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Influence of CD80 and CD86 Co-Stimulation in the Modulation of the Activation of Antigen Presenting Cells

Manzoor A. Mir and Javed N. Agrewala*

Institute of Microbial Technology, Chandigarh-160036, India

Abstract: The role of CD80 and CD86 costimulatory molecules is well established in the activation of T cells but not antigen presenting cells. Recently, many reports in literature have demonstrated categorically the influence of CD80 and CD86 in the activation of B cells and dendritic cells. Stimulation via CD80/CD86 in B cells can modulate their proliferation, IgG secretion and expression of pro-apoptotic and anti-apoptotic molecules, nuclear localization of NF-κB p50 subunit, phosphorylation of Rel A (p65) and IκB-alpha and increased oct-2 expression. In case of dendritic cells, it has been shown that signals induced via CD80 and CD86 enhance the production of IL-6 and IFN-γ which in turn, up-regulates the expression of the enzyme indolamine 2, 3-dioxygenase that results in tryptophan catabolism and affects T cell proliferation. Interestingly, it has been shown that co-stimulation through CD80 can restrict the survival of lymphomas and can also induce apoptosis in neural stem cells. Consequently, it may be concluded that CD28/CD152-CD80/86 interaction delivers a bi-directional co-stimulation, thereby not only having impact on the function of T cell, but also antigen presenting cells.

Keywords: Co-stimulation, bi-directional signaling, B cells, dendritic cells, CD80, CD86, CD40.

T CELL CO-STIMULATION

T cells are dependent on antigen presenting cells (APCs) for at least two distinct stimuli for their optimum activation and effector function. The first signal is provided by the engagement of clonotypic T cell receptor (TCR) with MHC-peptide complex on APCs. The second signal is non-MHC restricted and delivered by co-stimulatory molecules (Fig. 1) [1-4]. The significance of co-stimulatory molecules in T cell activation gained considerable impetus following the observation that occupancy of TCR alone is generally inadequate for exerting complete T cell activation. Thus an encounter with an antigen can lead to two quite distinct outcomes in T cells: proliferation and differentiation into effector cells; or inactivation or death. Which outcome occurs is determined by the appropriate delivery of co-stimulatory signals.

Till date, many co-stimulatory molecules viz. CD28, CD152 (CTLA-4), CD80, CD86, CD40, B7-DC, B7-H1, etc., are known to be expressed on the surface of T cells and APCs. But the best defined co-stimulatory molecules associated with APCs are two structurally related proteins, CD80 and CD86, both of which have been well documented in providing critical co-stimulatory signals to T cells by interacting with their specific receptors CD28/CD152 [5-9]. Interaction of CD28, which is constitutively expressed on T cells, provides a “positive” signal that results in optimal T-cell activation, cytokines production, clonal expansion, and prevention of anergy. In addition, CD28 signaling appears to be an important event in the prevention of cell death and promotion of the cell survival, presumably by up-regulation of anti-apoptotic Bcl-x(L) genes [10-15]. A deliberate interruption of the CD28-CD80/CD86 interaction has been shown to inhibit immune responses both in vivo and in vitro and results in T cell tolerance [3-9, 16]. After activation, T cells express CD152 which is homologous to CD28. CD152 functions to provide a “negative” signal to the T cell that inhibits cytokines production and arrests cell cycle progression thus limiting immune responses [17-19]. The role of
CD80 and CD86 is clearly established in the case of T cell co-stimulation but little is known about the co-stimulation on APC.

CAN CD80 AND CD86 DELIVER BI-DIRECTIONAL CO-STIMULATION?

Despite the fact that both CD80 and CD86 play a major role in providing co-stimulation to T cells, these molecules can also serve as counter-receptors that transduce signals to APCs upon engagement with CD28/CD152 (Fig. 1). The intracellular domains of CD80 and CD86 are quite distinct and could mediate differential signal transduction. Such signaling could influence the ability of APCs to function as effector cells [20-22]. However, whether the engagement of CD80 and CD86 molecules with CD28 and CD152 affect the function of the APC was poorly documented, till recently [23, 24]. Although there were indirect evidences suggesting that CD28-CD152/B7-signaling pathways may affect B cell responses and immunoglobulin synthesis [22-24]. Studies done with CD80−/− and CD86−/− mice do indicate the role of CD80 and CD86 in delivering bi-directional co-stimulation. The mice lacking CD80 and CD86 were found to be profoundly deficient in their ability to generate in vivo germinal center formation, Ig class switching, memory formation and affinity maturation through somatic hypermutation [23-26]. Thus, a role of CD80 and CD86 in the activation of B cells is plausible and need to be systematically addressed.

ROLE OF CD80 AND CD86 IN THE CO-STIMULATION OF B CELLS

Recently, we have for the first time investigated the impact of CD80 and CD86 on B cells [27]. Signaling in lipopolysaccharide stimulated B cells was delivered through CD80 and CD86 molecules by their respective antibodies (Abs). Exciting features observed during the study were that cross-linking of CD86 enhanced the proliferation and production of IgG1 and IgG2a antibodies. In contrast, anti-CD80 Ab could decline the proliferation and production of IgG1 and IgG2a antibodies. Importantly, anti-CD80 Ab could also retard the growth of B cells and up-regulated the expression of pro-apoptotic molecules (Fig. 2A,B). The involvement of Fas and FasL expression in B cells in inducing apoptosis was demonstrated by flow cytometry. In addition, the association of CD80 and CD86 molecules in the regulation of the activation of pro- and anti-apoptotic molecules (Fas, Fasl, FADD, FAP, FAF, TRAIL [FasL2L], TNFR [p55], TRADD, RIP, Bcl-w, Bfl-1, Bcl-x(L), Bak, Bax, Bcl-2, Bad, Caspase-3, Caspase-8) was examined by ribonuclease protection assay (RPA) in B cells [27]. We observed that signaling through CD80 molecule mainly augmented the levels of pro-apoptotic molecules, i.e. caspase-3, caspase-8, Fas, Fasl, Bak, and Bax and down-regulated the expression of Bcl-x(L) and Bfl-1 (Figs. 2,3). This suggests that CD80 signaling induces apoptosis via mechanism involving pro-apoptotic molecules rendering the cells more vulnerable to apoptosis and therefore restricting their proliferation. In contrast, signaling through CD86 increased the expression of anti-apoptotic molecules Bcl-w and Bcl-x(L) and down-regulated the expression of caspase-8 (Figs. 2,3). Thus, there may be a possibility that ligation of CD86 on B cells may promote their survival by increasing the expression of anti-apoptotic proteins. It is worth to mention here that we demonstrated the role of CD80 and CD86 not only in case of B cells isolated from the mice splenocytes but also for B cell lymphomas [27]. Since B cell lymphomas (WEHI-279 and A20) are 100% B cells, therefore rules out any involvement of cytokines secreted by the contaminating T cells in activating B cells. Thus, this finding accurately establishes that CD80 and CD86 not only costimulate T cells on ligation with CD28/CD152 but can also influence B cells through bi-directional co-stimulation, consequently not only modulating the activity of T cells but also of B cells (Fig. 1). After our report in 2002 [27], many evidences in literature started accumulating which demonstrate the role of CD80 and CD86 in bi-directional signaling of APCs [28-36, 46, 47, 55, 56]. Recently, in vitro studies showed that CD86 cross-linking on CD40 ligand and IL-4 activated B cell increases the rate and level of IgG1 expression, nuclear localization of NF-κB p50 subunit, phosphorylation of Rel A (p65) and IkB-alpha. It also increased oct-2 expression and binding to the 3'Ig H enhancer. These effects do not occur in CD86−/− B cells [33-36]. These results further support our findings that signaling through co-stimulatory molecules can also stimulate B cells.

