International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

Review Article **Open Access**

Impact of rickettsial bacterial disease on immune system

Amit Gupta[1] and Sushama R Chaphalkar[1, 2]*

[1]Department of Immunology, Vidya Pratishthan's School of Biotechnology (VSBT) Vidyanagari, Baramati, Pune, India
[2]Director, Vidya Pratishthan's School of Biotechnology (VSBT) Vidyanagari, Baramati, Pune, India

A B S T R A C T

Rickettsia belonging to the family *Rickettsiales*. The number of human cell types which is targeted by rickettsia i.e. macrophages are the members of innate immunity which is capable of enhanced or inhibit the inflammatory responses. In this paper, we discuss about the rickettsia negative and positive blood samples of human and studied its various immunological parameters i.e. CD3/CD4/CD8 population and peritoneal macrophages activation in mice. These studies directly or indirectly correlate with the innate as well as adaptive immune system.

Keywords: Rickettsia, *Rickettsiales*, peritoneal macrophages

A R T I C L E I N F O

CONTENTS

Article History: Received 30 November 2014, **Accepted** 31 January 2015, **Published Online** 15 March 2015

***Corresponding Author**
Dr. Amit Gupta
Assistant Professor, Vidya Pratishthan's School of Biotechnology (VSBT), Vidyanagari, Baramati, Pune, India
Manuscript ID: IJCTPR2450

PAPER-QR CODE

Citation: Sushama R Chaphalkar. Impact of rickettsial bacterial disease on immune system. *Int. J. Curnt. Tren. Pharm, Res.*, 2015, 3(2): 863-867.

1. Introduction

The Rickettsiae are coccobacilli, gram-negative cell wall (lack flagella) and is included in the bacterial family *Rickettsiaceae* of the order *Rickettsiales.* These are obligate intracellular parasites of eukaryotic cells [1]. These may be generally divided through binary fission and present or reside within the cytoplasm or nucleus of the cell that they invade. The rickettsiae frequently have a close relationship with arthropod vectors that may transmit the organism to mammalian hosts. The genomes of rickettsiae are very small of about 1.0-1.5 million bases [2, 3]. The genus *Rickettsia* includes many species associated with human disease including spotted fever group and the typhus group.

The rickettsiae that are pathogens of humans are subdivided into three major groups based on clinical characteristics of disease i.e. spotted fever group; typhus group and scrub typhus group [4, 5].

The rickettsiae's obligate intracellular existence in both mammalian and arthropod hosts serves as an excellent model for the study of complex host-parasite interactions. Number of cases of rickettsial diseases will never be diagnosed; these infections have been reported in epidemic form in human populations e.g. due to infection with *R. prowazekii*, 130 million cases of typhus during and immediately after World War I, causing nearly 3 million deaths and also associated with pathogen virulence and host-related factors (e.g., age, delayed diagnosis, hepatic and renal dysfunction, CNS abnormalities etc. Out of these rickettsiae's disease, classic epidemic typhus is the most severe disease with symptoms such as high fever (temperature more than 42 °C), extreme pain in the muscles and joints, stiffness etc [6, 7, 8]. During later stages of infection, patients showed neurologic symptoms. For the last ten years, researchers focused on rickettsiae disease and tried to increase the awareness about the disease and tried to maintain proper treatment as well as taken the preventive measures but these measures are so complicated because of the non-availability of effective and safe rickettsial vaccines. There is an urgent need of prophylactic as well as therapeutic vaccines for rickettsial disease because increased in the population growth and land use bring arthropods (insect vectors) and their associated pathogens into human habitations [6, 8]. In this short communication paper, our group focused on the infected human blood samples of rickettsiae disease and analyzed its infected cells on innate as well as adaptive immune system in mice. All these studies should be done as per the ethical guidelines.

