World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:8, No:8, 2014

The Identification of Anuran Glial Cells

Ibrahim M. S. Shnawa

***Abstract*—**Attempts were made to identify anuran glial cells. They were found as nervous tissue resident. Having stage dependent morphotype changes, whereby, appeared as an ovoid to oval in resting state and amoeboid mrophotypes in activated state, stained fairly with methylene blue and take up Pelikane blue 10% aqueous solution, as well as having the ability to phagocytize heat killed *Staphylococcus aureus*. They were delineated from the migrating peripheral monocytes by morphotypic and morphometeric differences. Such criteria were consistence with glial cells. Thus, the anuran glial cells are being identified in the frog *Rana ridibunda* Pallas 1771 and this animal can be of use as a simple model for the immunobiology of glial cells.

***Keywords*—**Amoeboid cell, bacterial phagocytosis, Glial cells, Resting.

I. Introduction

GLIAL cells are the tissue resident macrophages in the central nervous system [1]. They are the soon replier as a reactor to any inflammatory processes (of any nature). They undergo changes corresponding to the early stage of mobilization and phagocytic intervention [2]. Microglia have unique characteristics like; the expression of histocompatability molecules I&II, leukocyte common antigen, C3 receptor and Ig FC in reactive microglia and even to lesser extent in resting microglia [2]. At the down of the 21st century the physiological, patho-physiological and immunological importance of the micro-glial cells are hot topics for intense research interests [3].

The CNS may be regarded as properly specialized immune organ by the fact that as an intrinsic immune effector cells [3]. For the identification of microglia several criteria were followed such as; Lectin reactivity, antibody reaction, particle engulfment and the presence of surface receptors [1]. As in other vertebrates, frog has CNS microglia [4], [5]. The objective of the present work was to study the morphologic characters and the phagocytic potentials of frog glial cells.

II. Materials and Methods

A. Aquiculture

The present short term program (two days experimentation), the test frogs were kept in natural like environment in an aquaria bathed with their natural river water frequently changed to support normal physiologic needs.

B. The Test Natural Immune System

The *in vivo* test immune system was that of the anuran, the frog *Rana ridibunda*. It was collected from an inbred locally raised in moist gardens. The collect was including both male and female frogs. Their weights were ranging from 35–45 grams.

C. Dye Uptake

Pelikan blue ink (Germany CO,) in 10% aqueous solution was the test dye. The dye doses were 0.1 ml. injected through intra peritoneal (IP) intra spinal (IS) and intra-cranial-Intra spinal into the three test groups each of five. Half hour latter animals were paralyzed temporarily. Squash wet brain macerates were done and number of dye up taking cells were counted. [6]

D. Bacterial Phagocytosis

Heat killed *Staphylococcus aureus* suspension that match 10 IU WHO opacimeter grade, each frog receive 0.1 ml. amounts were injected via, intra spinal, intra spinal - intracranial, and intra cranial for each of the test frogs. The injected frogs were housed in aquaria at room temperature stand with for an overnight period. Squash brain tissue macerates were prepared and stained with methylene blue made three replicates for each frog in each group [7]. Bacterial phagocytic index were calculated as in the following formula

$$\text{Bacterial Phagocytic index} = \frac{\text{No.of cells engulfing bacteria}}{\text{Total numbers of the phagocytes}} \text{x}100$$

E. Glial Cell Mophometery

The morphometeric analysis for the glial cells was performed using ocular and stage micrometer [8]. In which one division at ocular oil emersion lens will be as in the following

$$C_{100}\ \text{x} = \frac{1}{100} \times 100 = 1$$

III. Results

A. Morphotypes

Resting cells were ovoid to slightly elongated oval in shapes. The activated glial cells were of amoeboid cell morphology whether, they were phagocytizing or not.

B. Morphometery

Resting cells were ranging 3-5 x 6-8 ums in size in wet mounts. In the dye up taking preparations, however, the amoeboid cells were with mean size of 7 x 12 μms. In comparison , the activated non-phagocytizing methylene blue stained were with mean size of 10 x 13 μms. And the bacterial phagocytizing were with mean size of 15 x 19 μms.

Shnawa, Ibrahim M.S. is with the Department of Biology, University of Babylon, Hilla, Iraq, (phone: 00964-7800188929; e-mail: ishnawa@yahoo.com).

C. Cyto –Chemistry

Simple methylene blue stained brain tissue macerates were showing the glial cell protoplasm as finely netted blue mesh, with purplish dark blue nuclei. The Pelikan blue taking cells were with faint sky blue colored protoplasm but paler than those stained with methylene blue preparations.

D. Phagocytosis

In pelican blue tissue macerates preparations, glial cells phagocytosis were noted in a rate of 1 to 5 cells/HPF in cerebrum tissue in the test groups. The bacterial phagocytosis preparations were showing the phagocytic percentages as; 16, 20, 21 for IC, IS_IC, and IS respectively while, bacteria-phagocyte cell associated were 3, 0, 2 accordingly Table III. Few dividing leukocyte forms were also noted.