It has also been demonstrated that increased expression of Bcl-x(L) but not Bcl-2 can prevent the apoptosis in B cell lymphoma. Moreover, signaling through CD40 up-regulated Bcl-x(L) and Bfl-1 and protected B cell lymphoma from apoptosis [37, 38]. The T cell co-stimulatory molecule CD28, which has a high and low binding affinity for CD86 and CD80 respectively, promotes T cell survival by up-regulating Bcl-x(L) and down-regulating FasL expression [39, 40]. Further, over expression of caspases is sufficient to cause apoptosis and Bax has been correlated with disease regression and shorter survival of B cell in chronic lymphocytic leukemia [41-45]. Importantly, co-stimulatory molecule mediated delivery of inhibitory signals may not only be responsible in the regulation of immune responses and maintaining self-tolerance but also for apoptosis of CD80-bearing tumor cells, thus indicating that ligation of CD80 by anti-CD80 Ab delivers effective signals for anti-tumor immunity [27-30]. Interestingly, recent studies have shown that anti-CD80 Ab therapy regresses the growth of lymphomas [46, 47].

Targeting CD80 bearing lymphomas with anti-CD80 can arrest their growth [27, 46, 47]. CD80 is transiently expressed on the surface of activated B cells and APCs including dendritic cells, but is constitutively expressed on a variety of non-Hodgkin’s lymphomas (NHLs), including follicular lymphoma. Thus, CD80 is an attractive target for lymphoma therapy [27, 46, 47]. Galiximab is a monoclonal antibody that binds and targets CD80 molecule. Preclinical studies indicate that targeting CD80 with this antibody has anti-tumor effects. In vitro, cross-linking of CD80 with anti-CD80 antibodies on lymphoma cells has been shown to inhibit cell proliferation, up-regulate pro-apoptotic molecules, and induce apoptosis [21, 27]. In vivo, anti-CD80 Ab also delays progression and prolongs survival in a human lymphoma xenograft/severe combined immunodeficiency (SCID) mouse model [48]. The clinical studies with anti-CD80 Ab therapy have shown promising results as well [46-48]. Phase I/II, single-agent, dose escalation, multiple-dose study demonstrated that galiximab administration can decrease tumor burden in nearly half of all patients [46, 47].
CD80 and CD86 Co-Stimulation

**Fig. (2).** Modulation of the expression of pro-apoptotic molecules by CD80 and CD86 signaling. Fig. 2A indicates the normal expression of pro-apoptotic molecules (red color symbols) on B cell lymphoma. Signaling through CD80 significantly up-regulate Fas, FasL, caspase-8, Bak, Bax, and FADD (green color symbols) and triggering through CD86 down-regulate caspase-8 (blue color symbols) (A). The WEHI-279 cells were cultured with anti-CD80 Ab, anti-CD86 Ab, and Rtlg and cross-linked with anti-Rtlg Ab. Total RNA was extracted from the cells by the guanidinium isothiocyanate-phenol-chloroform method using Trizol reagent. The expression was determined using the RiboQuant multiprobe RNase protection assay system. The figure depicts the summary of results of Suvas et al. [27]. The resulting resolved bands were imaged using a phosphor imager. The normalized quantity for each band was obtained by dividing with L32 housekeeping gene control. The fold change was calculated by dividing the value of normalized quantity of the experimental samples with that of cells cultured with medium alone and is expressed as mean ± S.D. obtained from two experiments (B). * indicates that p<0.001 employing students’ t test.

Therefore, clinical and experimental results successfully establish that signaling through CD80 can serve as a therapeutic intervention for treating cancer.

**ROLE OF BI-DIRECTIONAL CO-STIMULATION IN THE ACTIVATION OF DENDRITIC CELLS**

Several reports on the role of CD80 and CD86 have also been accumulating recently on dendritic cells [28-30]. Logue and Sha have indicated that CD28-B7 bi-directional signaling is a two-way street to activation of DC. It has been shown that CD28 induces immunostimulatory signals in DC via CD80 and CD86. Bi-directional signaling along the B7-CTLA-4 co-receptor pathway enables reciprocal stimulation of T cells and dendritic cells.

Binding of CD28 to CD80/CD86 ligands lead to enhanced production of IL-6 by dendritic cells [29, 48]. On the other hand, binding of CD152 to CD80/CD86 augments the secretion of IFN-γ, which in turn upregulates the expression of the enzyme indolamine, 2, 3-dioxygenase (IDO) in dendritic cells resulting in tryptophan catabolism and suppression of T cell proliferation [30, 49, 50]. Further, ligation of CD86 leads to anti-apoptotic signals [51].

It has been shown that CD86 is recruited to lipid rafts upon T cell-DC interaction. In this bi-directional interaction between CD28 on a T cell and CD86 on the DC, naive CD4+ T cells receive their co-stimulatory signal and the DCs appear to respond by reorganizing their CD86 to lipid rafts [52-54]. Although the functional significance of CD86 response remains to be determined, the fact that the ligation and recruitment of CD86 to lipid rafts is required for the appearance of phosphorylated serines in rafts supports the idea that this is a mutually beneficial interaction that modulates not only T cell, but also DC signaling. Cross-linking of B7-DC, a member of B7 family on DCs modulates the DC biology by increasing their survival, antigen presentation, IL-12p70 production and migration to lymph nodes [55-57]. These findings on DCs as well further confirm the interaction between CD80/CD86 and CD28/CD152 that delivers a bi-directional co-stimulation which activates both T cells and APCs.
Fig. (3). Modulation of the expression of anti-apoptotic molecules by CD80 and CD86 signaling. Fig. 3A indicates the normal expression of anti-apoptotic molecules (red color symbols) on B cell lymphoma. Signaling through CD80 significantly down-regulates Bcl-x(L) and Bfl-1 (green color symbols) and triggering through CD86 up-regulates Bcl-w and Bcl-x(L) (blue color symbols) (A). The WEHI-279 cells were cultured for 72h with anti-CD80 Ab, anti-CD86 Ab, and Rtlg and cross-linked with anti-Rtlg Ab. Total RNA was extracted from the cells by the guanidinium isothiocyanate-phenol-chloroform method using Trizol reagent. The expression was determined using the RiboQuant multi-probe RNase protection assay system. The figure depicts the summary of results of Suvas et al. [27]. The resulting resolved bands were imaged using a phosphor imager. The normalized quantity for each band was obtained by dividing with L32 housekeeping gene control. The fold change was calculated by dividing the value of normalized quantity of the experimental samples with that of cells cultured with medium alone and are expressed as mean ± S.D. obtained from two experiments (B). * indicates that p<0.001 employing students' t' test.

