Kerala Journal of Veterinary Science, Vol. 18, No. 1, June. 1987 P. 23—26

SEPARATION OF PERIPHERAL BLOOD LEUCOCYTES FROM CHICKEN BLOOD*

K. Umesh Kumar[1], S. Krishnaswamy[2] and T. Venkata Reddy[3]

Veterinary College, Tirupathy

(Received: April 27, 1987)

In many of the poultry diseases both cell-mediated immunity and humoral immunity play a key role in protection. For demonstrating cell-mediated immunity *in vitro* tests like leucocyte migration inhibition (LMI) and lymphocyte stimulation (LS) tests are useful. The success of these *in vitro* cell-mediated immunity techniques depend on the ability to separate leucocytes/lymphocytes from the peripheral blood of chicken.

Several methods were used to separate peripheral blood leucocytes from chicken. Coudert and Richard (1975) used distilled water lysis method to separate leucocytes from chicken blood. Chhabra and Goel (1981) used low speed centrifugation method to separate leucocytes from peripheral blood of chicken while assessing cell-mediated immune response of chickens to *Mycoplasma gallisepticum* infection, employing leucocyte migration inhibition test. Density gradient method was adopted by Nagaraja *et al.* (1982) to separate peripheral blood leucocytes of chicken for their use in leucocyte migration inhibition in chickens inoculated with *Salmonella typhimurium*. Confer and Adldinger (1981) used Bovine serum albumin (BSA) as a gradient to separate pesipheral leucocytes from chicken. A study was therefore undertaken to assess the usefullness of various techniques available for the separation of chicken leucocytes.

Materials and Methods

1 EDTA:

2 Egg albumin flakes:

3 Histopaque (containing ficoll type 400 and sodium diatrizoate) supplied by Sigma chemicals was used as gradient solution to separate leucocytes from peripheral blood of chickens.

* Part of the thesis submitted by the first author in partial fulfilment for the award of M. V. Sc. degree of A. P. Agricultural University, 1985.

Present Address:- 1. Vet. Asst. Surgeon, Animal Health Centre, Chittoor.
2. Director, Alved products, Madras.
3. Asst. Professor, College of Vety. Science, Tirupati.

4 RPMI–1640 medium supplied by Himedia, Bombay was used.

5 Hank's Balanced Salt Solution (HBSS): Ready made dehydrated powder supplied by Hi-media, Bombay was used.

6 Trypan blue of Central Research Laboratories, Bombay was used to count viable and dead leucocytes.

Separation of peripheral blood leucocytes from chicken:

1. Distilled water lysis method: Leucocytes were separated on the lines described by Coudert and Richard (1975) with slight modification. To 5ml of peripheral blood 10ml of sterile distilled water was added to lyse RBC. After gentle shaking for 20 seconds 2.5ml of sterile 4.5 per cent of Sodium chloride (pH 6.8) solution was added to restore isotonicity. Then the haemolysed blood was centrifuged at 450 g for 5 minutes. The supernatent was poured off and the pelleted white cells were washed in HBSS and then suspended in 1ml of RHMI-1640 medium.

2. Separation of leucocytes using ficoll density gradient solution: The method described by Nagaraja *et al.* (1982) was followed.

3. Egg albumin floatation method: The procedure described by Confer and Adldinger (1981) was followed except that 33 per cent egg albumin in saline was used as gradient solution instead of 35 per cent bovine serum albumin (BSA).

4. Low speed centrifugation method: Peripheral blood leucocytes were separated on the lines described by Chhabra and Goel (1981).

After separation of peripheral blood leucocytes by above methods, the viability of the cells in each method was checked by Trypan blue exclusion method. One part of 0.5% Trypan blue solution was added to one part of the cell suspension and the mixture was kept for ten minutes at 4°C. Viable and dead counts were made by counting unstained and stained cells respectively, using a haemocytometer and the total count of cells was calculated.