E. Migrating Monocyte

The migratory monocytes were found in a rates of 9, 28, and 5 for IC, IC_IS and IS respectively.

TABLE I
ANURAN GLIAL CELL MORPHOTYPES

Stage	Morphotype
Resting cells	Ovoid to slightly oval
Activated cells	Amoeboid

TABLE II
ANURAN GLIAL CELL MORPHOMETERY

Stage	Size in μms.
Resting	5 x 12
Activated dye uptaking	7 x 12
Activated non-bacterial phagocytozing	10 x 13
Activated bacterial phagocytozing	15 x 19

TABLE III
PLEKAN BLUE 10 % DYE UPTAKE BY THE ANURAN GLIAL CELLS USING DIFFERENT ROUTS HALF HOUR POST INJECTIONS

Rout	Number of cells uptaking dye
Intraperitoneal (IP)	1 – 2/ HPF
Intraspinal (IS)	3 – 5 /HPF
Intracranial (IC)	3 – 5 /HPF

TABLE IV
THE PHAGOCYTIC ACTIVITY OF THE ANURAN GLIAL CELLS TO *S. AUREUS* HEAT KILLED CELLS

Rout	Amoeboid non phagocytic	Amoeboid activated phagocytic	Extracellular test organisms	Dividing leukocytes	Monocytes/HPF
Intracranial(IC)	81:100(81%)	16:100(16%)	+	3:100(3%)	9
Intraspinal (IS)	79:100(79%)	21:100(21%)	+	-	5
Intracranial-intraspinal (IC-IS)	63:100(63%)	35:100(35%)	+	2:100(2%)	28

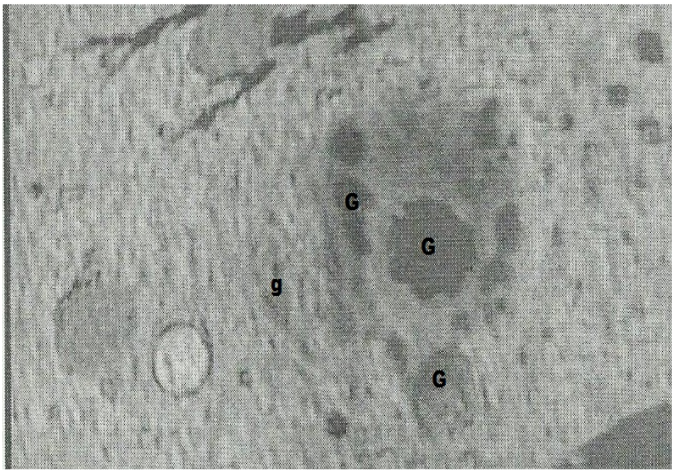

Fig. 1 Anuran glial cells (G) activated and (g) resting One millimeter on the photo equals, one actual micrometer size

IV. DISCUSSION

Microglia of nervous tissue parenchyma are part of the mononuclear phagocyte system, since they are bone marrow derived, inters central nervous system (CNS) and adopt the morphology of microglia. Such transition of monocyte to microglia in CNS was demonstrated and they bear antigens restricted to the mononuclear phagocyte system. Workers consider these cells as highly specialized and distinct population of phagocytes that lacks their antigens through down regulation processes to the limits that becomes undetectable. Microglia phenotype regulation in CNS occurred through three different possible ways as, Blood Brain Barrier (BBB), presence of their specific legend as well as the presence of down regulation factor [4]. Macrophages occur in the adult frog at the neck region, spleen, liver and brain [9]-[12].

Few works have been done about the anuran glial cells [13], [14]. The leopard toad *Rana pipiens* Schreber 1782 [15] and our common inbred frog *Rana ridibunda* Pallas 1771 [16] shared genomic fraction conserved for vertebrata, other fractions that are conserved for anura. Besides which there were segments in their genomes that are specific for the species or even for the variety. Such species or variety differences make differences in their transcriptomics and proteomics the second and the third dimentional aspects of the gene recent concepts ,a situation that inflects morphologic and immune physiologic differences [17], [18]. Thus, the author is of the opinion holds that these differences were fairly sufficient to make any contribution in the identification of glial cells of the common inbred frog *Rana ridibunda* is novel or might be even original contribution in this topic.

In previous works Shnawa [19], [20] has been found nil dye uptake and nil bacterial phagocytosis using cerebrum tissue macerates and cerebrum tissue sections in frog. Current study puts forward a model system for identification criteria of frog glial cells. These criteria are being mentioned in the followings

A. Nervous tissue microenvironment.
B. Stage dependent change in morphotypes
C. Characteristic size in accordance with cell immune-physiologic state.

D. Displays dye uptake phagocytosis
E. Displays bacterial phagocytosis
F. Have characteristic staining reaction with methylene blue.