INFLUENCE OF CO-STIMULATION ON MACROPHAGES

We have investigated the impact of co-stimulation on macrophages harvested after 96h of thioglycolate injected in BALB/c mice. The cells were incubated with anti-FcRγII/III Ab to block Fc receptors and then added to wells coated with anti-CD86 Ab and antibody matched isotype controls. In control wells, macrophages were cultured with medium alone. After 2h, non-adherent cells were removed and the wells were supplemented with RPMI-1640-FCS 10%. The cells were cultured for 48h and then harvested and stained for the expression of CD80 and CD86 with FITC-labeled anti-CD80 Ab and PE-labeled anti-CD86 Ab. The expression was analyzed by FACS Calibur flow cytometer and analysis was done on CellQuest. As compared to control cultures, where macrophages were cultured in the absence of anti-CD86 Ab, signaling through CD86 molecule employing anti-CD86 Ab significantly (p<0.001) increased the levels of CD80 and CD86 on macrophages (Fig. 4). No significant change was observed in the case of cells cultured with isotype matched control. We also observed that signaling via CD86 substantially (p<0.001) augmented the release of nitric oxide (NO) in a dose dependent manner (Fig. 5) (Mir M.A., unpublished data). The increase in the levels of CD80 and CD86 on co-stimulation through CD86 indicates that interaction of T cell with APC will lead to optimum activation of T cells. Maintaining optimum expression of co-stimulatory molecules is responsible in preventing cell death, especially during primary T cell activation [15]. The enhanced secretion of NO by macrophages on co-stimulation by CD86 molecule will help in the eradication of intra-cellular infections. Augmented release of NO by macrophages is known to be beneficial in case of many intracellular infections [58]. These results further substantiate our earlier findings [27] and now endorsed by other groups as well that signals delivered through CD80 and CD86 can affect the function of APCs [26-36, 55, 56].
Role of Bi-Directional Co-Stimulation in the Activation of T Cells

There are also some reports in the literature documenting the role of CD80 and CD86 displayed on T cells. However, the functional significance of their expression is not very well documented. CD86 but not CD80 is constitutively expressed on some resting T cells [58-60]. T cells that constitutively over-express CD86 have dramatically reduced allo-responses and graft verses host disease (GVHD) mortality. Conversely, CD80+ and CD86+ T cells led to accelerated allo-responses compared with wild type T cells as measured by GVHD mortality. It has been indicated that CD80 and CD86 expression on T cell down-regulates allo-responses via T:T interaction with CD152. It has been shown that suppression is restored by expression of full length but not truncated mutants that lack transmembrane and cytoplasmic domain [61-63].

Why CD80 and CD86 Behave Distinctly?

Findings from our and other laboratories have indicated that CD80 and CD86 can deliver distinct signals to APCs [27-30]. CD80 and CD86 share only 23% sequence homology and are type I transmembrane glycoproteins belonging to Ig supergene family encoded by separate genes [5, 64-66].

The differences in the biological function of CD80 and CD86 are likely to be related to the nature of their cytoplasmic tails and/or to the structure and potential oligomerization state of their extra-cellular regions, which may represent an important mechanistic determinant for the assembly and functional properties of signal transduction in both T cells and APCs [64, 65, 67]. Because CD80 exists predominantly as a dimer, cross-linking it with antibodies could result in the formation of an extended, ordered array as is observed in the crystal structure of the CD80:CD152 complex. On the other hand, for CD86, which exists as a monomer, the antibodies may simply induce clustering in which two CD86 molecules are bridged by a single bivalent monoclonal antibody. This proposed behavior would result in an increased local concentration of dimeric CD80 but a uniform, random distribution of cross-linked CD86 monomers. The organization of CD80 and CD86 ligands upon binding bivalent antibodies is likely to have relevance to the physiological state when the ligands bind to their preferred receptors, which has been suggested by recent studies to be CD152 for CD80 and CD28 for CD86 [64, 65, 67]. Further, CD86 behaves identical to CD28 in delivering positive co-stimulation. As compared to CD28, CD152 molecule binds to CD80 with 20-fold higher avidity [56, 64, 65, 68]. It is also known that signaling through CD152 can function to suppress the production of cytokines produced by T-helper cells that helps in the differentiation of
and chronic lymphocytic leukemia, Hodgkin's disease, and multiple myeloma) are also under consideration [41, 42].

Thus providing a novel insight into the mechanism where triggering through CD86 restricts their growth by modulating the expression of anti-apoptotic molecules [27]. The property of CD80 in delivering inhibitory signals may also be related to its faster association and slower off-rate for CD152 binding, thereby providing ample time to deliver signaling events necessary for restraining B cells and other APCs from being activated (Fig. 6C,D) [70]. The kinetics of rapid and high level of expression of CD86 and CD28 in early immune responses and the delayed expression of CD80 and CD152 on APCs and T cells, can be viewed as necessary activation and inhibitory signals, delivered by these molecules respectively, during the immune response (Fig. 6) [71, 72].

**ROLE OF BI-DIRECTIONAL CO-STIMULATION IN DISEASES.**

Many reports on in vivo studies reveal distinct role of CD80 and CD86 in diseases. We have for the first time reported that signals delivered via CD80 into B cell lymphomas restrict their growth by modulating the expression of anti-apoptotic molecules [27]. Thus providing a novel insight into the mechanism where triggering through CD80 could control the growth of lymphomas. These observations constitute a rationale for development of a therapy using anti-CD80 Ab for cancer cells that express CD80 molecule. Interestingly, after our report, clinical studies evaluated anti-CD80 Ab as a targeted therapy for lymphomas with promising results [41, 42]. Future studies in other CD80-positive hematologic malignancies (e.g., diffuse large B cell NHL, chronic lymphocytic leukemia, Hodgkin's disease, and multiple myeloma) are also under consideration [41, 42]. CD80 and CD86 have been shown to have differential effects on the development of spontaneous autoimmune diabetes in non-obese diabetic (NOD) mice. Antibody neutralization of CD80 has been shown to exacerbate diabetes, whereas neutralization of CD86 was shown to abrogate the development of diabetes [73]. Differential effects of signaling by CD80 and CD86 have also been demonstrated in other autoimmune models, including lupus nephritis in the MRL-lpr/lpr mice, experimental autoimmune encephalomyelitis (EAE). The animals lacking CD86 showed diminished renal Ig deposition and attenuated pathology but CD80 deficient animals developed more severe nephritis [74, 75]. Recently, a novel regulatory role for CD80-mediated intracellular signals in CD4+ T cells has been shown to have important implications in the relapse of experimental autoimmune encephalomyelitis [76]. It has been established that in vivo blockade of CD86 co-stimulation could suppress maternal immune attack to the fetus [77]. Further, cross-linking of CD80 on the surface of neural stem cells in vitro enhances apoptosis [32]. Besides this, CD80 and CD86 have been implicated to have opposing roles in regulation of xenotransplantation rejection, where CD80 drives cell-mediated rejection (CMR) and attenuates acute vascular rejection (AVR) while CD86 drives AVR. Remarkably, indefinite xenograft survival can be achieved by suppressing AVR with CD86 neutralization in combination of CsA therapy, which inhibits CMR [78]. An important question arises in the studies using Abs, whether the influence of anti-CD80 and CD86 Abs arise due to obstructing the interaction of T cells with APCs or due to signaling delivered by Abs. It is evident from the recently published work that triggering through CD80 can vis-à-vis deliver signals as well [24, 27, 41, 42].