Human negative and positive rickettsial blood samples were collected from *Mangal Pathology laboratory*, Baramati, District Pune, Maharashtra, India at different time intervals for flow cytometric studies. These human rickettsial diseases which are confirmed in *Mangal Pathology laboratory*, Baramati, Maharashtra, India on the basis of these strains OX-19, OX-2 and OX-K. If these strains showed agglutination it means the person is suffering from rickettsial disease. These rickettsial diseases are diagnosed through Weil-Felix test. The most common antigens used in the Weil-Felix tests are OX-K and OX-19 antigens. The OX-K antigen reacts with sera from scrub typhus patients and is used in the diagnosis of *Orientia tsutsugamushi* (OT) related infections, whereas OX-19 antigen reacts with sera from persons infected with typhus group rickettsiae as well as spotted fever rickettsiae. In India, scrub typhus is endemic and is reported more than 1 million cases. Generally, number of cases of scrub typhus occurs during visits to rural areas in endemic parts of India for activities such as camping, hiking, or rafting.

Rickettsiae (bacteria some of which can be transmitted to humans through bites of fleas, lice, ticks or mites) are usually injected directly from the saliva of ticks and mites as they feed on humans and in the case of fleas by contamination of bite sites by faeces. The symptoms of this disease is normally observed in human such as swollen lymph glands, fever, headache etc and is normally diagnosed through blood test or through biopsy (skin sample) from the bite area [4, 5]. Till now, there is no vaccine available for this disease, mostly drugs especially tetracycline antibiotic doxycycline which reduces the rate of reaction. One of the rickettsial diseases i.e. epidemic typhus normally occurs in Baramati region, Maharashtra, India where most of body lice are prevalent. The outbreaks of this rickettsial disease can be often during the colder months when infested clothing is not laundered. Generally, travelers at a higher risk for epidemic typhus include those who may work with or visit near areas with large shelterless populations, impoverished areas, shelter camps, and regions that have recently experienced war or natural disasters [6, 7, 8]. In an attempt to inject (intraperitoneally, 10^9 cells/ml) infected cells of rickettsial blood samples (lysed red blood corpuscles and wash two times with phosphate buffered saline) in Swiss mice, whole blood surface markers and peritoneal macrophages were processed for flow cytometric analysis at different time intervals. FACS and ELISA based assays were utilized to evaluate and quantify the interactions and activation of these rickettsial blood samples. The role played by cell surface markers and macrophages as key initiators and orchestrators of the immune response, is important to understand in rickettsial infections.

While the immune response against rickettsial disease is classified or described on animal models especially mice in terms of CD4/CD8 population, CD3 surface marker and peritoneal macrophages activation in mice. The *in vivo* effect of non-infected as well as infected rickettsial blood samples was evaluated in Swiss mice. Mice were immunized intraperitoneally with non-infected as well as infected samples of rickettsial disease on day 0 and 7^{th} day with human peripheral blood mononuclear cells (10^9 cells) in a final volume of 0.2 ml. On day 10, collect the EDTA blood samples were used for the estimation of surface markers and collect the peritoneal macrophages from the abdominal cavity. For flow cytometric analysis, whole blood (100 µl, 10^9 cells/ml) was taken from the retro-orbital plexus of mice in each tube. All these experiments will be done under the ethics rules and regulations.

FITC labeled CD3, CD8 and PE labeled CD4+ monoclonal antibody were added directly to 100 µl of cells. Tubes were incubated in dark for 30 min at room temperature. Subsequently, 2 ml of 1× FACS lysis solution was added at room temperature with gentle mixing followed by incubation for 10 min. The samples were spinned (300 – 400 × *g*) and the supernatant was aspirated and sample was given three washings of PBS (pH 7.4). The resulting stained cell pellet was resuspended in 500 µl of PBS and was run on a flow cytometer. The forward and side scatter gating applied for data acquisition on 10,000 events in FACS Calibur and fraction of FSC and SSC cell populations representing different phenotypes analyzed using cell quest

