INTRODUCTION
Survival of species depends upon an effective defense against a foreign invader. Host defense mechanisms have been evolving since the first organisms encountered one another, hundreds of million years ago and the distinction of self and non self is achieved by even the most primitive protozoa. With time, specialized cells and mechanisms evolved that separated phagocytosis from defense functions.

Broadly speaking an individual's reaction to a pathogen consists of a non adaptive or innate response and an adaptive immune response.

The innate immunity derives from all those elements with which an individual is born and that are always present and available at very short notice to protect an individual from challenges by foreign material. These elements include body surfaces as well as internal components. For example the skin, the mucous membranes and cough reflexes present effective barriers to environmental agents. Combined with these physical barriers, chemical influences such as pH, secreted fatty acids and lysozyme constitute effective barriers against invasions by many microorganisms. The innate response is not improved by repeated encounter with any particular pathogen.

On the contrary, the adaptive immune system can specifically recognize a pathogen when it first encounters and in case there is re-encounter with the same pathogen it will mount an enhanced response.

The blood cells are of two types, red and white blood cells. The adaptive immunity is effected primarily by the white blood cells. Both these cell types originate from the
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pluripotent stem cells in the bone marrow (Rosse et. al. 1976). The pluripotent stem cells are characterized by the expression of CD34 molecules and are capable of self renewal. Each pluripotent stem cell under the influence of different types of growth factors, can differentiate into lymphoid (in presence of IL3) or myeloid stem cells (in presence of GM CSF & IL3). The growth factors are provided by the bone marrow stromal cells (provide IL3, IL4, IL7, GM-CSF, M-CSF, & G-CSF), activated Th cells (provide IL3, IL4, IL5, IL6, IL9, GM-CSF, M-CSF, & G-CSF), and activated macrophages (provide growth factors like IL6, IL8, GM-CSF, M-CSF, &G-CSF).

The lymphoid stem cells are committed cells and give rise to T cell and B cell progenitor cells. The T cell progenitor gives rise to the Th and Tc cells (in presence of IL7, IL2 and IL4). On the other hand the B cell progenitor gives rise to the B cells (in presence of IL4, IL2, IL5 and IL6).

A small group of peripheral blood lymphocytes, do not express surface molecules, that distinguish T and B cells, are known as null cells. One of the functional population of cells, known as natural killer cells or NK cells, are cytotoxic in nature but are not MHC restricted unlike Tc cells. The Nk cells destroy tumor cells by a process known as antibody dependent cell-mediated cytotoxicity (ADCC).

The myeloid stem cells, on the other hand, depending upon the exposure to different types of growth factors derived from the bone marrow stromal cells, give rise to different types of progenitor cells. The granulocyte monocyte progenitor derived in presence of growth factors like IL3, GM-CSF, IL6, gives rise to macrophages and
neutrophils, in presence of GM-CSF, M-CSF; and GM-CSF, G-CSF respectively. The eosinophil progenitor derived in presence of IL3 and GM-CSF, gives rise to the eosinophils. IL3 and GM-CSF helps in formation of basophil progenitors and these in presence of IL9 or GM-CSF & IL4 give rise to mast cells and basophils respectively. IL3, IL11, GM-CSF & erythropoietin (EPO) help in the generation of megakaryocyte. Thus formed megakaryocytes, in the presence of GM-CSF, EPO & IL6 mature into platelets. Lastly the erythroid progenitor formed in presence of IL3, GM-CSF & EPO develops into RBC in presence of EPO (Dexter & Spooncer 1987).

The common CD antigens used to distinguish functional lymphocytes are as follows:

<table>
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<tr>
<th>CD Designation</th>
<th>B cell</th>
<th>T cell</th>
<th>NK</th>
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<tr>
<td></td>
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<td>Th</td>
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<td>CD2</td>
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<td>CD4</td>
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<td>CD8</td>
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<tr>
<td>CD11a/CD18 (LFA-1)</td>
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<td>CD16</td>
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<td>CD21 (CR2)</td>
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<td>CD28</td>
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<td>CD32 (Fcer II)</td>
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<td>CD35 (CR1)</td>
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<td>CD40</td>
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<td>CD45</td>
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<td>CD54 (ICAM-1)</td>
<td>+</td>
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<td>CD56</td>
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T cells are broadly divided into CD4+ & CD8+ T cells based on the CD4 or CD8 surface molecule expressed on them. The CD4+ T cells are helper cells and are further classified into Th1 & Th2 depending upon types of lymphokines secreted by them. The Th1 cells secrete IL2 & IFN-γ while the Th2 cells secrete IL4, IL5 & IL10 (Mosmann et. al. 1987b).

The CD8+ T cells are primarily cytotoxic in nature although some a subset of CD4 are also known to be cytotoxic in nature (Erb et. al. 1990).

Both the CD4+ & CD8+ T cells are αβ T cells i.e. the T cell receptor has αβ types of protein chains.

The other type of cells are known as γδ T cells. These cells instead of αβ TcR express γδ TcR. They are found at much lower number as compared to the αβ T cells. They do not express CD4 molecule characteristic of αβ T cells, although some express CD8 accessory molecule. The γδ T cells produce lymphokines characteristic of T cells bearing αβ TcR and also exhibit cytotoxicity. These cells are known to be activated by mycobacterial antigens and heat shock proteins. The γδ T cells have been implicated to play a role in forming a line of defense against common antigens of some bacteria (Hass et. al. 1993).