**APPLICATION OF REVERSE CO-STIMULATION**

Based on the pleotropic activities of the CD28/CD152-CD80/CD86 pathway mentioned above, it can be thus exploited for the potential clinical usefulness in immune intervention in diseases such as autoimmunity, transplant rejection, allergy and in the elimination of tumors that evade
Fig. (6). Interaction of CD80 and CD86 with CD28/CD152. The figures depicts the diagrammatic representation of interaction of CD80 (A, B) and CD86 (C, D) with CD28/CD152. On the basis of expression, binding affinities and crystal structure, CD80 shows better interaction with CD152, and thereby delivering bidirectional signaling, inhibiting the activity of not only T cells but also APCs. The interaction between CD80 on APC and CD152 on T cell begins at a later stage (after 48h) of activation due to their optimum expression by this time. The expression, and therefore interaction reaches maximum between 48h to 72h. However, it starts decreasing by 96h (A). The expression of CD28 reaches optimum level as early as 24h but the interaction between CD80 and CD28 begins only after 48h of activation and starts disassociation by 72h and looses interaction by 96h (B). The association of CD80 with CD152 is also considered better due its faster association and slower dissociation rates as compared to CD28 which shows slower association and faster dissociation kinetics. In contrast, CD86 shows better interaction with CD28, and therefore activating T cells as well as APC. The interaction between CD86 on APC and CD28 on T cell begins at early stage (after 12h) of activation due to their optimum expression. The expression, and therefore interaction reaches maximum between 48h to 72h. However, it starts decreasing by 96h (C). In contrast, expression of CD152 reaches optimum level by 48h and hence the interaction between CD86 and CD152 begins after 48h of activation and reaches maximum at 72h. By 96h it starts declining (D). The association of CD86 with CD28 is also considered better due to its faster association and slower dissociation rates as compared to CD152 which shows slower association and faster dissociation kinetics [64, 65, 72]. The shaded portion depicts strong association of CD152 with CD80 and CD28 with CD86.
immune surveillance [78-88]. Hence the interest lies in understanding the therapeutic manipulation of these co-stimulatory molecules. These molecules may provide a means to either promote immune responses against cancer or to reduce graft rejection and autoimmunity. CD80/CD86 signaling/blockade by CTLA4Ig has been studied in clinical trials as treatment for severe psoriasis vulgaris, rheumatoid arthritis, multiple sclerosis, lupus nephratitis and renal transplantation [81, 82]. Importantly, the experimental data showing distinct functions of CD80 and CD86 in regulating the immune response in various disease models underscore the need to design tailor-made therapeutic strategies for humans for preventing transplant rejection and treatment of autoimmune diseases. Some of the studies done recently, have established the therapeutic potential of antibodies against CD80 and CD86 [27, 41-42, 83]. However, it is imperative to evaluate their clinical significance in other diseases as well. In the case of autoimmune diseases and allergy, it will be worth to study the role of signaling through CD80/CD86 in promoting clonal deletion of highly pathogenic autoreactive B cells and T cells.

SUMMARY

Many studies published recently, have now established that the CD80 and CD86 co-stimulatory molecules expressed on the surface of the APCs do not merely have a unidirectional function of stimulating T cells on engagement with CD28 and CD152, but this interaction delivers bi-directional signals affecting the activity of APCs as well. Moreover, based on the kinetics of differential expression, affinity, association and dissociation rates and structure, indicates that CD80 and CD86 can differentially regulate the cells of immune system. In particular, CD80 deliver similar signals as delivered by CD152 for inhibiting the action of T cells. In contrast, CD86 costimulate APCs in an analogous manner as done by CD28 in activating T cells. Interestingly, the inhibitory role of CD80 on B cells, dendritic cells, neural stem cells and lymphomas have shown to induce apoptosis by upregulating the expression of pro-apoptotic molecules and down-regulating the expression of anti-apoptotic molecules. This inhibitory role of CD80 has been efficiently exploited to treat patients suffering from relapsed and refractory lymphomas. In addition, signaling through CD80 can be explored for inducing tolerance/apoptosis in autoimmune diseases, hypersensitivity reactions, allergies and transplantation.

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Signaling through CD80: an approach for treating lymphomas

Manzoor A Mir & Javed N Agrewal†
Institute of Microbial Technology, Sector 39A, Chandigarh-160036, India

Background: One of the mechanisms by which tumors evade the immune system is by downregulating the expression of costimulatory molecules like CD80 and CD86. The role of CD80 in the activation of T cells is well established, and current studies are exploring its role in cancer. Objective: To examine the possible role of CD80 signaling in generating an effective immune response against cancer cells. Methods: Many reports have described the influence of CD80 on the growth of B cell and follicular lymphomas. Signaling through CD80 in B cell lymphomas can retard their proliferation by upregulating expression of pro-apoptotic molecules and downregulating antiapoptotic molecules and can therefore induce apoptosis. Recently, a Phase III study of treatment with CD80-specific antibody has shown it to be quite effective in relapsed and refractory follicular lymphoma patients. Conclusion: This study shows that anti-CD80 immunotherapy may have a potent role in treating CD80-bearing cancer cells.

Keywords: cancer, CD80, costimulation, immunotherapy, lymphomas, vaccine


1. Introduction

Cancer is a major health problem worldwide and is one of the most prominent causes of morbidity and mortality in children and adults. The magnitude of the disease can be understood from the fact that in the year 2000 alone there were 7 million deaths from cancer, which is about 13% of total mortality [1]. The transformation of normal cells to cancer cells may arise due to dysregulation of oncogenes, tumor suppressors and/or stability genes. These transformed cells are sensed by the cells of the immune system, especially T cells, through specific receptors for an effective immune response. But unfortunately even after the interaction with T cells, an effective immune response is not generated because tumor cells have developed a number of mechanisms to resist recognition and elimination by immune system. Tumors evade immune responses by several mechanisms, like downregulating the expression of costimulatory and MHC molecules, producing immunosuppressive substances, not expressing tumor antigens and inducing tolerance to tumor antigens.

A successful immune response depends on a series of specific interactions between a T cell and an antigen-presenting cell (APC). Although the first essential step in this process is the binding of an MHC–peptide complex on an APC to the T cell receptor (TCR) on a T cell, this interaction alone is not sufficient to induce an effective and sustained response to a given antigen that leads to the optimum activation of T cells (Figure 1A) [2-4]. This response requires a second signal delivered by costimulatory molecules, without which, instead of activation it leads to anergy or apoptosis of T cells [5]. Many costimulatory molecules like CD80, CD86, CD40, heat stable antigen (HSA), intercellular adhesion molecule (ICAM–1), vascular cell adhesion molecule (VCAM–1), B7-DC/programmed death ligand-2 (PDL-2), B7-H1/programmed death ligand-1 (PDL-1) etc., are known to be
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Figure 1. Influence of CD80 in modulating the activity of cancer cells. When a normal cell presents peptide in context with an MHC molecule to T cells, delivery of CD80 signal generates an effective immune response (A). Efficient presentation of peptide in association with MHC but in absence of costimulation induces immunological ignorance in T cells and immune evasion in cancer cells (B). Presentation of tumor-peptide-MHC complex and expression of CD80 on tumor cells leads to its apoptosis and elicitation of antitumor immunity by the activation of T cells and bidirectional signaling (C).

6 expressed on the surface of APCs. But the best defined costimulatory molecules are two structurally related proteins known as CD80 (B7-1) and CD86 (B7-2). Both CD80 and CD86 have their ligands, CD28 and cytotoxic T lymphocyte associated antigen (CTLA-4), expressed on T cells. The costimulatory signals are necessary to ensure an effective immune response and are therefore provided by well-defined CD80/CD86:CD28/CD152 pathways. It has been established that CD80/CD86 contact with CD28 and CTLA-4 delivers signals that activate and inhibit cell activity, respectively.

It has been established recently, that CD80 and CD86 play a role in the activation of APCs. Stimulation via CD86 in B cells can modulate their proliferation, IgG secretion and expression of pro-apoptotic and antiapoptotic molecules, nuclear localization of NF-kB p50 subunit, phosphorylation of v-rel reticuloendotheliosis viral oncogene homolog A (Rel A) (p65) and 1xB-alpha and increased octamer-binding transcription factor 2 (oct-2) expression. Furthermore, in the case of dendritic cells, it has been shown recently that it enhances the production of IL-6 and IFN-γ which in turn, upregulates the expression of the immunoregulatory enzyme indolamine 2, 3-dioxygenase (IDO) that results in tryptophan catabolism, hence its depletion, and kynurinine 84 production, which produces the biological effect [6-8]. It has also been shown that silencing of SOCS3 by small interfering RNA (siRNA) makes CD28–Ig capable of activating IDO hence in the absence of SOCS3 CD28–Ig becomes immuno-suppressive and mimics the action of CTLA-4–Ig on tryptophan catabolism. These effects appear to result from a 90 combination of unopposed IFN-γ signaling and the occurrence of IFN-γ like actions by IL-6 [9,10]. However, in the case of CD80, it has been illustrated that signaling through this molecule can arrest the growth of lymphomas by upregulating expression of pro-apoptotic molecules and 95 downregulating antiapoptotic molecules [6,11,12].

