Manzoor Ahmad Mir (2009). ***Costimulatory molecules mediated regulation of the activation and differentiation of antigen presenting cells.*** Ph.D. Thesis. CSIR-IMTECH, Chandigarh/ Jawaharlal Nehru University, New Delhi: India.
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SUMMARY OF THE THESIS

The role of costimulatory molecules is very well established in the activation of T cells but nothing is very well documented in the case of antigen presenting cells (APCs) like dendritic cells (DCs), macrophages and B cells (Damle et. al. 1992, Suvas et. al. 2002, Orabona et. al. 2004). T cell activation is dependent upon signals delivered through the antigen specific T cell receptor (TcR) and costimulatory molecules. The best-defined costimulatory molecules to date are two structurally related proteins, CD80 (B7-1) and CD86 (B7-2) (Gordon et. al. 1996, Goldstein et al. 1996, Nakajima et al. 1998, Bergamo et. al. 1997). It has been suggested that CD86 participates in initiating immune response, whereas CD80 may be more important in sustaining or regulating immune responses. The upregulation of CTLA-4 on activated T cells parallels the kinetics of CD80 expression. Together with the higher avidities of CD80 ligands for CTLA-4 than CD28, has raised the possibility that CD80/CTLA-4 interactions predominate late to terminate immune responses (Turka et. al. 1992, Finck et. al. 1994, Schweitzer et. al. 1997). Although, both CD80 and CD86 play a major role in providing costimulation to T cells by binding to CD28, leading to their proliferation, cytokine production, and development of effector functions (Lenschow et. al. 1996). The binding to CTLA-4 provides inhibitory signals required for down regulation of the immune response (Greenwald et. al. 2001). CD80 and CD86 could also serve as counter-receptors that transduce distinct signal to the APCs upon engagement by CD28 or CTLA-4. The intracellular domains of CD80 and CD86 are quite distinct and could mediate differential signal transduction. Such signaling could alter the APCs ability to function as effector cells. The ability of APCs to deliver the costimulatory signal to T cells by CD80 and CD86 molecules is very well established (Shahinian et. al. 1993, Ronchese et. al. 1994, Snapper et. al. 1987). In contrast, whether the engagement of CD80 and CD86 molecules by CD28 and CTLA-4 also induces bi-directional costimulation that can affect the function of APCs is very poorly documented (Freeman et. al. 1995, Sethna et. al. 1994). Moreover, nothing is known precisely in the case of APCs proliferation, activation and differentiation by the engagement of CD80 and CD86 molecules. However, there is indirect evidence reported earlier that CD28-CTLA4/CD80-CD86 signaling pathways may affect B cell responses and the regulation of

immunoglobulin (Ig) synthesis (Ikemizu et. al. 2000, Bajorath et. al. 1994, Heath et. al. 1993). Recently, for the first time we demonstrated that costimulation through CD80 specifically inhibits the proliferation and IgG secretion by B cells and B cell lymphoma by up-regulating the expression of pro-apoptotic molecules caspase-3, caspase-8, Fas, FasL, Bak, and Bax and down-regulating the levels of anti-apoptotic molecule Bcl-x (L) (Suvas et. al. 2002). In contrast, costimulation through CD86 augmented the level of anti-apoptotic molecules Bcl-w and Bcl-x (L) and decreased the levels of caspase-8. Thus, the costimulatory signals not only influence the activation of T cells but by bidirectional costimulation they can also affect the activity of B cells.