software [9, 10]. In general, $CD4^+$ and $CD8^+$ T cells are important in protection against numerous intracellular infections, including rickettsioses and other immune lethal rickettsial challenge and significant blunting of rickettsial effector functions [11, 12]. In this study, the infected samples of rickettsia in mice showed rapid increase in CD4/CD8 ratio as compared to control **(Fig.1.).** Since mononuclear cells (especially monocytes) are over activated during acute infection, it is expected or well established that increase in the activation of T cell markers such as soluble CD4, soluble CD8 and CD3 cell surface marker. The activation of immunoregulatory T lymphocyte subsets (CD3 count) has been observed in rickettsial infection, being more evident in mice than in human. There are, however, as yet no well-defined cell surface markers to determine the rickettsial disease. Number of studies was performed to compare the cellular immune status in mice in order to observe the value of these parameters in the immunization as well as challenging stage of the disease. The result showed that there is slightly increased in CD3 count as compared to control **(Fig.2a).** In addition, cell mediated immunity which is mediated by T lymphocytes which played an important role to fight against intracellular or viral infections. Among the T lymphocytes, T helper cells induce B lymphocytes to secrete antibodies and cytotoxic T lymphocytes help phagocytes to destroy infection induced by pathogen and to kill intracellular pathogens or microbes [13, 14].

The macrophages from Swiss mice were collected from the peritoneal cavity and inoculated with infected as well as non infected samples of rickettsia of human peripheral blood mononuclear cells at different time intervals. The inhibiting effect of the rickettsial infected peripheral blood mononuclear cells of human on peritoneal macrophages in mice depended on the number of cells injected and duration of treatment. The data so far obtained suggest that the inhibiting effect of peritoneal macrophages was due to direct action of the rickettsia. In short, peritoneal macrophages collected from Swiss mice and observed the cells through flow cytometer, it is confirmed that the infected sample of human rickettsial showed reduction of peritoneal macrophages activation in mice as compared to control **(Fig.2b).**

Experimental evidence indicates that the there is rapid reduction of forward (shape and size) and side scatter (granularity) in case of rickettsial infected samples of mice as compared to control. Finally, it is confirmed that the macrophages play important role in host defense against rickettsial infections The macrophages have long been suspected to be the key cell in determining the outcome of rickettsia e.g. typhus in human. As per the results of flow cytometry, the relative inability of rickettsia to survive within the macrophages may indicate that the ability of rickettsia to multiply within and destroy nonimmune macrophages. This destructive activity of macrophages is associated with the ability of rickettsia to lyse the erythrocytes and is due to the action of a putative rickettsial phospholipase A activity [12, 13, 14]. Now a day, there is lot of progress in order to understand the mechanism and regulation of macrophage function, receptors and their signaling components for controlling the bacterial, viral and parasitic infection. With the advancement in the field of genomics, proteomics, immunology and availability of new technology it may be possible in near future to define the mechanism and regulation of macrophage activation and suppression.

