Role of Zebrafish C-Reactive Protein in infiltration of Macrophages during Bacterial Pathogenesis

Dr. Shuvasree Sarkar*
Visva Bharati University, Kolkata
Email :shuvasreesarkar@gmail.com

Liver is a major organ that helps in detoxification of foreign bodies, for example, bacterial cells, toxic chemicals and metals. During bacterial infection, macrophage plays an important part in minimising bacterial load from our body by clearing bacterial cells through phagocytosis. Several proteins are involved in inducing phagocytic activities of macrophages, especially C-reactive protein (CRP) plays a great role in this event. One important point is among different tissues, liver normally remains devoid of resident macrophages, therefore, an obvious question is then how does liver gets protected from bacterial infection. If we look into the cellular constituents of liver we would find that majority of them are hepatocytes and rest are blood cells, and satellite cells. In the present investigation, we intended to investigate whether hepatocytes itself is sufficient to minimize bacterial pathogenecity or other cells are involved. This will provide a better understanding about the contribution of hepatocytes and other cells in protecting liver during bacterial infection.

I have performed several experiments to understand the mechanism of reduction in bacterial pathogenicity in liver. In this context, we emphasized the role of macrophages in reducing bacterial load as these cells are directly involved in phagocytosis. Few important questions arise: i) Does liver tissue recruit macrophage during bacterial challenge for their protection? ii) If this is true, then what are the signals that trigger liver cells to recruit macrophage? iii) Which signaling cascades are triggered by both hepatocytes or macrophages and how these signaling molecules are associated in

* Dr. Shuvasree Sarkar, Post Doctoral Fellow from Visva Bharati University, West Bengal, Kolkata, is pursuing her research on "Functional Analysis of Zebrafish C-Reactive Protein (Crp) in Bacterial Pathogenesis: Interaction with Macrophage and Cytokines." Her popular science story entitled "Role of Zebrafish C-Reactive Protein in Infiltration of Macrophages during Bacterial Pathogenesis" has been selected for AWSAR Award.

reducing bacterial load? With this hypothesis, we obtained certain important findings which are enumerated below.

In zebrafish liver, role of macrophage during bacterial infection is still unknown as macrophage does not reside in the liver. During bacterial pathogenicity, macrophage plays a great role in destruction of bacterial cells by involving several proteins. Numerous proteins induced due to bacterial infection, trigger enormous signaling cascades for the onset of innate and adaptive immune responses. Among these proteins, CRP acts as a major acute phase protein that dramatically increases during bacterial infection to trigger immunological responses in host. In zebrafish liver, what is the role of CRP during bacterial infection? To understand this situation we conducted several experiments and found that zebrafish infected with *L.monocytogenes*, a gram positive bacteria, and *S.typhimurium*, a gram negative bacterium, showed a dose dependent increase of serum CRP as compared to control. The optimum dose at which *L.monocytogenes* and *S.typhimurium* significantly could induce CRP was found to be 10^{10} and 10^{6}, respectively. These findings clearly indicate that bacterial infection is directly associated with CRP level in infected zebrafish. Apart from these, we also tested CRP level at different time intervals in infected zebrafish and observed that at 24hrs maximum CRP induction. An obvious question here is how is CRP induced with increasing bacterial infection? (A: See change. Is it ok?)

To have an idea on how bacterial infection could cause high CRP level in serum of infected zebrafish, we came to know that CRP is majorly synthesized in liver in mammals however, in fish scant information is available in this direction. The expression of CRP increased in liver though maximum was noted in serum of infected zebrafish. The differential availability of CRP in liver tissue lysate and serum are may be due to the fact that CRP is a secretary protein and once it synthesized in liver, quickly comes into the serum. Since few proteins regulate CRP expression, we found that expression of CRP enhanced because of elevated expression of IL6 and IL1b in liver. Elevated CRP in infected zebrafish is a marker for bacterial pathogenesis and may be associated with toxicity.

The above findings clearly indicate an association between CRP synthesis and the involvement of IL6 and Il1b therein. To have an idea whether elevated CRP in infected zebrafish could cause toxicity, we performed further experiments. We expected that as CRP level elevated within 24hrs there may be a chance of high toxicity in infected zebrafish. To examine this, we measured the MDA level and found a marginal rise in its level against control in gram positive *L.monocytogenes* with in 48hrs. However, MDA level greatly enhanced in *S.typhimurum* infected zebrafish within the same period as observed with *L.monocytogenes* suggesting maximum superoxide production during this period. Since MDA is a direct measure of superoxides, we expected that increasing superoxides triggers synthesis of endogenous anti-oxidants, which will help in neutralising the toxicity. One of the important anti-oxidants is glutathione which remains decreased in *L.monocytogenes* treated zebrafish until day 2 though after that it gradually increased and almost reached to the control at day 4. The reason for initial depletion of GSH may be due to scavenging of enhanced superoxides that is produced within day 2.

One important point is that liver majorly consists of hepatocytes and CRP is synthesized primarily by these cells while CRP induction requires IL6 and IL-1β which are marginally produced

by macrophages. An obvious question here is how CRP could be synthesized in significant amount by hepatocytes, is/are other cell/s involved here? To understand this, we measured availability of macrophage in the zebrafish liver tissue. It should be noted that normally zebrafish liver does not have macrophages and we found that expression of a macrophage marker-F4/80 greatly enhanced in *L.monocytogenes* infected zebrafish liver thus indicating that macrophage might be infiltrated during bacterial infection within 24 hrs. This was further confirmed by immunofluorescence study where we observed few cells apart from hepatocytes were labelled with FITC-labelled macrophage specific marker, F4/80. The results are further validated by FACS analysis where we determined the percent of macrophages in control in infected zebrafish liver. It could be evident from Fig 5 that in control liver percent of F4/80 labelled macrophage is about 12.1% and this elevated in *L.monocytogenes* infected zebrafish liver to 17.3% as shown in quadra dot plot of FACS. Similar profile was noted in histogram where F4/80 positive cells in liver enhanced from control to treated zebrafish from 1 to 2.1%. Therefore, from these results it is clear that macrophage infiltrates in infected zebrafish liver**.**

Results from these investigations clearly demonstrates that macrophage infiltrates during bacterial infection in zebrafish liver and that possibly triggers induction of CRP. Earlier there was no report on macrophage availability in zebrafish liver; this is the first time we observed that macrophage enters in zebrafish liver. This event is associated with induction of CRP in infected fish, thus there might be a relation between these two events. However, the precise mechanism of this phenomenon is not yet clear and I am continuing my research in this area.