After our report (Suvas et. al. 2002), many groups world wide have endorsed the concept of bidirectional costimulation. Recently, Schmidt, et. al., has shown that targeting death receptor-6 (DR-6) on B cells can enhance B cell expansion, survival and humoral responses. Consistent with this, increased nuclear levels and activity of nuclear factor kappa-B (NF-κB) transcription factor, c-Rel, and elevated Bcl-XL expression were observed (Schmidt et. al. 2003). Nguyen, et al., have shown that cross-linking B7-DC with the antibody directly potentates dendritic cell function by enhancing antigen uptake, DC presentation of major histocompatibility complex–peptide complexes, promoting DC survival and increasing secretion of interleukin (IL)-12p70 (Nguyen et. al. 2002). Radhakrishnan, et al., have demonstrated that B7-DC cross-linking restores antigen uptake and augments antigen presenting cell function by matured dendritic cells (DCs). Lumsden, et al., have shown that CD80/86 and CD40 are required on B cells for T-dependent antibody responses (Lumsden et. al. 2003). Mukherje, et al., have shown that CD80 and CD86 expression on mouse $CD4^+$ T lymphocytes enhance their cell-cycle progression and survival (Mukherje et. al. 2002). Grohmann, et al., have shown that upon recognition of CTLA-4 on T cells by B7 expressed on APCs, both the cells could become activated, leading to changes in the functional state not only of T cell but also of the APCs (Grohman et. al. 2002).

Recent reports indicate that CD86 stimulation on a B cell activates the classical NF-κB pathway, leading to a protein kinase C $(PKC\text{-}3)^3$–independent phosphorylation and degradation of IκBα and subsequent nuclear localization of

P_{50}/P_{65}. CD86 is also reported to increase the phosphorylation of P_{65} in a PKC dependent manner (Yamamoto et. al. 2004). It has been reported that cross-linking CD40 on B cells rapidly activates NF-κB (Podojil et. al. 2004). Many studies have shown the significance of CD80 and CD86 in influencing the activity of B cells, stem cells, DCs but nothing is known about the role of these molecules in case of macrophages.

Macrophages are one of the potent APCs and also a host for a number of intracellular pathogens especially of *M. tuberculosis*. Hence, to understand the activation of macrophages in context with costimulation needs to be evaluated. Cell surface interactions between T cell costimulatory receptors CD28/CTLA-4 and CD40 with their cognate ligands CD80/CD86 and CD40L respectively on macrophages involve a potentially complex network of signals. One aspect of this complexity that is not well understood is the directionality, and possible bidirectional signaling through specific "receptor-ligand" interactions. This question is particularly relevant in the case of two highly important sets of molecular interactions (CD80/CD86 on macrophages and CD28/CTLA-4 on T cell) that can function in T cell-macrophage communication. The ability of macrophages to deliver the costimulatory signal to T cells by CD80/CD86/CD40 molecules is very well established. In contrast, whether the engagement of CD80/CD86 and CD40 molecules by CD28/CTLA-4 and CD40L respectively also induces bi-directional costimulation that can affect the function of macrophages is not well understood. Moreover, nothing is known precisely in the activation and differentiation of macrophages by the engagement of CD80, CD86 and CD40 molecules.

In the present study, we have attempted to analyze distinct role of CD80, CD86, CD40 costimulatory molecules in the activation of macrophages and their influence on the survival of intracellular pathogens. The following five major findings have emerged from this study. **1)** Costimulation through CD80, CD86 and CD40 augmented the release of NO, IL-1, IL-6 and TNF-α; **2)** CD80, CD86 and CD40 worked synergistically with IFN-γ in the production of NO, IL-1, IL-6, TNF-α and IL-12; **3)** CD80 and CD86 modulated the expression of CD14, CD206, CD80, CD86, MHC-I and MHC-II molecules; **4)** triggering of CD86 and CD40 but not

CD80 increased the uptake of soluble and particulate antigens; **5)** engagement of mainly CD80 reduced the survival of intracellular pathogens.

Costimulatory molecules differ in their expression pattern on APCs and the relative expression of CD80 and CD86 is critical in determining the nature and extent of the immune response (Lenschow et. al. 1995, Baskar et. al. 1995, Corry et. al. 1994). Whereas DCs constitutively express both costimulatory molecules, macrophages display CD86 only and resting B cells express low levels of CD86 (Azuma et. al. 1993). In the present study, we observed that thioglycollate elicited peritoneal macrophages differentially expressed CD80, CD86 and CD40. We first did experiment to see if signaling through these molecules is influencing the viability of macrophages. Interestingly, no change was noticed. Similar results have been reported in case of B cells (Suvas et. al. 2002).