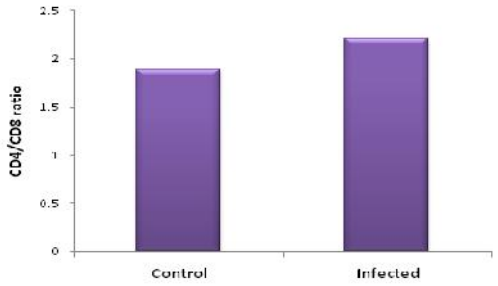
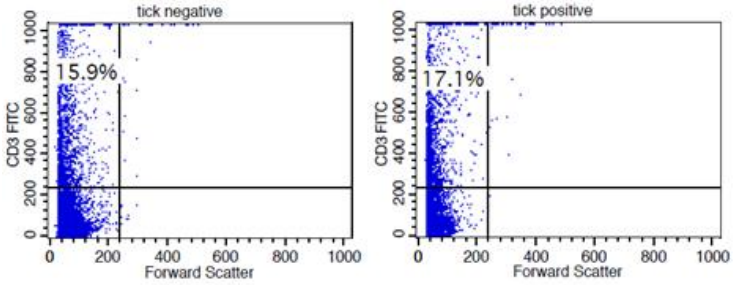
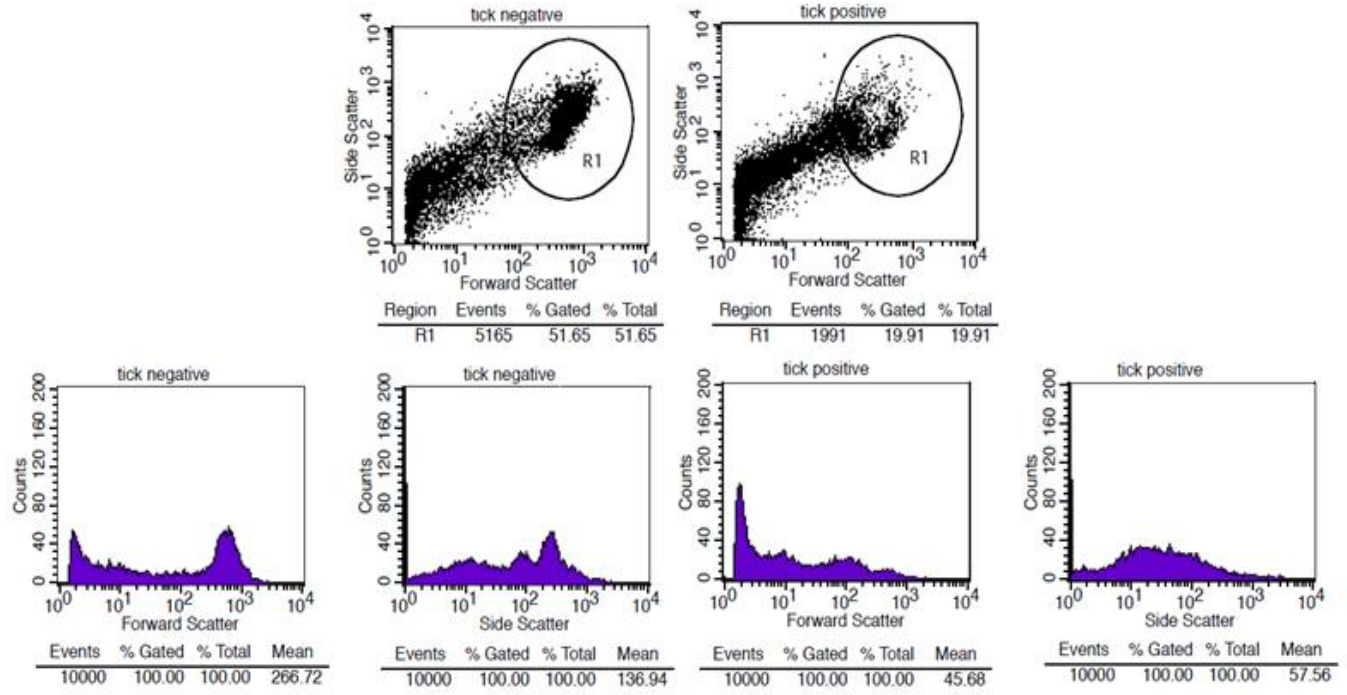


Figure 1: Effect of human infected and non infected ricketssial PBMC (10_9 cells) in whole blood of mice. Staining of whole blood with T cell surface marker CD8 (FITC conjugated monoclonal antibody) and CD4 (PE-conjugated monoclonal antibody). Whole blood were lysed with red blood cell lysing buffer and washed the samples two to three times with phosphate buffered saline and then analyzed the cells through flow cytometer (FACS Calibur).



