

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 IJABR 2024; SP-8(3): 12-15
www.biochemjournal.com
 Received: 03-01-2024
 Accepted: 05-02-2024

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Comparative assessment of cytospin preparation of a few lymphoid organs of pig

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i3Sa.680>

Abstract

The study of cell suspension preparation is important for detecting neoplastic conditions of the organs concerned. Cell suspensions were prepared from lymph nodes, Peyer's patches and spleen. The cell suspensions were centrifuged in Labofuge-400 and cytospots were prepared on micro slides. The cytospots were stained with Giemsa and Leishman's stain and were compared. The lymph nodes, Peyer's patches and spleen cytospots were predominantly occupied by lymphocytes which were of different sizes and shapes. Besides, mucus-secreting goblet cells were seen in Peyer's patch cytospot. In splenic cytospot, other leukocyte varieties were also noticed like the neutrophils and the eosinophils. It was observed that Giemsa staining gave better results in all cases compared to Leishman's stain in terms of better contrast between cytoplasm and nucleus. Many types of cells with different morphology were seen in all the preparations. However, it was difficult to identify the cells employing normal microscopy and normal Giemsa or Leishman's staining. The same cytospots may be subjected to immuno-staining under immunocytochemistry to differentiate various types of cells.

Keywords: Cytospin, cytospot, lymphoid organ, pig

Introduction

Cytology of biological fluid as well as cell suspension preparation from different organs might be a useful diagnostic tool for detecting many diseased conditions (Day *et al.*, 1995, Pradhan *et al.*, 2006) [6,8]. Cyto-centrifuges are mainly used to prepare cytospot of the samples and are stained for studying cell morphology and distribution. The procedure of cytospot preparation is of special clinical importance in human medicine (Grierson *et al.*, 2005) [7]. Nowadays, fine needle aspiration biopsy or fine needle aspiration cytology is a widely used diagnostic tool to diagnose many diseases including the neoplasm (Cozzolino *et al.*, 2012) [5]. Further, the cytospot preparations may be used for fluorescent antibody labelling and other procedures to give confirmatory diagnosis of diseases. For detecting the diseased condition by examining the cells in cytospin preparation, it is mandatory to study the normal morphology of the cells in the smears. Besides, the procedures for the preparation of cytospot may vary from organ to organ depending upon the number of cells present per unit volume of the organ and the presence of other tissue debris. There should be a standard procedure to stain the cytospot to check probable artefacts and to detect the normal architecture of the cells in terms of normal morphology of the nucleus and the cytoplasm.

The diseases of the lymphoid organs are numerous in the domestic animals and many other diseases affect the lymphoid system directly or indirectly. Hence, to detect the diseases of the lymphoid organs, cytospin preparation might help to a considerable extent. Present investigation was intended to study normal cellular population of lymph nodes, Peyer's patches and spleen in cytospin preparation and also to standardize one effective protocol for staining of cytospin preparation.

Materials and Methods

The present study was conducted on 6 (six) healthy adult pigs irrespective of age and sex. The lymphoid organs were collected in ice from freshly slaughtered animals from slaughterhouses in and around Guwahati city. The samples were preserved in deep freeze maintained at -20 degree centigrade.

The experiment was carried out in the Department of Anatomy and Histology, College of Veterinary Science, Khanapara, Guwahati-781022, Assam.

The tissue pieces were cut into smaller pieces under aseptic condition. They were ground in stainless steel wire grit and were washed with 2% BSA-PBS. The solution was further diluted by PBS at pH 7.2 and was transferred to centrifuge tubes and stored in deep freeze. Cell concentration was determined by Neubaure's counting chamber and the following procedure (Chauhan and Agarwal, 2008; Turksma *et al.*, 2013)^[4,9] was followed:

1. Washing was carried out for 10^5 cells in cold 2% BSA-PBS twice and dilute in 100 μ L of cold 1% BSA-PBS and was kept in ice.
2. The slides and filters were placed into appropriate slots in the cytospin with the cardboard filters facing the center of the cytospin. It was ensured that each filter and slide pair was flushed with each other and that the hole in the filter was in the proper position so that cells reached the slide.
3. 100 μ L of each sample was put into the appropriate wells of the cytospin.
4. The lid of the cytospin over the samples was carefully placed and spin at 3000 rpm for 2 minutes.
5. Filters were removed from the slides without contacting the smears on the slides.
6. Slides were examined under microscope and were allowed to dry at room temperature overnight.

