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Research Article

The Application of Erythrocyte Rosette Test to Characterize T-Like Lymphocytes in the Mud Catfish *(Clarias gariepinus)*

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ABSTRACT

The Erythrocyte rosette test, a technique used to characterize T-lymphocytes using sheep red blood cells was evaluated to demonstrate T-like lymphocytes sub-population in the peripheral blood of the mud catfish (*Clarias gariepinus*). Forty eight young adult mud catfish weighing between 100-150g each were used in the study. The effects of thymectomy and injected anti lymphocyte serum on the degree of erythrocyte rosette formation were assessed. Rosette of sheep red blood cells around the mud catfish lymphocytes were observed in all experimental groups. The numbers of rosette formed were significantly higher ($p<0.05$) in the untreated group as compared to the treated group.

Keywords: Erythrocyte Rosette Test, T-like lymphocytes, Mud catfish.

1. INTRODUCTION

The Erythrocyte rosette (E-rosette) test, is a technique used to characterize T-lymphocytes using sheep red blood cells (SRBCs). The principle is that the suspected receptor bearing cells are mixed with the signal cells which carry the corresponding receptor building substance on their surface. That substance is either naturally occurring on it or artificially coupled to it. The receptor bearing cell will then bind the signal cells around their surface and form rosettes. Human lymphocytes can be classified according to their surface markers, thymus derived (T) lymphocytes may be identified by spontaneous rosette formation with sheep red cells [1].

The E-rosette test has been used extensively in human medicine to diagnose a wide variety of diseases [2] and to elucidate many experimental immunopathological mechanisms [3]. Peripheral blood lymphocytes from normal and thermally injured patients were studied for their ability to form rosettes with sheep red blood cells by active E-rosette assay at different times post injury and it was suggested that active rosette forming cells in the total peripheral blood may be used as an index for the early identification of patients at a greater risk of mortality [4]. Patients with neurological or infectious diseases examined for blood T lymphocyte percentage by the standard method using rosette formation with untreated sheep erythrocytes and a technique using 2-amino-ethyl-thio-isouronium bromide hydrobromide (AET)-treated E, with fetal calf serum in the medium have been reported [5]. E- Rosette tests for the quantization of the total number of T lymphocytes were performed with untreated as well as AET-treated erythrocytes, in assays with and without fetal calf serum, lymphocytes from patients with active and stable multiple sclerosis and with infectious mononucleosis as well as from healthy individuals were examined. It has been demonstrated that E-rosette-forming and human thymus-lymphoid tissue antigen-positive lymphocytes are of thymus origin [6]. The interaction of lymphocytes with other cell types is critical for immune function, and provides excellent opportunities to study the cell biology of dynamic cell-cell and cell-extracellular matrix interactions [7-10]. Since fish lymphocytes from all organs posses surface membrane immunoglobulin [11], immunofluorescent technique will only identify them as B-like lymphocytes, however peripheral T-lymphocytes and thymoctes form rosettes with SRBCs which B lymphocytes do not [12]. Thymus factor has been tested by its action on spleen rosette-forming cells from adult thymectomized mice [13]. Therefore the E-rosette test was evaluated in this study to demonstrate T-like lymphocyte sub-populations in the peripheral blood of the mud catfish. The effects of thymectomy and injected anti lymphocytes serum (ALS) on E- rosette formation were also assessed.

2. MATERIALS AND METHODS

Fort eight (48) young adult mud catfish (*Clarias gariepinus)* weighing between 100-150g each obtained from stock were randomly divided into two groups (Group 1 and Group 2) of

twenty four each. Group 1 was injected intra-peritoneally with ALS raised from rabbit and their thymuses were cauterized using an electric soldering to burn off completely each thymus (Thymectomy) while group 2 was not given any treatment. See table 1.

Table 1: Treatment given to two Sample Groups of Young Adult C. gariepinus

Fish Group	Treatment	Dose	Death Record
1	Injected with ALS and Thymectomy	0.2ml ALS	4
2	No treatment given	none	none

Four fish were sampled from each group every week for lymphocytes separation from the blood of each fish. At the end of the second week, the remaining fish in group 1 were given a second dose of ALS injection. Lymphocytes were separated from the blood of two fish from each group every week. The lymphocytes were used for the E-rosette test.

