of mice were injected with $0.5–2 \times 10^6$ viable hybridoma cells in 1 ml of cell culture medium and were inspected daily to assess ascitic fluid production by swelling. 5 ml of physiological saline (0.15 M NaCl) were injected into the peritoneal cavity with a 25 gauge needle and the mice were allowed to rest for a few minutes. Then diluted ascitic fluid was withdrawn from the peritoneal cavity by insertion of a 22 gauge sterile needle. Cell-free diluted ascitic fluid was stored frozen at $-20\,^{\circ}C$.

Detection of monoclonal antibody

Anti-CRP was detected by enzyme immunoassays on solid-phase immobilized CRP was previously described (Iturralde and Coll, 1984). Briefly, a concentration of 0.25 μg/50 μl of CRP were added to the wells of polystyrene plates, dried overnight and then washed. The ascitic fluids were diluted in 0.2 M sodium borate, 75 mM NaCl, 2 mM $CaCl_2$, 0.01% merthiolate, 1% bovine serum albumin, 0.05% Tween 20, pH 8 and 100 μl added to the wells. After 1 h incubation and washing, 100 μl of peroxidase-labelled anti-mouse IgG (Nordic, The Netherlands) were added and incubated for 30 min. Color development was carried out using *o*-phenylenediamine and the wells were measured in an SLT EAR 400 FW spectrophotometer (unpublished observations). Titer was defined as the dilution of ascitic fluid which gave an absorbance of 1 in the above mentioned assay.

Results

Time required to withdraw diluted ascitic fluid

The time taken to withdraw ascitic fluids from those mice in which harvest of ascitic fluid was difficult (those who showed very little or no swelling) was decreased by about 1/3 to 1/4 by prior injection of saline. The average time required for injection of saline, extraction and centrifugation of diluted ascitic fluid in processing 10–40 mice was 4–6 min per mouse. About 5–7 ml of clean induced ascitic fluid was obtained per mouse per extraction. The handling of 5–7 ml of diluted ascitic fluid for centrifugation in order to remove

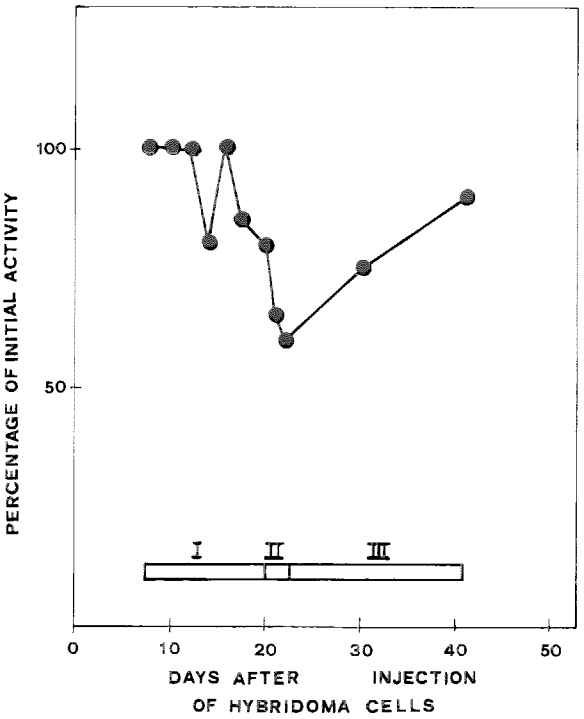

Fig. 1. Relative titer of diluted ascitic fluid after draining with different frequencies a solid tumor-bearing mouse. A mouse was injected with anti-CRP hybridoma cells (six injections throughout a month of about 100000 cells/injection). It was then drained as indicated in the materials and methods section every 2 days (I), every day (II), or every 8–11 days (III). The mouse had a solid tumor as evaluated by dissection on day 41. Anti-CRP activity was measured as indicated in the materials and methods section by further diluting the ascitic fluids 1/50. Averages from duplicates were used to calculate the titer recovered with respect to the titer on day 0 after the last injection of hybridoma cells.

lipids (top of the centrifuge tube) and cells and debris (bottom of the centrifuge tube) was faster and easier than the handling of 0.1–2 ml of the ascitic fluid obtained otherwise.

Production and yield

In hybridoma injected mice with ascites tumors the production of ascitic fluids lasted an average of 4 days from the first evidence of swelling, producing a total of 5–10 ml of ascitic fluid per mouse. In hybridoma injected mice with none or very little swelling after 10–20 days, following prior injection of physiological saline the diluted ascitic fluid production lasted for 20–30 days, producing a total of 60–80 ml of about 1/3 diluted ascitic fluid (equivalent to 20–26 ml of ascites fluid).

Fig. 1 shows the variation of relative titers of diluted ascitic fluids from one solid tumor bearing mouse after draining at different times. The titre was maintained between 80–100% of the initial titer during the first 14 days by draining every other day. After this, daily draining reduced the titers to 60%. After resting the mouse for 8 days or more antibody titers were restored to the initial level.

Fig. 2 shows that the recovery of the antibody activity in diluted ascitic fluid with respect to the activity in serum varied from 8 to 92% with an average of 33% for 12 different mice and different anti-CRP hybridomas. The titers in ascitic fluid and serum were about equal (data not shown).

