Shweta Jain (2010). ***Influences of Toll like receptors and Costimulatory Molecules in B Cell Activation and Antigen Presentation.*** Ph.D. Thesis. CSIR-IMTECH, Chandigarh/ Jawaharlal Nehru University, New Delhi: India.
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SUMMARY OF THE THESIS

Whenever a pathogen infects a host, the innate immune system acts as the first line of defence and protects the host by releasing soluble mediators, recruiting cells at the site of infection leading to phagocytosis and killing of the bug. Thereafter, the baton is passed onto the adaptive immune system which brings about a more robust and "specific" immune response and additionally endows the host with "immunological memory". The earlier notion that the two arms of the immune system work in seclusion, does not hold true any longer. A flexible continuum exists between the components of the innate and the adaptive immune system that play resolute roles in maintaining the immune homeostasis and bridging these two arms of the immune system (Hoebe et al, 2004). Some prudent examples of such players are TLRs, the complement system, antimicrobial peptides, interferons, defensins, dendritic cells, macrophages, B1B cells, NKT cells etc (Medzhitov & Janeway, 1997). Moreover, this crosstalk between the two arms of the immune system is bidirectional. For example, the engagement of TLRs bolster the antigen presentation abilities of macrophages which, in turn, upregulate their surface MHC molecules and secrete cytokines such as IL-1, IL-6, TNF-α, etc., which brings about an acute phase response (Medzhitov, 2001).

Optimal activation of B cells depends upon the integration of signaling pathways that involve molecules of both innate and adaptive immune system. Knowledge in this area is fragmentary. However, it has started to evolve with the emerging facts through studies on B cells elicited concomitantly through BCR and TLRs (Dye et al, 2007). Little is known about the outcomes of BCR independent signaling on B cells.

In the present study, we attempt to dissect the role and probable involvement of molecules of innate and adaptive immunity in regulating the functions of APCs such as B cells. With the growing body of evidences indicating that combinatorial effects of TLRs with

molecules of adaptive immunity such as BCR, CD38, etc., have an immense impact on the modulation of B cell activity. Therefore, in the current study, we sought to investigate whether TLRs can club with costimulatory molecules to enhance or impede B cell activity (Ahonen et al, 2004; Chaturvedi et al, 2008). The idea of choosing costimulatory molecules came from well-acclaimed studies from our group indicating distinct roles of CD86 and CD80 in modulating B cell functions (Suvas et al, 2002). While CD86 differentially activates B cells in terms of their growth, survival, immunoglobulin isotype secretion, CD80 has totally opposing effects on B cells. TLRs are present on all APCs including B cells and they activate B cells differentially owing to their disparity in expression on various B cell subsets (Bekeredjian-Ding & Jego, 2009). Whereas signaling through TLR-3, 4, 7 and 9 activates B cells, chronic signaling through TLR-7 induced unresponsiveness leading to a phenomenon called "TLR-tolerance". The "slumber" of TLR-tolerance can be wrecked by stimulating B cells with TLR-7 and BCR sequentially (Poovassery et al, 2009). Moreover, signaling through TLRs and CD38 induces strong proliferative signals in murine B cells and leads to their activation (Manjarrez-Orduno et al, 2007). Much has been "said and done" on the role of TLRs 4, 3, 7 and 9 on B cells but the precise role of TLR-2 is still not very well deciphered.

Using a system of highly purified B cells with no T cell contamination, we report here that cross-linking of surface CD40 or CD86 with their respective antibodies coupled with triggering through TLR-2 ligand affects B cell functions significantly. We testify that synchronous signaling through TLR-2 and CD40 enhances B cell proliferation in a dose responsive and time dependent manner. Additive effect of TLR-2 and CD40 in B cell proliferation at shorter time durations indicates that synchronous signaling reduces the time threshold of activation of

B cells. Moreover, B cells stimulated through CD40/CD86 in association with TLR-2 demonstrated an enhanced activation phenotype in terms of augmented expression of B cell activation markers as compared to controls. The increased activation status was further ascertained by the fact that these cells have expanded size and ability to form blasts as compared to individually stimulated or unstimulated controls. Further, such cooperatively stimulated B cells underwent class switch recombination better than controls and secreted elevated levels of immunoglobulin isotypes.

The most notable feature of concomitantly stimulated B cells is the augmentation in their abilities as APCs. B cells are considered as poor APCs because they cannot take up soluble antigens as efficiently as DCs. However, B cells have immense ability of receptor mediated endocytosis through their somatically rearranged highly specific BCR. Synergistic stimulation through CD40/CD86 and TLR enhanced the capacity of pinocytosis and receptor mediated endocytosis of B cells. In addition, such B cells also display an improved skill to help and activate CD4 T cells.

The aforesaid findings corroborated well with microarray data of global gene modulations as a result of concomitant signaling. Genes involved in apoptosis of B cells were downregulated and those involved in survival and activation were upregulated. Moreover, genes responsible for somatic hypermutation saw a downhill route, whereas those of isotype secretion exhibited an uphill trend. In addition, genes involved in endocytosis and T cell activation were upregulated as a result of concomitant signaling. This authentication of gene regulation with the physiological parameters persuaded us to conclude categorically that synergistic stimulation through TLR and costimulatory molecules have a positive effect on the overall B cell functions and activities. Additionally, a slight upregulation in some of the negative regulators

in response to such signaling implicate that concomitant signaling does not lead to B cell activation in an uncontrolled manner, rather it aids in maintaining an overall immune homeostasis and accord.

Cross linking of the surface receptors such as CD40 has been shown to integrate several downstream signaling molecules, which act in cohesion to bring about cellular activation (Cambier et al, 1994). Similarly, TLRs also engage several downstream adaptor molecules that relay the emanating signal and lead to terminal activation of B cells (Kawai & Akira, 2006). Whether concomitant signaling brings these downstream molecules together in a reduced spatiotemporal manner, or it by-passes some of the "runners in the relay" for quick and enhanced response, or whether some new players are involved in such a phenomenon, are some of the questions which are "peeking out of the nest" at the moment. Investigation into the molecular mechanisms involved in the dynamics of dual-signaling phenomena will improve our understanding in this area. Moreover, the convoluted heterogeneity among molecules of the innate and adaptive immune system opens avenues to investigate and develop understanding of their communication networks. How they operate, what they do and how they are regulated are some of the key questions which, if answered, may give an opportunity to comprehend their roles in immunity and may present possibilities for therapeutic intervention in various diseases.