Macrophage interactions with specific phagocytic or endocytic ligands are influenced markedly by cytokines and non phagocytic adhesive interactions with the extracellular matrix which promote complex changes in patterns of macrophage gene transcription and protein expression. The net outcome of these interactions can be a full-blown activation phenotype of macrophages. Similarly, complex interactions with other plasma membrane ligands/molecules may result in the activation of macrophages represented by the respiratory burst or proinflamatory cytokine production (Gordon et. al. 2003). Production of NO by macrophages determines their activation status and we know that macrophages can be activated to secrete NO by IFN-γ (Ehrt et. al. 2001, Goerdt et. al. 1999) or in response to bacteria and bacterial products (Boldrick et. al. 2002, Nau et. al. 2002). Therefore, in present study we next monitored the role of CD80, CD86 and CD40 in eliciting the production of NO. It is of interest to mention that CD80, CD86 and CD40 costimulation enhanced the secretion of NO. Indicating that engagement of costimulatory molecules can activate macrophages and thereby leading to secretion of NO. We also noticed that signaling through CD80, CD86 and CD40 synergy with IFN-γ and further amplified the secretion of NO. Although it is known that, macrophages on stimulation with LPS or IFN-γ produce copious amounts of NO (Snyder et. al. 1992) but this is the first report

that signaling through CD80, CD86 and CD40 can also induce its secretion. Further, it also indicates that there may be operating many alternative pathways for release of NO and this may not solely depend on LPS and IFN-γ. Such synergistic concept is consistent with *in vivo* studies showing the dependence on both microbial signals and endogenous CD40L to induce NO secretion (Schulz O et. al. 2000). Hence it can be concluded that in addition to innate, classical (IFN-γ) and alternate (IL-4/IL-13) activation pathways macrophages can also be activated through costimulation **(Fig. 1d)**.

Proinflammatory cytokines and other inducible effector responses are known to be regulated mainly by macrophages. These cytokines not only regulate macrophages but also other cells of the immune system (Gately et. al. 1998, Trinchieri et. al. 1995, Ma et. al. 2001, Snijders et. al. 1998). How costimulatory molecules regulate these Proinflammatory molecules is unknown. The activated macrophage plays an important role in the initiation and subsequent amplification of a variety of immune responses (Snick et. al. 1990, Kishimoto et. al. 1992). Thus, a better understanding, how to manipulate the macrophage-derived Proinflammatory cytokines production would be beneficial in cases where modulation of immune response was needed. It is a very well established phenomenon that either IFN-γ secreted by T cells or triggering through Toll-Like Receptors (TLRs) is responsible for the activation of macrophages and thereby leading to release of proinflammatory cytokines (Gordon 2003). Interestingly, we observed that engagement of CD80, CD86 and CD40 molecules can also significantly elicit macrophages to produce Proinflammatory cytokines like IL-1, IL-6, TNF-α and IL-12. This observation concretely indicates that macrophages also secret proinflammatory cytokines through bidirectional signaling on engagement of costimulatory molecules. Hence this may not be a mere function of molecules involved in innate immunity or cytokines released by T cells. The production of Proinflammatory cytokines by macrophages is usually noticed as a result of inflammation. However, they are also secreted in response to pathogens and their products. Following bacterial (by LPS) or immunoglobulin (by Abs) ligation of CD14 (LPS receptor), (Nockher et. al. 1997) or CD64 (IgG receptor) (van de Winkel et. al. 1993) on macrophage, these cytokines can be released in the *milieu* where they influence a number of cell types. In this study, we also observed that

IFN-γ, which is the classical activator of macrophages, shows synergisy with CD80, CD86 and CD40 signaling. This indicates that there exist multiple pathways in stimulating macrophages to release Proinflammatory cytokines. Macrophages display diverse sets of receptors on their surface. Although, many of these receptors tie into common signaling pathways, each has its own distinctive influence on the elicited intracellular responses. Therefore, it is not surprising that synergy exists in the activation of macrophages between various molecules expressed on its surface. Studies reported in past have demonstrated synergy in TLR-7 and CD40 signaling (Ahonen et. al. 2004). Further, simultaneous activation of TLR-3 (by double-stranded RNA) and TLR-9 (by CpG DNA) induced NO, IL-12, TNF-α and IL-6 production by murine macrophages (Whitmore et. al. 2004).