Clinical studies have demonstrated that administration of anti-CD80 antibody can decrease tumor burden in patients suffering from lymphomas [13,14]. CD80 is expressed transiently on the surface of activated B cells and other APCs, including 100 dendritic cells, but is constitutively expressed on a variety of non-Hodgkin’s lymphomas (NHLs), including follicular lymphomas. Thus, CD80 is an attractive target for lymphoma therapy. Targeting CD80 on non-Hodgkin’s lymphomas with anti-CD80 antibody can arrest their growth and therefore 105 can serve as a therapeutic intervention for treating cancer.

2. Role of CD80 in immunity against cancer

In the absence of costimulation, recognition of antigens by T cells may not cause any response, even if tumor cells 111
express MHC molecules and tumor specific antigens [13]. CD80 is a costimulatory molecule known for its role in T cell activation and also in regulating normal and malignant B cells activity [11]. Surface CD80 is expressed transiently on activated B cells, macrophages, and dendritic cells. Surprisingly, CD80 is downregulated on most of the cancer cells and the loss of CD80 alone is sufficient to allow them to escape the attack of the immune system and to impart anergy and apoptosis in tumor-infiltrating T cells [16]. Hence most human malignancies that lack CD80 expression have been suggested to evade immune surveillance and therefore contribute for failure of immune recognition [17]. However, follicular lymphomas express CD80 and therefore could potentially be targeted by CD80 immunotherapies.

Lack of either CD80 or MHC-I must be sufficient to allow tumor cells to evade immune response but it has been shown in some tumors that high expression of MHC-I and absence of costimulatory molecules renders them resistant to lysis by cytotoxic T lymphocytes (CTLs) (Figure 1B). It has been shown that high expression of MHC-I but lack of CD80 does not allow cytolysis of target cells by CTLs ex vivo [16]. In contrast, CD80 transfected tumor cells become susceptible to lysis by CTLs ex vivo [18]. This suggests that CD80 expression on tumor is important for antitumor CTL effector function. Expression of CD80 on tumor cells confers direct presentation capacity to prime naive CTLs in vivo [19]. Although priming of antitumor CTLs can also take place in absence of CD80, but the effector T cells are not produced without the expression of CD80 on tumor cells [20]. Interestingly, CD80 has also been shown to enhance the memory response in CTLs [21,22]. Transfection of tumor cells with CD80 will help in generating long-lasting immunity. Therefore, this approach may lead to the development of a successful vaccine against cancer.

The expression of CD80 is required for the sustained predominance of CD8+ T cells within a tumor (Figure 1C). Transfection of CD80 cDNA into human erythroleukemia (HEL-DR+) cells induces the allogeneic response of purified T cells from both cord blood and peripheral blood of adult donors, demonstrating that CD80 expression could lead to accessory-cell-independent activation of naive T cells. It has also been demonstrated that the expression of CD80 by HEL-DR+ cells was required to initiate and to sustain the T cell response [23]. Furthermore, in the P815 tumor system, CD86 was substituted for CD80, interestingly, no specific CTL activity was observed. Even though in the tumors that express CD86 there is a need for CD80 expression by host APCs for efficient eradication [24,25]. Thus, it is apparent that transfection of CD80 in tumor cell serves as a co-factor for IL-2 production and in prevention of anergy by TCR ligation [26,27]. This suggests that expression of CD80 on cancer cells contributes towards the activation of T cells responsible for imparting anticancer immunity.

CD80 costimulation can direct the CD8 T cells to produce IL-2, proliferate and acquire cytolytic activity [28]. This helper independent generation of CTLs may have practical application in the development of tumor-specific immunotherapy [29,30]. So it is clear that downregulation of CD80 costimulatory molecule by various cancer cells acts as an effective immune evasion mechanism (Figure 1B). This has been reported in carcinomas, multiple myeloma's, leukemia's and transplantable malignancies etc. Some of the colon carcinomas such as MC38, and melanomas such as B16, also lack the expression of CD80. This is evident from the fact that silencing of CD80 expression results in tumorigenicity and transfection of CD80 leads to decreased tumorigenicity [31].

The expression of CD80 on tumor cells also enhances natural killer (NK) cell recognition and lysis of tumors, which plays an important role in tumor immunity [32]. CD80 expression triggers in vitro NK-cell-mediated cytotoxicity. The CD80 gene product functions as a triggering signal for NK-cell-mediated cytotoxicity and the strength of this response is such that it overrides the inhibitory signals mediated by MHC-I molecules [32,33]. The efficient control of solid allogeneic tumors by NK cells depends on co-delivery of both CD80 and MHC-I on the tumor cells. The co-delivery is required for optimal expansion and effector function of NK cells in response to both melanoma and plasmacytoma that express allogeneic MHC-I. The two signals required for T cell function can also regulate NK cell immunity and reveal an important similarity between the innate NK cell response and the adaptive T cell response. Furthermore, it has been reported that NK cells and CD8+ T cells can eliminate CD80-expressing tumors that are resistant to IL-12 gene therapy [34-36]. Hence expression of CD80 on tumor cells may also have an important bearing on NK cell effector function.

3. CD80 gene transfection of tumor cells to generate vaccine, and elicitation of antitumor response

It is now evident that CD80 plays an important role in immunity against cancer cells. Thus, it is apparent that expression of CD80 on cancer cells can enhance the immunity substantially (Figure 1B, C). Expression of costimulatory molecule CD80 via somatic gene transfer represents one approach to improve the immunogenicity of 210 tumor cells. A tumor cell engineered to provide optimal costimulation would then, directly induce T cell activation, proliferation, and differentiation into effector cells, following recognition of the antigen.

Transfection of CD80 gene into various cancer cells, RMA, EL-4, P815, E6B2, B16, K1735 etc., has reduced or eliminated subsequent tumor growth in syngeneic mice and can lead to the establishment of protective immunity against challenges with CD80-negative tumor cells [19,30,37,38]. The positive effects of CD80 gene transduction have also been thoroughly explored in a rapidly spreading tumor, B16.
lithal hematopoietic leukemia cell line which is non-immunogenic by itself. CD80 gene transfection in this cell line resulted in loss of tumorigenicity and induction of protective immunity against subsequent challenges with the parental CD80+ cell line in immunocompetent syngenic mice [18]. CD80 transfection has been shown to inhibit lymph node metastasis by the mechanism of enhanced immunogenicity [39]. Transfection of CD80 in colon cancer cells resulted in increased immunogenicity and tumor rejection. In contrast, silencing of CD80 expression in colon cancer cells resulted in lack of tumorigenicity [31]. The CD80 transfection into EL-4 cells, T cell thymoma cell line, showed that CD80 plays an essential role in mediating in vivo antitumor rejection. Furthermore, not only the presence but also the levels of CD80 expression can affect the tumor growth [40]. The cells generated by introduction of the CD80 gene can work as prophylactic or therapeutic vaccines against tumors that lack CD80 by means of eliciting a strong CTL and NK cell response, which is desired in antitumor immunity [37,41].