- On comparison with the identification criteria documented by other workers [13], [14], there were agreements and contradictions. The presence of few dividing leukocytes may be attributed to the bacterial injection insults [12]; while, presence of the peripheral blood monocyte in brain tissue macerates may due to derangements of BBB [13], or due to macrophage mobility and niche dysfunction during inflammation [21].
- There is a possibility that this current frog model can be of use as an indicator for environmental immune toxicity as that found in leopard toad *Rana pipiens* [22], [23].

V. Conclusion

Frog *Rana ridibunda* Pallas 1771 glial cells are being identified and differentiated from migratory peripheral blood macrophage arriving to the nervous tissue due to blood brain barrier through heat killed bacterin injection insults. Frog as a laboratory animal model may be of use for detection of glial cell immunobiology, and having the potential to be used as a model system for environmental immunotoxicity as that found in *Rana pipiens*.

Acknowledgment

The author wish to appreciate the technical help extended by the under graduate honor students Mr. Ahmed Joda, Mr. Yaser ALMolaa and Mr .Mohamed Kais.

References

[1] Bignami A and Dahl D, Glial cells in the central nervous system and their reactions to injury ,R G Landes Company,1994,Austin.
[2] Castellano B, Gonzalez B and Neto-Sapedro M. Undedrstanding Glial Cells. Walter Kluwer Academic Publisher, 1998,USA,7-110.
[3] Rock BR,Cekker G, Hu S, Sheng WS, Cheeran M,et al., Role of micrglia in central nervous system infections. Clin. Micrbiol.Rev. 2004,vol.17(4) pp 942-956.
[4] Lara JM, Velasco A,Lillo C, Jimeno D ,and Aijon J. Characterization of glial cells in the teliost retinal pathway.In,CastellanoB,Gonzalez B and Neto-Samppedro M eds .Understanding Glial cells.Kluwer Academic,1998,USA.
[5] Stteit WJ.Micrglial response to brain injury.Toxicol,Pathol.2000, vol.28(2),pp 28-30.
[6] Kinmonth JB .Lymphography .Lymphology1977vol. 10 ,pp.102-106..
[7] Kwapinski JBJ Methodology of Immunochemical and immunological Researchs .Wiley-inter science.1972,New York.
[8] Cruickshank R,Duguid JB ,Marimoin B and Swain RHA .Medical Microbiology12th ed,Vol.2.Practice of Medical Microbiology.Churchill-livingstone,1975,NY.
[9] Perry VH. Macrophage and The Nervous System .R G Landes Company.1994,Austin.
[10] Cooper EL. General Immunology Pergamon Press,1982,Oxford.
[11] Manning MJ and Turner RJ .Comparative Immunobiology. blackie,1976,London.
[12] Pastoret PP,Giriebel P, Bazin H, Govaerts A. Handbook Of Vertebrate Immunology ,Academic Press 1998,USA.
[13] Perryman EK. Stellate cells as phagocytes of pars distalis. Cell and Tissue Res. 1983vol.,231,pp143-155.
[14] Miller RH and Liuzzi FJ .Regional specialization of radial glial cells of adult frog spinal cord.J.Nephrol.1986,vol. 15,pp187-196.
[15] David HM,Thomas PW.Phylogeny of the new world true frogs.Mol.Phylogen.Evo.2004,34(2):299-314.
[16] Darrel RF.Pelophylax ridibudus(Palla,1771).Amphibian species of the world 5.6,an online reference. American Mueseum of natural History,2013.
[17] Yang W,Qi Y ,Bi K,Fu J.twards understanding genetic basis of Adaptation to high level-elevation life in poikilothermic species:A Comparative transcriptomic analysis of two ranid frogs,Ranachensis And Rana kukunoris.BMC Genomics 2012,vol.13.pp 588-609.
[18] Zhou W-w,Wen Y,Fu J,Xu Y-b,Jin J-q,Min M-q,Che J,Zhang Y-P.Speciation in Rana chensinesis species complex and its relationship to uplift of Qinghai-Tibetan plateau.Mol.Ecol.2012,vol.21 pp 960-973.
[19] Shnawa IMS . Regional anuran lympho graphy.Baby.Uni.J.2002 vol.,8(3) pp745-749.
[20] Shnawa IMS .Anuran nonspecific cellular immune functions. Baby.Uni.J.2002vol,7(3) pp.745-749.
[21] Pettet MJ , Nahrendrorf M and Swirski FK. The journey from stem cell to macrophage .The Year In Immunology. Ann.N.Y.Acad.Sci., 2014, Harvored.
[22] Brodkin MA ,Madhoun H, Rameswaran M and Vatrnick I.Atrazine is an immune distruptor in adult northern leopard frog *Rana pipenis*. Env.Tox.Chem.2007,vol.26(1) pp 80-84.
[23] Gilbertson M-K, Haffner D ,Droullard KG , Albert A, and Dixon B. Immunosuppression in northern leopard frog Rana pipenis induced by pesticides .Env.Tox.Chem. 2003,vol.22(1) pp 101-110.