Since towering augmentation in the yield of proinflammatory cytokines and NO was noted, hence it was of concern that this may alter the viability of macrophages, consequently may have impact on their function. Importantly, engagement of CD80, CD86 and CD40 could not cast any impact on the viability of macrophages. Thus it was safe to intrepidly conduct experiments in response to signals delivered through CD80, CD86 and CD40.

Phagocytosis is a basic biological function of macrophages. Recently, it has been published that CD86 knock out ($CD86^{-/-}$) mice displayed a defect in antigen uptake and diminished mannose receptor expression (Yadav et. al. 2007). Moreover, signaling through CD40 has also been shown to restore the ability of mature DCs to acquire exogenous antigen (Radhakrishnan et. al 2005). Hence we also thought whether CD80 and CD86 might also be involved in influencing the antigen uptake by macrophages. Intriguingly, signaling through CD86 and CD40 significantly enhanced the uptake of both soluble (ovalbumin) as well as that of particulate (*M. tuberculosis, M. microti, E. coli, etc*) antigens by macrophages. The recent recognition that engagement of CD40 can synergize with other DC-activating signals to enhance cellular functions (Ahonen et. al. 2004) and mDCs restores the ability to acquire exogenous antigen (Radhakrishnan et. al 2005) supports our observation. Further, the fact that engagement of CD40 or B7-ĐC or CD86 with Abs can induce similar effects, suggest overlap in the downstream

signaling pathways. Although anti-CD80 Ab treatment, activated the macrophages and enhanced the production of NO and cytokines in contrast, it failed to regulate the antigen uptake by macrophages. Thereby it signifies that only CD86 but not CD80 is involved in the antigen engulfment by macrophages. It is likely that CD80 may be inducing different signals in terms of NO induction and antigen uptake in macrophages. Moreover, downstream signaling pathways for NO (iNOS) induction and antigen uptake (PKC-ε) are quite distinct (Ghosh et. al. 2008, Cheeseman et al. 2006, Rosenberger et. al. 2003, Larsen et. al. 2002, Manji et. al. 2002).

We know that macrophages use a wide range of unique receptors to acquire exogenous antigens, which include both endocytic and phagocytic receptors like mannose (CD206), CD14 (LPS receptor), complement and Fc (CD16/32) (Schlesinger et. al. 1990, Ernst et. al. 1998). Mannose receptor recognizes a wide range of gram positive and gram negative bacteria, yeasts, parasites, etc., (Zamze et. al. 2002, Stahl et al. 1998, Ernst et. al. 1998). The expression of mannose receptor on monocytes can be enhanced by cytokines like IFN-γ. Therefore, we realized that difference in the uptake of antigen by CD80 and CD86 signaling may be having some relation in the expression of these receptors. Consequently, we checked the influence of costimulation through CD80, CD86 and CD40 on the expression of CD14 (LPS receptor) and CD206 (mannose receptor) on the surface of macrophages. Our results demonstrated that signaling delivered through CD86 and CD40 upregulated the display of both CD206 and CD14 molecules. It has been shown that CD86 deficiency affects the phenotypic and phagocytic characteristics of APCs. $CD86^{-/-}$ mice displayed a defect in antigen uptake and diminished expression of mannose receptors (Yadav et. al. 2007). As we have already observed that signaling through CD86 and CD40 enhanced the uptake of bacteria like *M. tuberculosis, M. microti* and *E. coli,* so the increase in the expression of these receptors on the surface of macrophages by CD86/CD40 signaling can be correlated with the augmented engulfment of antigen/bacteria by macrophages. Several reports in literature suggest a correlation between PKC-ε levels in APCs with their phagocytic ability (Larsen et. al. 2002, Cheeseman et. al. 2006). Membrane diacylglycerol, which is responsible for PKC membrane translocation and phagocytic prime is reduced on CD86 deficiency and signaling

through CD86, has been shown to upregulate this membrane diacylglycerol in B cells (Kin et. al. 2006). Hence, signaling through CD86 may play an important role in PKC homeostasis and thereby phagocytosis by macrophages.