Antitumor effects of CD80 expressing tumor cells can be mostly attributed to costimulatory activity resulting in activation, proliferation and generation of CTLs [41-43]. CD80 expression may protect T cells from activation-induced cell death upon recognition of tumor cell targets [44,45]. CD80 expression on tumor cells enhances their recognition by NK cells, hence causing enhanced lysis of tumor cells, which in turn makes the antigen available for the cross-priming pathway, thereby generating systemic immunity to parental tumor. CD8+ T cell precursors are directly primed by CD80 transfected tumors, which provide signal 1 and 2 to the T cells [20,41]. Indicating that CD80-transfected cells serve directly as APCs in vivo for the priming of naive CTLs. It is quite interesting that the anticancer modalities like mitomycin-C, melphalan, γ-irradiation etc., exert their immunopotentiating effect in tumor bearers by upregulating CD80 gene expression [46]. However, the mechanism by which surface expression of CD80 on tumor cells enhances the antitumor immunity seems to be the same as that depending on the maturation state of DCs regulating immune response by CD80 expression, as the low expression of CD80 on immature DCs suppresses T cell immunity in a CD80-dependent fashion and high CD80 expression by mature DCs promotes immunity [46,47].

Considering the importance of CD80 costimulation in the regulation of immune responses against cancer, the manipulation of this molecule to increase immunity represents a promising therapeutic and prophylactic approach. It has been recently shown that primary tumor cells resected from cancer patients can be transfected effectively with CD80-SA (streptavidin) and that such cells serve as APCs to induce autologous antitumor T cell responses [48]. CD80 has been co-administered with various cytokine genes to elicit antitumor activity. A single intratumoral co-administration of CD80 with the IL-12 gene, by electroporation, elicited specific antitumor CTL 277 response and eradicated the tumor from mice. This co-administration also maintains the high level of endogenous CD80 of tumor cells through, STAT-1 expression [49]. The 280 coexpression of CD80 and IL-12, administered by intratumoral injection with an adenovirus vector, effectively elicited antitumor immunity in a nonimmunogenic, therapy-resistant, mouse pancreatic cancer model [50]. Tumor cells transfected with CD80 and IL-7 genes induced CD28 285 and CD25 on tumor-infiltrating T cells and triggered strong antitumor responses [51]. A dual modified tumor cell vaccine using CD80 and superantigen Staphylococcus enterotoxin A (a potent inducer of CTL activity and cytokine production) elicited significantly stronger antitumor responses in vivo [52]. It has been shown that when IFN-γ transfectant immunotherapy was used for cancer treatment; it promoted tumor progression. But interestingly, when a combination of CD80 and IFN-γ transfection immunotherapy was used, the cancer-promoting effect of cellular vaccination was completely abolished and instead the cancer vaccine potency to eradicate tumors increased [53]. CD80 expression on tumor cells has been shown to eliminate resistance to IL-12 gene therapy [53]. CD80+ MHC-II+ transfected tumor cells were used as a 300 potent vaccine for stimulating tumor rejection in tumor-bearing mice [54]. Transfection of CD80 gene into human hematopoietic malignant cell lines that are deficient in CD80 expression empowers their antigen presentation potency for activation of antitumor T cells. Repairing the 305 deficiency in the CD80 co-stimulatory pathway in tumor cells can be a novel immunotherapeutic approach for human hematopoietic malignancies [55].

The combined expression of CD80 and IL-2 in acute myeloid leukemia blasts show increased stimulation of both 310 alloimmune and autologous T cells. The stimulated lymphocytes secrete higher levels of Th1 cytokines and show specificity [56]. Phase I clinical trails of a CD80 gene modified autologous tumor cell vaccine in combination with systemic IL-2 have shown promising results in patients with metastatic 315 renal cell carcinoma [57]. A tri-cistronic viral vector co-expressing IL-12 (IL-12p40 plus IL-12p35) and CD80 showed enhanced CTL and proliferative response in a myeloma tumor model [58]. Apoptotic uveal melanoma cells expressing CD80 were efficient at inducing an immune 320 response and served as potent immunogens. This strategy could prove to be a novel adjuvant immunotherapy for the disease [59]. It has also been shown that CD80 vaccination after removal of a tumor is useful for the prevention of tumor recurrence. The model of CD80 enhancing tumor 325 immunity is that exogenous CD80 acts as a costimulatory molecule to directly interact with CD28 on T cells, thus resulting in the activation of CD8+ T cells against tumor cells. Not only this but CD80 on tumor cells can also interact directly with CD28 on NK cells and activate them to inhibit tumor growth [36,41].
Figure 2. CD80 delivers death signals in lymphomas. Signaling through CD80 in B-cell lymphomas triggers signaling that leads to apoptosis by upregulating the expression of pro-apoptotic molecules and downregulating the expression of antiapoptotic molecules.

4. Signaling through CD80 can inhibit the growth of lymphomas by upregulating the expression of pro-apoptotic molecules

Recently, we demonstrated that crosslinking of CD80 by anti-CD80 antibodies retarded the growth of B cell lymphomas and favored the upregulation of pro-apoptotic molecules (Figure 2). It was observed that signaling through CD80 molecules mainly augmented the levels of pro-apoptotic molecules, i.e., caspase-3, caspase-8, -Fas, FasL, B cell leukaemia/lymphoma associated protein 2 antagonist/killer 1 (Bak), and B cell leukaemia/lymphoma associated protein 2-associated X protein (Bax)) and downregulated the expression of antiapoptotic molecules (i.e. B-cell leukaemia/lymphoma x (Bcl-x(L)) and B cell leukaemia/lymphoma associated protein 2 from fetal liver (Bfl)-1). This suggests that CD80 signaling induces apoptosis via mechanism involving pro-apoptotic molecules, consequently rendering the cells more vulnerable to apoptosis and therefore restricting their proliferation. It has been shown that overexpression of caspases is sufficient to cause apoptosis. The pro-apoptotic molecule (Bax) has been correlated with disease regression and shorter survival of B cells in chronic lymphocytic leukemia [60-63]. Resistance to apoptosis is also one of the mechanisms employed by tumor cells for evasion and it is not only relevant for tumorigenesis and resistance to chemotherapy but also influences immunosurveillance and immunotherapy. It is important to mention here that co-stimulatory-molecule-mediated delivery of inhibitory signals may not only be responsible for the regulation of immune responses but also for apoptosis of CD80-bearing tumor cells. These studies very categorically demonstrates that tumor regression is not only the consequence of CD80-mediated activation of CTLs and NK cells but signaling through CD80 can also trigger death of lymphomas [11,64,65].


The development of monoclonal antibodies, biological therapies that target tumor-associated antigens specifically, gives hope for improvement of survival in many cancers. Monoclonal antibodies make an important contribution to the therapeutic treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, breast cancer and colorectal cancer. These antibodies can bind to their receptors on tumor cells specifically and block the binding of endogenous ligands, which leads to inhibition of phosphorylation of receptor-protein tyrosine kinases and inhibition of downstream signaling events [66]. Monoclonal antibodies that act through this mechanism include: trastuzumab, which targets v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (ERBB2); cetuximab, which targets EGFR; alemtuzumab a lymphocyte-depleting humanized monoclonal antibody with efficacy in the treatment of chronic lymphocytic leukemia targeting CD52 [67] and bevacizumab, which blocks angiogenic signaling by binding to and sequestering the ligand of VEGF receptor (VEGFR) [68].