Results and Discussion

When Ficoll–sodium diatrizoate (Histopaque) was used to separate leucocytes the total yield of cells was 97% and viability was 96%. In egg albumin floatation method yield of cells was 98% with a viability of 97% and further very slight traces of RBC were seen. When low speed centrifugation method was followed the yield of cells was 98% with a viability upto 98–99% and no traces of RBC were found. Distilled water lysis method of separation of leucocytes was not at all suitable in poultry. The results are furnished in Table.

When egg albumin was used to separate leucocytes, the yield of cells was good. But there was slight contamination with RBC and as it required several washings to remove the egg albumin, this method was not suitable. The ficoll – diatrizoate method which was originally used for the separation of human lymphocytes (Boyum, 1968) had also been used for separation of peripheral blood leucocytes by Nagaraja *et al*. (1982). Though this method resulted in the yield of cells upto 97%. the viability was less than that of egg albumin floatation method. In Avian system the distilled water lysis method of separation of leucocytes was not practicable because the nuclei of birds' erythrocytes were not destroyed and it was difficult to differentiate them from some mononucleate cells such as small lymphocytes (Coudert and Richard, 1975). But this method had been found useful for the separation of leucocytes and LMI test in cattle (Naylor and Little, 1975) and in sheep (Sreenivasulu, 1983).

Method of separation	% yield of cells	Viability in %	Traces of RBC
1. Ficoll – diatrizoate	97	96	—
2. Egg albumin floatation method	98	97	Slight
3. Low speed centrifugation	98	98–99	—

The low speed centrifugation method was found to be good as both the yield and viability were good, when compared to other methods. Further it was found to be more economical since no chemical was needed unlike in other techniques. Hence low speed centrifugation method of separating the peripheral blood leucocytes in poultry was considered better and practicable.

Summary

The success of application of *in vitro* CMI techniques to assess cell – mediated immunity depends on the ability to separate leucocytes/ lymphocytes from the peripheral blood. Various methods like distilled water lysis method, Egg albumin floatation method, density gradient method using Ficoll – diatrizoate and low speed centrifugation method were tried, for the separation of leucocytes in poultry. Out of all these low speed centrifugation method for the separation of leucocytes was found to be better, economical and practicable in avian system.

സംഗ്രഹം

കോഴികളുടെ രക്തത്തിലുള്ള വെളുത്ത കോശങ്ങളെ വേർതിരിച്ചെടുക്കാനുള്ള വിവിധ മാർഗ്ഗങ്ങളെ പറ്റി പഠനം നടത്തി. ഇതിൽ ലോ സ്പീഡ് സെൻട്രിഫ്യുഗേഷൻ നല്ല മാർഗമാണെന്നു കണ്ടു.

Acknowledgements

The authors are thankful to the Principal, College of Veterinary Science, Tirupathi for providing facilities to carry out this research work.

References

Boyum, A. (1968) Separation of leucocytes from blood and bone marrow. Isolation of mononuclear cells and granulocytes from human blood. *Scand J. Clin. Lab. Invest* **21**: 77–89

Chhabra, P. C. and Goel, M. C. (1981) Immunological response of chicken to *Mycoplasma gallisepticum* infection. *Avian dis.* **25**: 279-293.

Confer, A. W. and Adldinger, H. K. (1981) The *in vivo* effect of levamisole on phytohaemagglutinin stimulation of lymphocytes in normal and Marek's disease virus inoculated chickens. *Res. Vet. Sci.* **30**: 243-245.

Coudert, F. and Richard, J. (1975) The purification of lymphocytes in chicken blood. *Poult. Sci.* **54**: 59-63.

Nagaraja, K. V., Newman, J. A. and Pomeroy, B. S. (1982) Leucocyte migration inhibition in chickens inoculated with *Salmonella typhimurium*. *Amer. J. vet. Res.* **43**: 916–918.

Naylor, P. E. and Little, T. W. A. (1975) A simple method for isolating leucocytes from bovine blood and their separation into lymphocytes and granulocytes. *Res. Vet. Sci.* **18**: 336–338.

Sreenivasulu, D. (1983) Standardisation of the *in vitro* techniques to assess the cell-mediated immune responses to vaccination against Jhones disease in sheep and Brucellosis in cattle. Thesis submitted to A. P. Agricultural University, Hyderabad.