The expression of MHC molecules can also be very well correlated with antigen presentation by APCs. MHC-I and MHC-II molecules participate in the activation of CD8 and CD4 T cells, respectively, leading to adaptive immune responses. Processing of exogenous antigens such as internalized bacteria, typically generate peptides that bind MHC-II molecules. In contrast, endogenouly processed antigen are displayed in context with MHC-I. However, bacteria as well as other exogenous antigens can also be processed for peptide presentation on MHC-I molecules (Wick et. al. 1999). Infection with flaviviruses has been shown to upregulate the cell surface expression of MHC-I and MHC-II molecules (Abraham et. al. 2006). Moreover, the role of macrophages in stimulating naive T cells to initiate an immune response *in vivo* is unclear. This may be due to insufficient surface expression of MHC and costimulatory molecules needed to trigger naive T cells. Presentation of antigens from intracellular bacteria on both MHC-I and MHC-II is important in generating effective immunity to these pathogens. MHC class II molecules (MHC-II) are crucial for macrophage function, and it has been shown that M-CSF induces a rapid redistribution of MHC-II from the MHC-II containing compartments to the plasma membrane. Signaling through LPS or TNF-α causes an increase in MHC-II synthesis and surface distribution (Baron et. al. 2001). Further phenotypically, CD86 deficiency led to the prevalence of a nonimmunogenic APC repertoire because PKC-ε represents a component of CD86 mediated signaling inside APCs (Yadav et. al. 2007). Based on the results of the present study, it may be conjectured that enhanced phagocytosis of ovalbumin and bacteria on engagement of CD86 and CD40 macrophages may be correlated with the upregulated expression of CD14, CD206, MHC-I and MHC-II. Together these data provide strong evidence that CD86 and CD40 not only activates T cells but also delivers bidirectional signals into macrophages and can therefore directly modulate the release of cytokines, NO, antigen uptake, expression of CD14, CD206, MHC-I and MHC-II. It may be mentioned here that the purity of the macrophages used in this study was more

that 96%, hence the chances of cytokines released by contaminating T cells in contributing the activation of macrophages are quite aloof.

Another interesting observation of our study is that though the infection with bacteria reduced the viability of macrophages, but the delivery of costimulatory signals enhanced their survival. Recently, it has been reported that CD86 plays a role in the survival of DCs because enhanced apoptosis of DCs was seen under CD86 deficiency (Yadav et. al. 2007). Further, signaling through B7-DC, a costimulatory molecule expressed on the surface of DC promoted the survival of DC (Nguyen et. al. 2002). Interestingly, CD86 engagement activates the NF-κB pathway, which plays an important role in DC survival and reduced NF-κB activity has been reported in $CD86^{-/-}$ mice (Orabona et al. 2004, Kriehuber et al. 2005). Our data also suggest that CD86/CD40 signaling plays a critical role in promoting the survival of macrophages; the results are in concurrence with the previous studies in DCs (Nguyen et. al. 2002, Orabona et. al. 2004, Kriehuber et. al. 2005).