(A)



(B)

Figure 2: Effect of human infected and non infected ricketssial PBMC (10_9 cells) in peritoneal macrophages of mice. A) Staining of whole blood with T cell surface marker CD3 (FITC conjugated monoclonal antibody). B) Peritoneal macrophages collected from the abdominal cavity of mice. Whole blood and peritoneal macrophages were lysed with red blood cell lysing buffer and washed the samples two to three times with phosphate buffered saline and analyzed the cells through flow cytometer (FACS Calibur).

2. Conclusion

Future studies should be considered into other infectious diseases including rickettsial as well. Inspite of the fact, we focused on adaptive humoral and cellular immunity in relation to medicinal plants to better address or understand the protective immunity.

3. Acknowledgement

We would also like to thanks Dr Gandhi, Mr. Shekhar (*Mangal Pathology laboratory* and Mr. Devdan Chopade, VSBT, Barmati, District Pune, Maharashtra, India) for collection of rickettsia infected human blood samples.

4. References

1. Walker DH, Valbuena GA and Olano JP. Pathogenic mechanisms of diseases caused by Rickettsia. Ann NY Acad Sci 2003; 990:1-11.
2. Valbuena G, Feng HM and Walker DH. Mechanisms of immunity against rickettsiae: new perspectives and opportunities offered by unusual intracellular parasites. Microbes Infect 2002; 4: 625-33.
3. Feng HM and Walker DH. Mechanisms of intracellular killing of Rickettsia conorii in infected human endothelial cells, hepatocytes and macrophages. Infect Immun 2000; 68: 6729 - 6736.
4. Hechemy KE, Paretsky D, Walker DH, Mallavia (eds): Rickettsiology: Current Issues and Perspectives. Vol 590. NY Acad Sci, New York, 1990.
5. Walker DH (ed): Biology of Rickettsial Disease. Vols I and II. CRC Press, Boca Raton, FL, 1988.
6. Dobler G, Wolfel R. Typhus and other rickettsioses: emerging infections in Germany. Dtsch Arztebl Int 2009; 106 (20): 348– 354.
7. Hendershot EF, Sexton DJ. Scrub typhus and rickettsial diseases in international travelers: a review. Curr Infect Dis Rep 2009; 11(1): 66–72.
8. Raoult D, Parola P, editors. Rickettsial Diseases. New York: Informa Healthcare USA, Inc; 2007.
9. Gupta A, Khamkar PR and Chaphalkar SR. Immunomodulatory and anti-inflammatory activity of aqueous extract of leaf, stem and root of *Ficus religiosa* on human whole blood and peripheral blood mononuclear cells. International Journal of Medicine and Pharmaceutical research 2014; 2(4): 732 - 739.
10. Gupta A, Khamkar PR and Chaphalkar SR. Anti-inflammatory activity of aqueous extract of *Mimusops elengi* on human whole blood and peripheral blood mononuclear cells. International

Journal of Current trends in Pharmaceutical research 2014; 2(5): 494 – 501.

11. Amber IJ, Hibbs JB, Taintor RR and Vavrin Z. Cytokines induce an l-arginine-dependent effector system in nonmacrophage cells. J Leukocyte Biol 1988; 44: 58–65.
12. Davis CE, Belosevic M, Meltzer MS and Nacy CA. Regulation of activated macrophage antimicrobial activities. Cooperation of lymphokines for induction of resistance to infection. J Immunol 1988; 141:627–635.
13. Gupta A, Khajuria A, Singh J, Singh S, Suri KA, Qazi GN. Immunological adjuvant effect of Boswellia serrata (BOS 2000) on specific antibody and cellular response to ovalbumin in mice. International Immunopharmacology 2011; 11(8):968 – 975.
14. Khajuria A, Gupta A, Malik F, Singh S, Singh J, Gupta BD, Suri KA, Suden P, Srinivas VK, Ella K, Qazi GN. A new vaccine adjuvant (BOS 2000) a potent enhancer mixed Th1/Th2 immune responses in mice immunized with HBsAg. Vaccine 2007; 25 (23): 4586 – 4594.