Rituximab is a highly specific mouse/human chimeric IgG1 antibody that targets CD20 a cell-surface protein expressed on 95% of B cell lymphomas, has shown efficacy in patients with various lymphoid malignancies, including indolent and aggressive forms of B cell non-Hodgkin’s lymphoma (NHL) and B cell chronic lymphocytic leukemia (CLL) [69]. It inhibits cell proliferation and directly induces apoptosis [70]. It has been shown that patients can be treated safely and effectively with multiple courses of rituximab without induction of human antichimeric antibodies (HACA), cumulative myelosuppression, adverse haematological effects and serious or opportunistic infections that are related to standard chemotherapy or radiotherapy [69,71].
Galiximab is a chimeric, anti-CD80, IgG1 antibody that targets CD80, which is constitutively expressed on a variety of lymphomas like diffuse large B cell NHL, CLL, Hodgkin's lymphoma, and multiple myeloma. Because of the favorable safety profile of galiximab and its lack of induction of myelosuppression which is associated with typical standard chemotherapies, radiotherapy and other antibody therapies, it is an attractive agent for lymphoma therapy [72-74]. Galiximab has also shown promising results when used in combination with rituximab.

These monoclonal antibodies bind to their targets specifically on the surface of cells and do not circulate in the plasma as free protein that could competitively inhibit antibody binding to lymphoma cells. Other properties of these monoclonal antibodies like long half-life, high specificity and high safety standards compared with other cancer therapeutics together with their ability to bind to and modulate key players in signaling pathways that drive malignant transformation and enhance antitumor immune functions make them highly desirable therapeutic agents.

6. Anti-CD80 antibody therapy in the treatment of lymphoma patients

The observation published by our laboratory on the induction of apoptosis on stimulation through CD80 [11] has constituted a rationale for development of an anti-CD80 therapy for lymphomas. Preclinical studies indicate that targeting CD80 with anti-CD80 antibody has antitumor effects [11-14,77,79]. Anti-CD80 antibody therapy delays progression of lymphoma and clinical studies have shown promising results [13,14,78,79]. Recently, Biogene has developed an anti-CD80 antibody known as galiximab. Galiximab is a chimeric, anti-CD80, IgG1 lambda monoclonal antibody with human constant regions and primate (Cynomolgous macaque) variable regions. It is structurally indistinguishable from human antibodies and, therefore, is unlikely to be significantly immunogenic in humans. Some characteristics of the antibody are summarized in Table 1. This makes it more suitable for potential repeated dosing in lymphoma patients. The specificity of galiximab binding to human CD80 has been validated in competitive binding studies with several lymphoma cell lines. Preclinical in vitro and in vivo studies evaluated galiximab as a targeted therapy for lymphoma, with promising results. A Phase I/II, single-agent, dose escalation, multiple-dose study demonstrated that galiximab administration can decrease tumor burden in nearly half of all patients suffering from relapsed and refractory follicular lymphoma [14].

Treatment of relapsed/refractory follicular lymphoma with galiximab in an open-label, multicenter, Phase I/II monotherapy trial in 37 patients with median age of 56.5 years who received different doses of antibody has shown no dose-limiting toxicities (DLT) and a favorable safety profile. The most common related adverse events (AEs) experienced were grade one or two (headache, fatigue and nausea) with only two cases of grade three deep venous thrombosis and one case of grade 3 axillary pain. Only one (3%) of 37 patients showed 465 cytopenias which was also considered to be unrelated to galiximab but related to the studied disease. Clinical results of this study have been summarized in Table 2 [14,15]. Furthermore, in a study covering 242 patients receiving galiximab as part of multiple-dose psoriasis studies it has shown that targeting of CD80 with other lymphoma therapies. Hence galiximab can be 490 combined safely with a standard course of rituximab for treatment of relapsed or refractory, follicular lymphoma. Based on these observations a Phase III, randomized study has been initiated to evaluate the clinical benefits of rituximab and galiximab for treatment of relapsed or refractory follicular lymphoma [14]. They found that this combination therapy does not alter the pharmacokinetics or immunogenicity but instead has the potential to avoid or delay chemotherapy or its associated toxicity or to amalgamate with other lymphoma therapies. Hence galiximab can be 490 combined safely with a standard course of rituximab for treatment of relapsed or refractory, follicular lymphoma. Based on these observations a Phase III, randomized study has been initiated to evaluate the clinical benefits of rituximab therapy versus combination therapy [15]. A 495 Phase I/II study of galiximab in combination with rituximab therapy in patients with relapsed or refractory disease has shown promising preliminary results for clinical activity without added toxicity compared with single-agent rituximab alone [80]. The future plans in this direction include 500 evaluating maintenance therapy with the combination treatment of galiximab and rituximab in follicular NHL and galiximab, rituximab, plus chemotherapy for follicular and diffused aggressive NHL.

7. Conclusion

As established by clinical and experimental results, it is quite evident that anti-CD80 antibody treatment is effective against relapsed and refractory follicular lymphoma. Future studies in other CD80-positive hematological malignancies (e.g., diffuse large B cell NHL, chronic lymphocytic leukemia, Hodgkin's lymphoma, and multiple myeloma) should also be taken under consideration. Thus, anti-CD80 antibody immunotherapy may have a potent role in the...
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treatment of CD80-bearing cancer cells because their binding can modulate the key molecules in the signaling pathway and enhance antitumor response. Considering the importance of CD80 signaling in the regulation of immune responses against cancer, the manipulation of this signaling pathway to increase immunity against cancer represents a potential therapeutic approach.

8. Expert opinion

Understanding the mechanisms by which tumor cells escape immune surveillance will help us to establish new and effective approaches to vaccination and immunotherapy. One of the most important immune evasion mechanisms employed by tumor cells is the downregulation of the CD80 costimulatory molecule. The success of novel cancer therapies depends on the identification of functional targets that play an essential role in tumor growth and metastasis, survival and evasion from immunosurveillance. Anti-CD80 therapy can be used to target tumors expressing CD80. The clinical success of anti-CD80 antibody for the treatment of refractory follicular lymphoma has stimulated great interest in the promise of antibody therapeutics for cancer. The qualities of galiximab, like long half-life and high specificity and safety compared with other cancer therapeutics together with its ability to bind to and modulate key players in signaling pathways that drive malignant transformation and enhance antitumor immune functions makes it a highly desirable therapeutic agent.

Most tumors do not regress but continue to grow in spite of the presence of spontaneous or antigen induced immune responses, due to downregulation of costimulatory molecules like CD80. The existence of systemic immune responses may not by itself be sufficient to deal with the complex nature of tumor-host interactions because factors such as insufficient costimulation to induce T cell response may further contribute to the lack of effective immunity. It is now well established that T cells are rendered anergic due to the lack of costimulatory molecule(s) expression by tumor cells. Tumor cells are able to induce antigen-specific tolerance or anergy on the basis of MHC-I-restricted antigen presentation without the expression of costimulatory molecules. This unresponsiveness, however, can be reversed when tumor cells are genetically modified to express costimulatory molecules. A plethora of studies suggest that the insertion of genes encoding CD80 into tumors generally increases their immunogenicity and can be used as vaccine. Recently, fusagene vectors were developed to encode multiple gene products like CD80 with cytokines or MHC molecules as fusion proteins from a single cistron to increase the immunogenicity of target tumor cells. The vectors generated could be used in immunotherapy for the treatment of multiple myeloma, leukemia and other cancers, as they have been shown to stimulate allogeneic mixed lymphocyte

Table 1. Characteristics of anti-CD80 antibody (galiximab) in the treatment of lymphomas.