2.1. The E-Rosette Test

Four sheep served as donors and blood was obtained fresh each week. Sheep red blood cells (SRBCs) were first treated with 2-amino ethyl-iso-thiouronnium bromide hydrobromide (AET) reconstituted with phosphate buttered saline (PBS) pH7.2, at 1:40 dilution (25mg/ml). The treated SRBCs were washed twice with PBS at 200 x g for 5 minutes and kept at 4^0C before use.

The E-rosette test was performed as follows: 0.1ml of lymphocytes from blood of fish in group 1 or group 2 was added to 0.1ml of a well mixed 2% AET treated SRBC suspension (5% SRBC suspension diluted 1:2.5 with tissue culture medium) and absorbed fetal bovine serum (FBS) in a microtube. Tubes were spun at room temperature for five minutes at 6,500rpm in an MSE microfuge. Tubes were then incubated for 12-18 hours at room temperature. After incubator, the pellet of cells were either left undisturbed while with a Pasteur pipette, a pinch of cells were gently agitated in order to re-suspend the settled cells and break up cell buttons into single cells and rosettes. On drop of 1% methylene blue was added to each microtube to stain the cell suspension.

Observation and counting of rosettes were performed under a light microscope to which a camera was attached for photographing rosette cells. Rosettes were enumerated using the "Battlement method" of differential leukocyte count [14]. The mean number of rosettes counted out of 200 lymphocytes (rosette and non rosette) was recorded. Only cells with three or more tightly adherent SRBCs were scored as rosettes.

3. RESULTS

Rosettes of SRBCs around the mud catfish lymphocytes were observed in the two fish groups. There were differences however in the response of fish to the treatment given as shown in table 2. The number of rosettes forming lymphocytes was significantly higher ($P < 0.05$) in all samples from group 2 (not treated) when compared to group 1 (treated with ALS and thymectomy). Higher rosettes counts were obtained in the second and third weeks for group 2. The mean E-rosette count for group 2 is 11.43 ± 2.57. Rosettes counts for group 1 declined gradually. The mean E-Rosettes count for group 1 is 8.3 ± 0.84.

The proportion of lymphocytes staining pale with methylene blue (live cells) varied in each preparation and there was correlation in the counts between the number of live cells and rosettes formed. All the rosettes were formed around the pale staining cells (live cells). The formation of rosettes varied from a circumscribed form as shown in plate 1 to a three dimensional form in plate 2. Treatment of SRBCs with AET did not increase or decrease rosettes formation but rosettes were stable for a longer period to be examined and photographed.

Table 2: Time related E-rosette formation by lymphocytes from untreated group (Group 2) and ALS injected, thymectomized group (Group 1).

	Mean E-rosette Count	
Week	Untreated (Group 2)	ALS injected and Thymectomy group (Group 1)
1	10.6 ± 2.4	10.0 ± 3.1
2	13.0 ± 2.9	9.0 ± 2.2
3	14.0 ± 4.7	8.0 ± 2.4
4	9.4 ± 5.4	7.8 ± 1.8
5	10.2 ± 3.0	7.6 ± 2.8
6	11.4 ± 4.0	7.4 ± 3.2

Plate 1: Photographic enlargement (x 10) of a rosette to show the usual ring around arrangement (circumscribed) of SRBCs around a lymphocyte.

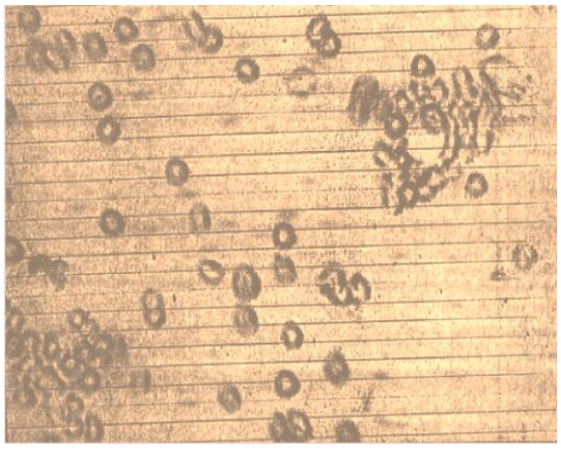
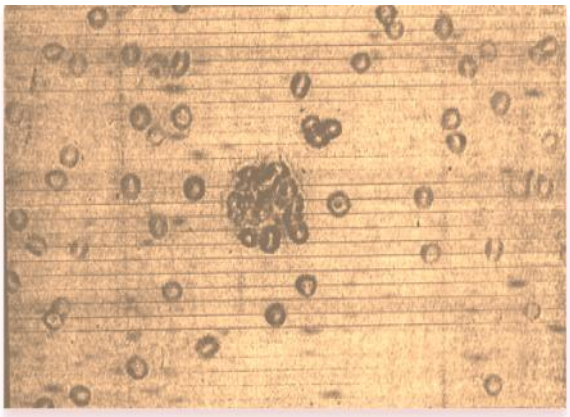