Table I shows the comparison of volumes, titers and total anti-CRP activity in diluted ascitic fluid with respect to ascitic fluid from the same mice. Mice with ascites or solid tumors are also compared. In the conventional method, volumes per mouse per extraction varied according to the type of tumor and the volume of ascites tumors varied greatly. The volumes from the mice used in this experiment were approximately 2.2 ml (ascites tumor) and 0.4 ml (solid tumor), both volumes measured after centrifugation and elimination of

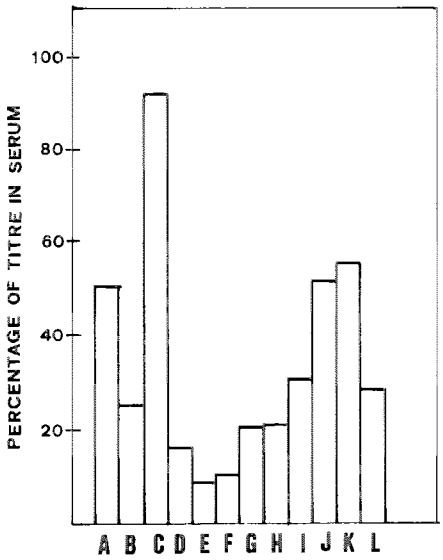

Fig. 2. Recovery of anti-CRP titer in diluted ascitic fluids with respect to the titer in serum in solid tumor-bearing mice. Serum and diluted ascitic fluid were obtained simultaneously from 12 mice injected with different anti-CRP hybridomas (A–L). Two dilutions of each serum and ascites were assayed for anti-CRP activity as indicated in the materials and methods section. Averages from duplicates were used to calculate the percentage of titer in serum using the formula: (titer in diluted ascitic fluid/titer in serum) × 100. Serum was obtained from the tail vein, diluted ascitic fluid was obtained as indicated in the materials and methods section.

TABLE I

VOLUME, TITER AND TOTAL ANTI-CRP ACTIVITY IN ASCITIC FLUIDS OBTAINED BY CONVENTIONAL OR DILUTION METHODS FROM MICE WITH ASCITES, OR SOLID TUMORS

Mice were drained first by conventional procedures, after 2 days they were injected with 5 ml of saline and the diluted ascitic fluids were obtained. Mouse 1 was injected with a hybridoma which grew as an ascites tumor but had a low anti-CRP titer. Mouse 2 was injected with a hybridoma which grew as a solid tumor but had a high anti-CRP titer. The volume of ascitic fluid was measured approximately after centrifugation. Titer was calculated by assaying the fluids at four dilutions as indicated in the materials and methods section. Total anti-CRP recovery was obtained by multiplying the volume of ascitic fluid (ml) by the reciprocal of the titer.

Method used to obtain ascitic fluid		Mouse 1 (ascites tumor)	Mouse 2 (solid tumor)
Conventional	ml	2.2	0.4
	Titer	1/2500	1/80000
	Total	5500	32000
Dilution	ml	6.3	5.1
	Titer	1/1000	1/7500
	Total	6300	38250

both pellet and top parts of the centrifugated tube. In the dilution method, the volumes were more homogeneous (5–6 ml). Titers varied with the dilution factor in each case but the total amount of anti-CRP recovered did not show significant differences compared with the conventional method.

Discussion

Injection of physiological saline into mice before the harvest of ascites was used to extract diluted ascitic fluids from hybridoma tumor-bearing mice.

This method was used, in general, to obtain diluted ascitic fluids from anti-CRP producing hybridoma-injected mice with either poor growth of the hybridoma or solid tumors. Of nine anti-CRP hybridomas grown in BALB/c, only three had high titers of anti-CRP activity. Of those three, two grew poorly in BALB/c and these had the highest titers (Iturralde and Coll, 1984; unpublished observations). After identification of these hybridomas as producing useful monoclonal antibodies, the solid tumors were excised and injected into other mice. No easy identification and follow up of these hybridomas could possibly have been made without the method of harvesting ascitic fluids described in this article.

The production of monoclonal antibodies by growing hybridomas in mice is still the preferred method when large-scale production is needed in spite of the efforts to develop cell culture techniques (Reuveny et al., 1986a, b; Vélez et al., 1986). The method described offers several advantages and can be used with all kinds of tumor bearing mice: (a) reduced time needed to withdraw ascitic fluid because of increased abdominal pressure (this shorter time also reduces the pain for the animal); (b) facilitated processing, mainly the extraction and the elimination of cells, debris and fat, by centrifuging increased volumes; (c) an increased number of extractions can be performed in every animal, probably because the extraction of more hybridoma cells slows down the deterioration of the mice (the physical recovery of drained mice was very apparent), and (d) identification of solid tumor-bearing mice which produced good titers of anti-CRP antibodies. Some of these tumors after they had been excised and retransplanted grew as ascites but others did not, but still produced good titers in diluted ascitic fluids. In addition to permitting the extraction of diluted ascitic fluids from tumor-bearing mice with little or no ascites, this method can also be used to facilitate extraction of ascites tumor bearing mice (Table I).

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