The slides having cytosps were subjected to routine staining by Giemsa and Leishman's stains (Chauhan and Agarwal, 2008)^[4].

Giemsa stain

Giemsa stain in liquid form was procured from Fisher Scientific. The staining solution was kept at 4⁰ C and was diluted 10 times before use. The staining of dry cytospot preparations was done in the following steps:

1. The slides were fixed for 3-5 minutes in methyl alcohol and were air-dried.
2. Working solution of Giemsa stain was prepared by adding 1 mL of Giemsa stain to 9 mL neutral distilled water (pH 6.8).
3. The slides with the cytospot were placed in Coplin jar having diluted stain for 30-45 minutes.
4. The slides were washed with neutral water and dried to examine under microscope.

Leishman's stain

The Leishman's stain was procured from Fisher Scientific. The powdered dye was mixed with the absolute methyl alcohol to make an even suspension. It was kept in dark at 4°C for 2 weeks before use.

1. The air-dried cytosps were placed horizontally on the staining tray and a few drops of undiluted stain were allowed to react for 2-3 minutes.
2. The stain was diluted by double the amount of buffered distilled water and mixed.
3. The stain was allowed to stay for 10-15 minutes.
4. Excess stain was washed by distilled water until the film of cells had a pinkish tinge.

5. The slides were allowed to dry and examined under microscope.

Results and Discussion

The cytospot staining of lymph nodes were characterized by the presence of lymphocytes (Figs 1, 2, 3). The nuclei were large and occupying the most part of the cells. The cytoplasm was scanty. There were other cells also which were devoid of identifying features and might be the epithelial cells. The lymphocytes were small and large. Many of the lymphocytes were clumped together. The contrast was more in the case of Giemsa's stain and might be superior to Leishman's stain. Aulbach *et al.*, (2010)^[1] also studied the optimization of procedure for fine needle biopsies of lymph nodes for immunostaining and could determine intact neoplastic cell concentration within a short period.

The cytospin preparations of Peyer's patch revealed a wide variety of cells (Figs 4, 5). Mostly lymphocytes of various sizes and shapes were seen. The lymphocytes had large nuclei and were basophilic and the cytoplasm was scanty. Some other cell varieties were also noticed which might be epithelial cells of various types. Mucus cells stained purple and were roughly round with uneven boundaries. The contrast of cells in the case of lymphocytes was more prominent with the Giemsa stain. The nuclei were bluish purple and the cytoplasm was eosinophilic. Castellaneta *et al.*, (2004)^[3] identified interferon- α producing (plasmacytoid) dendritic cells in Peyer's patches by staining cytospot.

The cytospin preparation of splenic pulp revealed different types of numerous cells (Fig. 6, 7, 8). At low magnification, erythrocytes were seen and under oil immersion lymphocytes were visible. Eosinophils and neutrophils were also observed in a few fields. In the case of splenic preparations also, Giemsa stain gave better results in terms of good contrast. Barrouin *et al.*, (2006) standardized cytological and immunochemical methods for the analysis of fine needle aspirates where they used these methods to determine the cellularity of the splenic pulp and the cytosps were prepared from spleen and stained with haematoxylin & eosin stain and Wright's stains. Following the procedures of immunochemistry, they could diagnose Leishmaniasis by larger neutrophil & monocytes/macrophage relative count and lower lymphocytic relative count.

