PROCEDURE NO. 46312

COLLECTION AND GROWTH OF CANINE LYMPHOCYTES FROM PEYER'S PATCHES

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SUMMARY: A procedure is described for culturing lymphocytes from Peyer's patches of the canine small intestine. A description is provided for the identification and removal of the lymphoid tissue (Peyer's patch), preparation of the tissue, separation of the lymphocytes from the tissue, and subsequent culturing of the lymphocytes. This procedure can be performed in 8 h, provides adequate antibiotic doses to prevent bacterial contamination, and describes how to remove excessive intestinal mucus from the preparation. This technique can be used for the preparation of lymphocytes from other lymphoid organs.

Key words: gut lymphocyte; Peyer's patch; blastogenic response; fecal contamination; local gut immunity; lymphoid organs.

I. INTRODUCTION

Lymphocytes used in immunologic studies are usually collected from peripheral blood, however lymphocytes from the lymphoid organs can provide a means to study local immunity. Recent attention has been turned towards the importance of gastrointestinal (gut) immunity, which in the past years has been difficult to study. The lymphoid organs associated with gut immunity are the Peyer's patches, which are elevated, circumscribed areas measuring about 2 by 1.5 cm and are found in higher concentration between the proximal duodenum and distal ileum. These areas are rich in rapidly dividing lymphocytes (both T and B cells) and in the dog average 22 in number (1). The mucoid nature of gut lining and contamination from the fecal material discourages the study of this cell population in vitro. Our interest in gut immunity of the dog has resulted in the development of a simple technique for the isolation of lymphocytes from the Peyer's patches and subsequent application towards other lymphoid organs, such as the thymus, spleen, and mesenteric lymph node. A comparison was made between the recovery rate of viable lymphocytes of Peyer's patches and other lymphoid organs.

II. MATERIALS

A. Chemicals and culture medium
70% Ethanol solution
NH₄Cl, 0660, J. T. Baker¹
NaHCO₃, 3506¹
EDTA, 8993¹
Hanks' balanced salt solution (HBSS), No. 450-1200, GIBCO²
Penicillin/streptomycin, N-0507-6¹
Fetal bovine serum, No. 200-6140²
Leibovitz media, 430-1300²
RPMI 1640 with 25 mM HEPES and L-glutamine, 380-2400²
Dulbecco's phosphate buffered saline (PBS), 450-1300²
Mycostatin, 10,000 U/ml, 600-5320²
Gentamycin, 50 mg/ml, No. 17-740 Microbiological Associates³
Ficoll-Paque, No. 17-0840-02, Pharmacia⁴

B. Glassware and plastics
Plastic petri dishes, 100 × 15 mm, No. 25384-070 VWR Scientific, Inc.⁵
20 ml Syringes, No. BD5661⁶
Oak Ridge tubes, No. 21009-342⁶
Disposable syringe needle, 18 gauge × 1/2, No. BD5196⁶
96-Well tissue culture cluster, 6.4 mm diam well, with sterile cover, No. 3596 Costar
Glass beakers, 200 ml
Glass beakers, 500 ml, with single and double layers of sterile gauze taped over the top

C. Surgical instruments
Forceps, straight, No. IR-373, Irex Surgical Instruments
Scalpel handle, No. 3, 08-913-5; scalpel blade, No. 15, 08-916-5D, Fisher Scientific
Scissors, straight, 7½”, No. 160-300 Tieman

D. Animals
Dogs of many breeds, approximately 1 yr old, 15-20 lb, and with short hair, obtained from the Franklin County Dog Pound, Columbus, OH

E. Equipment
Electrocution cables
AO light microscope, series 150 No. 41710-131
Quincke (Babcock) spinal needle, stainless steel, 18 gauge × 6”, No. 7326, Popper and Sons, Inc.
Rubber bulb; 1 ml capacity, No. 56311-027; 1½ oz. capacity, No. 56315-083
CO2 incubator, Forma Scientific
pH Meter, model 13500, No. 123602, Beckman
Centrifuge, model TJ-6, No. 340508
Water bath, model 84, Precision Scientific Co.
Laminar flow sterile hood, Banker Co.
Brass mesh screen, 60 to 150 mesh units, No. S 1225-6, American Scientific Products

III. PROCEDURE
A. Preparation of solutions
1. Hanks’ balanced salt solution media
   Prepare the media as directed by the manufacturer and add: fetal bovine serum (50 ml/liter media), penicillin/streptomycin (20 ml/liter media), mycostatin (20 ml/liter media), and gentamycin (1.5 ml/liter media).
2. Leibovitz media
   Prepare the media as directed by the manufacturer and add: penicillin/streptomycin (20 ml/liter media), mycostatin (20 ml/liter media), gentamycin (1.5 ml/liter media) and EDTA (0.5 g/liter media).
3. RPMI media
   Prepare as directed by manufacturer and add: penicillin/streptomycin (10 ml/liter media), mycostatin (10 ml/liter media), and gentamycin (1 ml/liter media).

B. Collection of organs
1. After a 2 min electrocution of the dog, wet the animal’s fur coat with alcohol and, with a scalpel, cut and lay back the skin on the chest and abdomen.
2. Flush fecal material from the nonperforated gut with PBS. Identify and remove approximately 9 to 12 Peyer’s patches from the proximal portion of the small intestine with sterile tissue forceps and scissors by careful dissection in a circumscribed fashion.
3. Wash the sections of gut tissue excessively with PBS and in 200 ml beakers containing HBSS with antibiotics and 5.0% FBS placed on ice for 15 to 30 min.

C. Cell preparation
1. Trim the Peyer’s patches of excess connective tissue. Place the 60 ISO unit mesh screen with pan on ice. Push the inside surface of each patch through the screen with a 12 cc plastic syringe barrel. Then wash the top and bottom surfaces of the screen with 100 ml cold HBSS.
2. Collect the cell suspension, then aspirate through an 18 gauge needle and filter through a single layer of sterile gauze taped over the top of a beaker, which is then washed with 10 ml cold HBSS. Repeat this filter procedure using a double thickness of sterile gauze taped over the top of another beaker and again rinse with 10 ml cold HBSS.
3. Centrifuge the cell suspension in Oak Ridge tubes for 10 min at 1000 to 1500 rpm at room temperature. Aspirate the supernatant fluid.
4. Wash the cells in 20 ml cold Leibovitz media with antibiotics.
5. Resuspend the cells in 20 ml cold Leibovitz media in a sterile Oak Ridge tube and underlay with 10 ml of Ficoll-Paque using a sterile spinal needle. Centrifuge the gradients at room temperature at 400 Xg for 15 min.
6. Remove the interface layer containing the gut lymphocytes with a 10 ml pipette and wash three times in 20 ml Leibovitz media for 10 min at 1000 to 1500 rpm at room temperature.
7. Finally, resuspend the cells to 1 × 10⁶ viable lymphocytes/ml of RPMI 1640 with 25 mM HEPES, L-glutamine, and antibiotics.