<table>
<thead>
<tr>
<th>Antibody name</th>
<th>Type of antibody</th>
<th>Target</th>
<th>Dose</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galiximab or IDEC-114</td>
<td>Human-primate dimeric IgG1A</td>
<td>CD80 (B7-1)</td>
<td>125 – 500 mg/m²</td>
<td>Fatigue, asthenia, nausea, headache, dizziness, vomiting etc. Most grade 1 and 2</td>
</tr>
<tr>
<td>Anti-CD80 antibody</td>
<td></td>
<td></td>
<td>and half life of 2 – 4 weeks in serum</td>
<td></td>
</tr>
</tbody>
</table>

Most tumors do not regress but continue to grow in spite of the presence of spontaneous or antigen induced immune responses, due to downregulation of costimulatory molecules like CD80. The existence of systemic immune responses may not by itself be sufficient to deal with the complex nature of tumor-host interactions because factors such as insufficient costimulation to induce T cell response may further contribute to the lack of effective immunity. It is now well established that T cells are rendered anergic due to the lack of costimulatory molecule(s) expression by tumor cells. Tumor cells are able to induce antigen-specific tolerance or anergy on the basis of MHC-I-restricted antigen presentation without the expression of costimulatory molecules. This unresponsiveness, however, can be reversed when tumor cells are genetically modified to express costimulatory molecules. A plethora of studies suggest that the insertion of genes encoding CD80 into tumors generally increases their immunogenicity and can be used as vaccine. Recently, fusagene vectors were developed to encode multiple gene products like CD80 with cytokines or MHC molecules as fusion proteins from a single cistron to increase the immunogenicity of target tumor cells. The vectors generated could be used in immunotherapy for the treatment of multiple myeloma, leukemia and other cancers, as they have been shown to stimulate allogeneic mixed lymphocyte
Signaling through CD80: an approach for treating lymphomas

Table 2. Clinical results of patients with relapsed or follicular lymphoma showing reduction in tumor lesions and clinical adverse events after treatment with anti-CD80 antibody, galiximab.

<table>
<thead>
<tr>
<th>% reduction in lesions</th>
<th>Number of patients (%)</th>
<th>Adverse event type</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 – 100%</td>
<td>17 (49%)</td>
<td>Lymph node pain</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥ 50%</td>
<td>6 (17%)</td>
<td>Dysepsia</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥ 50% but with disease progression</td>
<td>2 (6%)</td>
<td>Vomiting</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>New lesions</td>
<td>1 (3%)</td>
<td>Axillary pain</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Non-measurable disease progression</td>
<td>1 (3%)</td>
<td>Dysguesia</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Not available for measurement</td>
<td>4 (12%)</td>
<td>Loose stools</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Headache</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Out of 35 (100%) patients 31 (89%) were available for assessment [13].

Table 3. Clinical results of patients with moderate to severe plaque psoriasis showing reduction in psoriasis area and severity index (PASI*) and improvement in physicians global psoriasis assessment (PGA) after treatment with an anti-CD80 antibody, galiximab.

<table>
<thead>
<tr>
<th>Study days after treatment of patients with galiximab</th>
<th>Number of patients assessed after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>29</td>
<td>36</td>
</tr>
<tr>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td>57</td>
<td>71</td>
</tr>
<tr>
<td>99</td>
<td>127</td>
</tr>
</tbody>
</table>

% of patients showing ≥ 50% reduction in PASI*:

| % of patients showing ≥ 50% reduction in PASI* | 3   | 10  | 7   | 15  | 20  |
| % of patients showing ≥ 75% reduction in PASI* | 3   | 5   | NA  | 10  | NA  |

*PASI is calculated in patients after treatment by visually assessing the extent of psoriatic involvement in four main body areas (head, trunk, upper-extremities and lower extremities) weighted by % of total body area and the severity of signs in those areas [78].

Taking all time points and all dose groups, 20 out of 35 patients (i.e., 57%) achieved a PGA rating of Good and 7 patients (i.e., 20%) achieved a PGA rating of Excellent.

NA: Not available.

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Declaration of interest

The authors declare no conflicts of interest.
B7 interactions in function of natural killer cell response


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Affiliation
Manworo A Mir & Javed N Agrewala1

1Author for correspondence
Institute of Microbial Technology, Sector 39A, Chandigarh-160036, India
Tel: +91 172 2636680;
Fax: +91 172 2690585, 2690632;
E-mail: javed@imtech.res.in
Influence of Immunomodulation of CD80 and CD86 Costimulatory Molecules in the Infectious Diseases.

Javed N. Agrewala and Manzoor A Mir

Institute of Microbial Technology, Sector 39A, Chandigarh-160036.

Abstract. Recently, we have given a concept of bi-directional costimulation. We have demonstrated that engagement of CD80/86 with CD28/CTLA-4 not only influence T cells but equally effects the activity of antigen presenting cells. Further, we showed that signaling through CD80 can retard the growth of B cell lymphomas by upregulating the expression of pro-apoptotic molecules and downregulating anti-apoptotic molecules. In continuation of our previous study, we now show that triggering through CD80 and CD86 can effectively activate macrophages and hence can influence the survival of intracellular pathogens.

Introduction

The role of costimulatory molecules is very well established in the activation of T cells (1). T cell activation is dependent upon signals delivered through the antigen specific T cell receptor and accessory receptors on the T cell. The best-defined costimulatory molecules to date are two structurally related proteins, CD80 (B7-1) and CD86 (B7-2) (2-5). These two B7 ligands, CD80 and CD86 can augment immune responses by binding to CD28 and down regulate responses by binding to CTLA-4. It has been suggested that CD86 participates in initiating immune response, whereas CD80 may be more important in sustaining or regulating immune responses. The up-regulation of CTLA-4 on activated T

*Address correspondence: Dr. Javed N. Agrewala, Institute of Microbial Technology, Sector 39A, Chandigarh-160036, INDIA. Email: javed@imtech.res.in, Tel: ++91-172-2636680, Fax: ++91-172-2690585, 2690632.
Dietary Polyphenols in Modulation of the Immune System

Manzoor A. Mir and Javed N. Agrewala
Institute of Microbial Technology, Chandigarh-160036, India

Abstract

Polyphenols are a diverse group of naturally occurring compounds with multiple biological functions. Polyphenols like curcumin, resveratrol, quercetin, catechin, chlorogenic acid, myricetin and apigenin can modulate the immune response through their potent antioxidant and anti-inflammatory mechanisms. In addition, many polyphenols can regulate immunological reactions by modulating pro-inflammatory cytokines, chemokines, adhesion molecules, NF-κB, inducible enzymes, etc, or by influencing the activity of cells of the immune system. For instance, it has been shown that resveratrol and curcumin suppress the immune system by mainly modulating the expression of CD28/CTLA-4 and CD80 costimulatory molecules. Curcumin also blocks the cyclosporin A-resistant CD28 pathway of T-cell activation and induces apoptosis. It inhibits allergic encephalomyelitis by blocking IL-12 signaling and exerts inhibitory effects on the production of IL-8, IL-1β, and TNF-α. Tea polyphenols have been shown to scavenge reactive oxygen and nitrogen species and reduce their damage to lipid membranes, proteins and nucleic acids in cell-free systems. Green tea polyphenols also prevent ultraviolet-B induced cyclobutane pyrimidine dimers, which are considered to be mediators of ultraviolet-B induced immune suppression. In conclusion, although the exact manner through which polyphenols produce their effects is not fully understood, they have potential for use as drugs to correct immune system disorders like allergies, autoimmune diseases, inflammation and hypersensitivity.

* Address correspondence: Dr. Javed N Agrewala, Institute of Microbial Technology, Sector 39A, Chandigarh 160036, INDIA. Email: javed@imtech.res.in, Tel: ++91-172-2636680, Fax: ++91-172-2690585, 2690632.