It is well recognized fact that cognate signals and certain cytokines can induce and up-regulate the expression of CD86 on APC. Signals triggered within monocytes by intracellular organisms, factors released by them, and interactions between the intact pathogen and molecules on the surface of macrophages may be involved in the process of regulation of CD86 expression. It has been shown that CD86 deficiency causes a reduction in survival, maturity and prevalence of a nonimmunogenic APC repertoire indicating that CD86 is delivering activating signals (Yadav et. al. 2007). Since we also observed that CD86 is encouraging macrophages for antigen uptake and cell survival, hence we checked the expression of CD86 on the infected macrophages. Even though, bacterial infection downregulated the expression of CD86 on the surface of infected macrophages, it is of interest that triggering through CD86 augmented the expression of both CD80 and CD86. Certain intracellular organisms, such as *M. tuberculosis, M. leprae* and *L. donovani* either fail to up-regulate or actually decrease the expression of costimulatory molecules (Kaye et. al. 1994, Agrewala et. al. 1998, Saha et. al. 1994, Bonato et. al. 2001). CD86 is also downregulated on cell lines RAW264.7 and J774A.1 infected with murine cytomegalovirus (Loewendorf et.

al. 2004). Infection of macrophages with *T. cruzi* and DCs also causes downregulation of CD40 and CD86 co-stimulatory molecules (Planelles et. al. 2003). This may represent strategies used by the pathogens to avoid recognition, induce anergy, or cause immunosuppression. Thus, intracellular organisms can have a major influence on antimicrobial immunity through modulating the expression of costimulation. Further, signaling through CD86 may have an important implication for the efforts to establish a vaccine against intracellular pathogens, since it is suggested that vectors that induce/up-regulate the expression of costimulatory molecules, especially CD86 on APC will help to generate a protective immune response (Subauste et. al. 1998).

Nitric oxide production by phagocytic cells has been identified as a major pathway of microbial elimination (Nathan et. al. 1994). Nitric oxide and its derivatives are considered to be the major players in the killing of intracellular pathogens. It is well established that NO is an important bioactive molecule exerting its biological functions like host defense, tumor cytotoxicity, etc., in diverse ways in many cell types without mutual interference (Barnes. et. al. 1995, Snyder et. al. 1992). In addition to paracrine and juxtacrine effects, macrophage NO also has an autocrine effect. Induction of NO is critically important for macrophage mediated microbicidal activity. IFN-γ is a major inducer of NO release and antimicrobial function (Nathan et. al. 2000). Our results showed that CD80, CD86 and CD40 signaling alone, as well as in synergy with LPS and IFN-γ enhanced the production of NO by macrophages. Therefore we became curious to monitor the impact of costimulation on the production of NO by macrophages infected with *M. microti* and *M. tuberculosis.* This further establishes that on T cell and APCs interaction, costimulation not only activates T cells but by two-way signaling it can also evoke series of events necessary for the activation of APCs as well. Further, signals delivered through CD80, CD86 and CD40 are powerful enough to overcome the impingement of intracellular pathogens in suppressing the activation of macrophages.

We have very categorically demonstrated that signaling through CD80, CD86 and CD40 significantly elevated the yield of TNF-α, IL-1 and IL-6 by macrophages infected with *M. tuberculosis* or *M. microti*. Macrophages not only control

intracellular infection by getting stimulated by IFN-γ and other established pathways, but also by signaling delivered through costimulatory molecules. To best of our knowledge, this is the first report indicating the significance of costimulatory molecules CD80, CD86 and CD40 in eliciting the release of proinflammatory cytokines by infected macrophages. Proinflammatory cytokines like IL-1, IL-6 and TNF-α, secreted chiefly by macrophages play fundamental role in the stimulation of macrophages, regulation of the immune response and protection against mycobacterial infections. TNF-α has long been regarded as a protective cytokine involved in antimicrobial immunity. Mice defective in TNF-α production or lacking TNF-α signaling quickly succumb to *mycobacterial* infection. The control of infection depends on TNF-α, as mice treated with anti-TNF-α antibodies succumb to infection. Thereby indicating that TNF-α is critical and non-redundant in controlling the *mycobacterial* infection (Jacobs et. al. 2007). IL-1 is responsible for the generation of early-phase protective immunity against *M. tuberculosis* and macrophages from IL-$1^{-/-}$ mice show reduced production of NO (Yamada et. al. 2000). Further, IL-6 has a number of important functions including augmentation of B lymphocyte differentiation, stimulation of hematopoiesis, and amplification of inflammatory responses (Kishimoto et. al. 1992). IL-6 deficient mice rapidly die due to *L. monocytogenes* infection and lethal tuberculosis was observed in IL-6 deficient mutant mice (Dalrymple et. al. 1995, Ladel et. al. 1997).