Plate 2: Photographic enlargement (x 10) of a rosette to show rosette in almost a 3-dimensional form.



4. DISCUSSION

This study is a contribution to the mud catfish (*Claris gariepinus*) T and B like cells dichotomy using the E-rosette markers. The result indicated that the mud catfish posse's readily demonstrable T-like cells in the peripheral blood, confirming similar studies on frogs [15] rosette formed consisted of a central lymphocyte and a number of adherent SRBCs.

Sheep erythrocytes formed rosettes with lymphocytes obtained from the blood of untreated mud catfish is similar to the finding of rosette forming cells in all lymphoid organs and peripheral blood of unimmunized mice [14]. All rosettes were stabilized when the red blood cells were pretreated with AET. The numbers of rosettes forming lymphocytes were observed to be significantly higher ($P < 0.05$) in the untreated group as compared to the group injected with ALS and had their thymuses removed, this could be due to the fact that E-rosette forming lymphocytes are of thymus origin [6], and immunosuppressive effect of the injected ALS.

Therefore as in man, it would appear that the E-rosette test could be applied to several problems and research such as the search for a T-cell marker (using SRBC), diagnosis of auto-immune diseases, evaluation for the potency of ALS and immunosupressors in the mud catfish. If these clinical application should applicable to the distinction of lymphocytes sub-population in the mud catfish, the effects of T-cell manipulation on these parameters would suggest further that a T-cell sub-population is in circulation and plays an immunological role in the mud catfish and that complete characterization into T-and B-cell dichotomy exists in the mud catfish.

Further studies for evidence that T-like lymphocytes exist in the mud cat fish may be tried through responsiveness to the mitogens *concanavalin* A and *phytohaemagglutinin*.

5. REFERENCES

1. Jondal M, Holm G, Weigzell H. *J Exp Med*, 1972; **136**: 207-215.
2. Sea J, Borella L. *New Eng J Med*, 1985; **292:** 828.
3. Bianco C, Nussenzweig V, Lay WH, Mendes NF. *Nature*, 1971; ***230*(5295):***531–532*.
4. *Singh* H, Herndon *DN, Stein MD. Burns, 1988;* **14** ***(2):*** *85-90.*
5. Naess A, Nyland H. *Acta Pathologica Microbiologica Scandinavica Series C: Immunology,* 1982; **90C**: 327–330.
6. Yata J, Tsukimoto I, Tachibana T. *Clin Exp Immunol*, 1973; **14**(3): 319–326.
7. Gowans JL, Knight EJ. Proc *R* Soc *Lond B Biol Sci. 1964; **159:257***–282.
8. Mosier DE. *J Exp Med*, 1969; **129:**351.
9. Lipsky PE, Rosenthal AS. *J Exp Med*, 1975; **141:**138-154.
10. Martz E. *Contemp Topics Immunobiol,* 1977; **7**: 301.
11. Ellis AE. *J Micro Dis Fish RJ Roberts ed,* 1982; 1-29.
12. Brain P, Gordon J, Willets WA. *Clin Exp Immunol,* 1970; **6**: 681-688.
13. Baker FJ, Silverton RE, Luckcock ED. An Introduction to Medical Laboratory Technology, 4th *edition.* Butterworth, London; 1966.
14. Bach JF, Dardenne M. *Transplant proc*, 1972; **4 (3):** 345-350.
15. Nagata S, Tochinai S. *Dev Com Immunol,* 1978; **2**: 637.