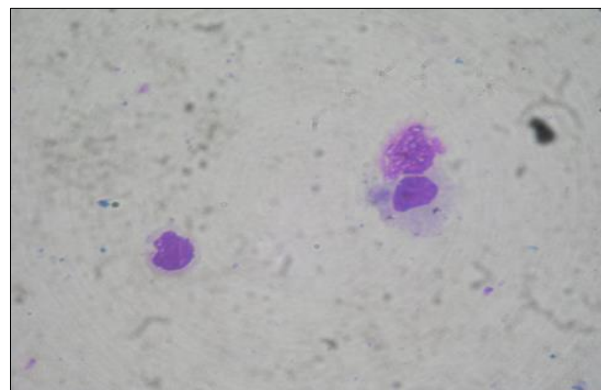


Fig 1: Cytospot of lymph node. Note the lymphocytes (arrow), stain Giemsa, x1000

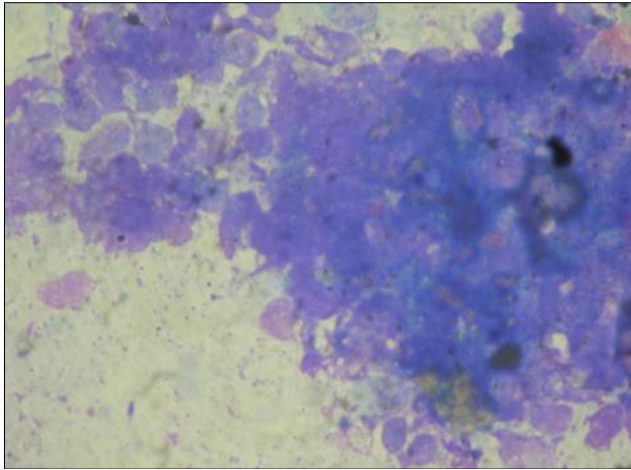


Fig 2: Cytospot (lymph node). Note the clumping of lymphocytes and other cells. Stain Giemsa, x1000

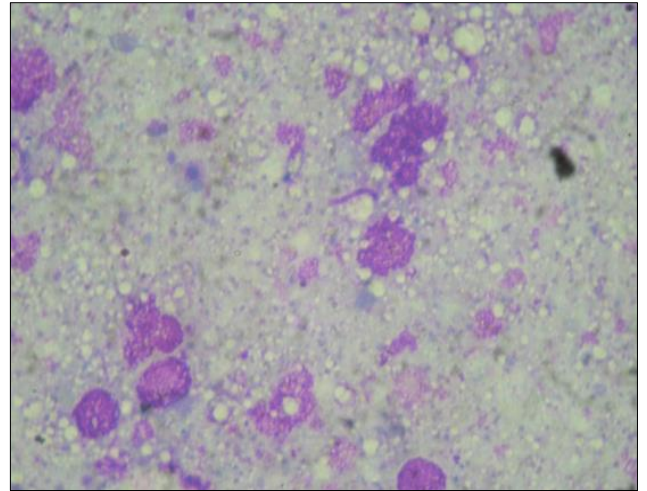


Fig 5: Peyer's patch cytospot. Stain Leishman, x1000

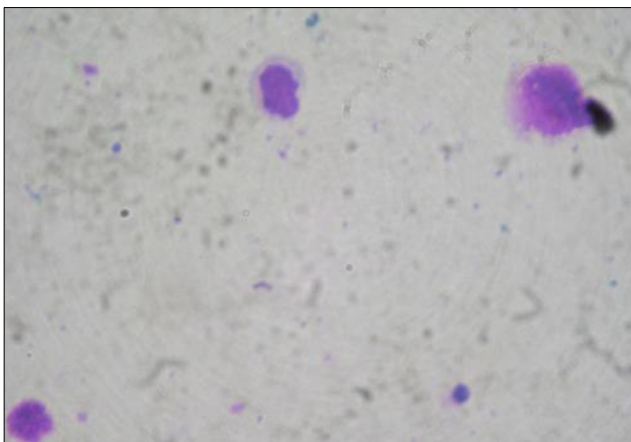


Fig 3: Lymphocytes (arrow) in lymph node cytospot. Stain Leishman, x1000

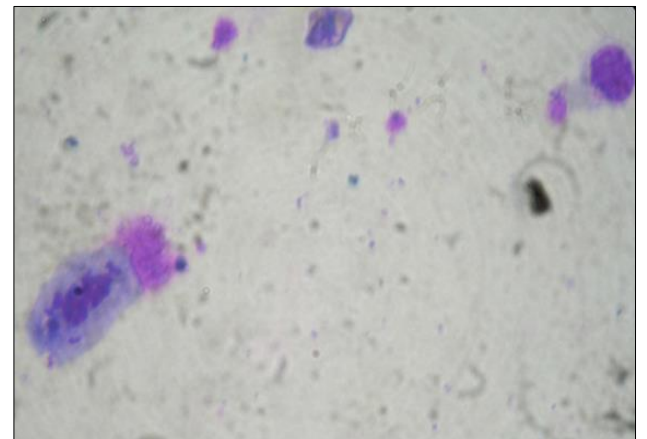


Fig 6: Cytospot of spleen. Note the lymphocytes (black arrow), neutrophil (white arrow). Stain Giemsa x1000