Macrophages are the host for a number of pathogens especially the deadly pathogen *M. tuberculosis,* which infects one third of the world population and every year causes about 8-10 million deaths (WHO bulletin 2007). We observed that costimulation through CD80, CD86 and CD40 can enhance the release of NO and proinflammatory cytokines like IL-1, IL-6 and TNF-α by infected macrophages. These parameters are favorable in controlling the intracellular infection. Therefore, finally we examined whether infected macrophages could be induced to kill intracellular pathogens when stimulated with CD80, CD86 or CD40. Intriguingly, macrophages activated through CD80, CD86 and CD40 showed enhanced microbicidal function and reduced survival of *M. tuberculosis* H37Rv. Similar results were reproduced with *M. microti* and *S. typhimurium* (data not shown). It may be conjectured here that microbicidal activity demonstrated

may be a cumulative effect of augmented release of NO and IL-1, IL-6 and TNF-α by the infected macrophages on engagement of CD80, CD86 and CD40. It is worth to mention here that maximum inhibition in the growth of *M. tuberculosis* and *M. microti* was observed when triggering was done *via* CD80, as evidenced by lesser number of CFU. CD86 and CD40 were comparatively less effective. In contrast, CD86 and CD40 induced better phagocytosis of bacteria as compared to CD80. Consequently, reduced frequency of CFU in the infected macrophages can be explained due to the lesser number of bacteria engulfed on CD80 signaling. To best of our knowledge, this is the first report indicating the role of costimulation in restricting the growth of intracellular infections in macrophage.

In last decade, sharp rise in the TB cases have been reported mainly due to i) failure of BCG as a vaccine, ii) appearance of AIDS, iii) emergence of multi-drug resistant strains of *M. tuberculosis*. Hence, there is an urgent need and challenge for scientific community to develop alternative strategies to defeat the problems linked to the reemergence of TB. The plan of action mentioned in the current study is novel and may overcome the above mentioned problems responsible for rise in TB cases, since this a novel strategy of modulating the immune system and thereby may curtail the growth of intracellular bacteria like *M. tuberculosis*. Further, this method will have no fear of emergence of resistance in the bacteria associated with drug treatment. In past, we have very elegantly demonstrated the role of CD80 in inhibiting the growth of B cell lymphomas by upregulating the expression of pro-apoptotic molecules and down regulating the display of anti-apoptotic molecules (Suvas et. al. 2002). Based on the study of Suvas et al., Biogene has developed a strategy to treat patients suffering from relapsed and refractory follicular lymphomas (Czuczman et. al. 2005, Leonard et. al. 2007). Therefore, results based on the current study also indicate potential implication in treatment of TB patients. Further, this strategy can be successfully employed even for patients suffering from drug resistant TB. Furthermore, this approach may not be limited to *M. tuberculosis* but also to other intracellular infections including AIDS, leishmania, malaria, etc. This approach can be tried as an alternate approach or in association with drug therapy for the treatment of TB.

Finally, major conclusions drawn from the current study are that signals delivered through costimulatory molecules: i) not only responsible for optimum activation of T cells but through bidirectional signaling can also influence macrophages; ii) can modulate the secretion of proinflammatory molecules; iii) can regress the growth of intracellular pathogens like *M. tuberculosis, M. microti, etc.;* iv) this novel strategy can be effectively exploited to develop immuno-therapy either using humanized antibodies against CD80, CD86 and CD40 or CD28 fusogenic proteins for the treatment of intracellular pathogens like *M. tuberculosis, HIV, L. donovani, T. cruzi,* etc.; v) since this approach is based on modulating the immune system of the hosts rather than targeting the pathogen; hence it significantly diminishes chance of emergence of drug resistant strains of bacteria.