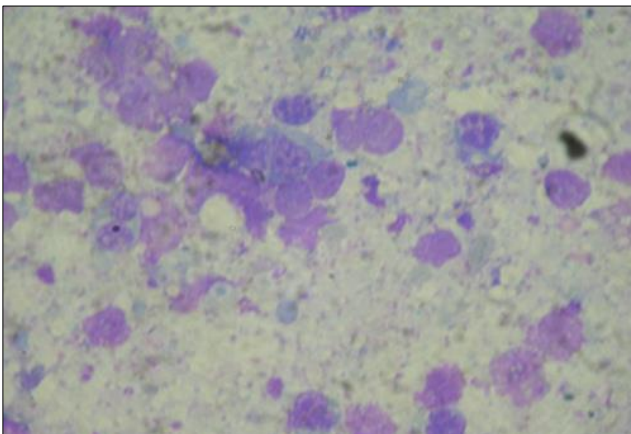


Fig 4: Lymphocytes (black arrow) in Peyer's patch cytospot. Goblet cells (white arrow) are eosinophilic and numerous. Stain Giemsa, x1000

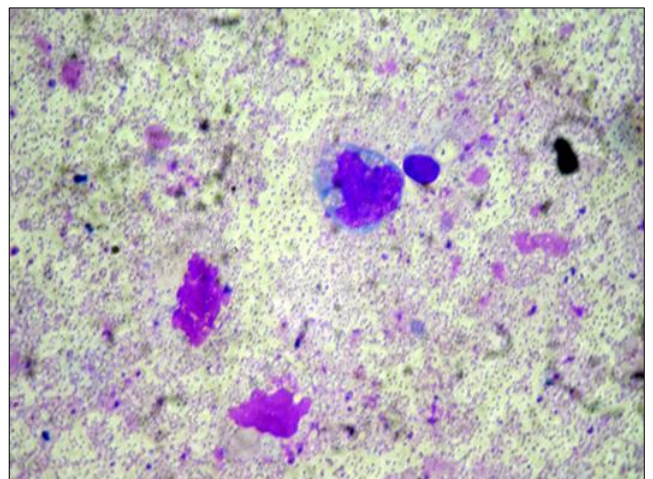


Fig 7: Cytospot of spleen. Stain Leishman, x1000

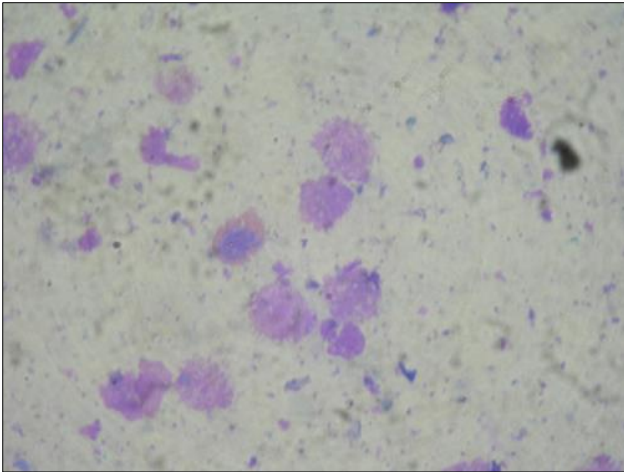


Fig 8: Eosinophil (arrow) in the cytospot of spleen. Stain Giemsa, x1000

Conclusion

The lymph nodes, Peyer's patches and spleen cytosots were predominantly occupied by lymphocytes which were of different sizes and shapes. Besides mucus-secreting goblet cells were seen in Peyer's patch cytospot. In splenic cytospot, other leukocyte varieties were also noticed like the neutrophils and the eosinophils. It was observed that Giemsa staining gave better results in all cases compared to Leishman's stain in terms of better contrast of cytoplasm and nucleus. Many types of cells with different morphology were seen in all the preparations. However, it was difficult to identify the cells using normal microscopy and normal Giemsa or Leishman's staining. The same cytosots may be subjected to immuno-staining under immunocytochemistry to differentiate various types of cells.

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