T cells play a pivotal role in mediating immune response to a foreign invader. The immune response mounted by them can be of humoral or cell mediated type. T cells generated from the thymus are naive or virgin and are of CD45 RA+ (Thomas 1989., Rothstein et. al. 1992). These T cells migrate to the lymph nodes where their activation
takes place. Before this event occurs the antigen has to be presented to Th cells, by an antigen presenting cell (B cell, dendritic cell or macrophage) in association with MHC-II. This complex is recognized by the T cell receptor present on Th cells (Brodsky et al. 1991., Engelman et. al. 1981., Swain et. al. 1983). There is another pathway for antigen presentation, which is the endogenous or cytosolic pathway. In this case the antigen peptides are presented in association with MHC class I molecules. The recognition here is by the CD8+ T cells which are mainly cytotoxic in nature (Brodsky et. al. 1991).

Hence, cellular collaboration is an important feature of the immune system and it involves the recognition of cell surface associated molecules on one cell by specific receptor on the other. Recognition of some cell surface molecules stabilizes cellular interactions (e.g. CD2 on T cells & LFA-3 on APC, LFA-1 & ICAM-1 or ICAM2) (Parnes 1989), while recognition of others is involved in the transduction of regulatory signals (CD45 on T cells & CD22 on APC, CD28 on T cells & B7 on APC). Cellular interactions regulate activation, differentiation, suppression and lysis.

The adaptive immune response is characterised by cognate interactions between the T cells and professional antigen presenting cells like B cells, macrophages and dendritic cells. The outcome of these interactions is cell mediated immunity or humoral immunity. The interaction between Th cells and macrophages induces secretion of cytokines, expression of cytokine receptors and proliferation of Th cells. The lymphokines from Th cells namely IFN-γ activates macrophages to overcome and kill interacellular pathogens. When cellular interaction occurs between Th cells and resting
B-lymphocytes, this induces Th cell interaction and leads to B cell proliferation, differentiation and production of specific antibodies of different isotypes.

**ACTIVATION OF T CELLS:** Once the above interaction has taken place the entry of naive T cell from G0 to G1 phase of cell cycle takes place. In other words the T cell is activated. There is induction of synthesis of IL-2 along with expression of high affinity IL-2 receptor. The binding of IL-2 to its receptor triggers the progression of T cell through the rest of the cell cycle. The T cells, thus activated, undergo clonal expansion giving rise to thousands of progeny bearing the identical receptor for antigen.

This interaction leads to a series of biochemical events which culminates in the transfer of information from the cell surface into the nucleus finally leading to the transcription of IL-2 gene. The signaling event apart from TcR-CD3 complex involves other molecules like CD4 or CD8 and CD45 molecules. TcR complex consist of α and β chain (Allison et. al. 1987) apart from the three members known as the CD3 complex (Allison et al 1985). The CD3 complex includes 25-28 kD glycosylated γ chain, 20 kD δ chain and 20 kD nonglycosylated ε chain. In addition it contains a homodimer of 16 kD nonglycosylated ζ chain in 90 % cases and in 10 percent cases a heterodimer of ζ and a 22 kD nonglycosylated η chain (Samelson et. al. 1985., Ottegen et. al. 1986). The α and β of TCR consists of a short cytoplasmic tail and hence it is unable to mediate signal transduction. The cytoplasmic domain of each CD3 consists of a sequence known as antigen recognition activation motif (ARAM). Each ζ,
chain has three ARAM sequences while the \( \gamma, \varepsilon \) and \( \delta \) chain contain one each. The \( \varepsilon \) and \( \zeta \) chain of CD3 complex consist of protein tyrosine kinases known as p59\(\text{bn}\) (Samelson et. al., Gauen et al 1992) and ZAP70 (Zeta associated protein of 70 kD) respectively (Chan et. al. 1991). The cytoplasmic domains of CD4 and CD8 are associated with a protein tyrosine kinase called p56\(\text{ck}\) (Barber et. al. 1989., Veillette et. al. 1988). The cytoplasmic domain of CD45 has a protein phosphatase activity having two domains and catalyses dephosphorylation of p56\(\text{ck}\) and p59\(\text{bn}\) and hence these two protein tyrosine kinases are activated. They phosphorylate not only \( \varepsilon \) and \( \zeta \) chains of CD3 but also other cellular proteins and phospholipase C (PLCy1). Once PLCy1 is phosphorylated it hydrolyses phosphoinositol 4,5 biphosphate into diacylglycerol and 1,4,5 biphosphate (IP3) (Rhee and Choi 1992).

The diacylglycerol activates protein kinase C (PKC) which in turn dephosphorylates NF-\(\kappa\)B. The other breakdown product IP3 triggers the release of calcium from intracellular stores of endoplasmic reticulum and mitochondria leading to the activation of calmodulin dependent protein phosphatase known as calcineurin. Calcineurin dephosphorylates NF-ATc (Meuer and Resch 1989., Clipstone and Crabtree 1992., Klausner and Samelson 1991., Mustelin and Altman 1989).

The dephosphorylated nuclear factor NF-\(\kappa\)B and NF-ATc enter the nucleus and bind to IL-2 enhancer region called \( \kappa\)B-RE and ARRES respectively leading to the transcription of IL-2 gene (Crabtree 1989). This process is also mediated by signal transduction. The cytoplasmic domain of CD28 contains tyrosine residues that become phosphorylated as a consequence of CD28 binding to B7. There are evidences to
suggest that lck and fyn mediate this process. This leads to transcription factor called CD28-RC which binds to CD28-RC enhancer region of IL-2 gene leading to optimum production of IL-2. (Chan et. al. 1994; Perlmutter et. al. 1993).

**STAGES OF ACTIVATION OF CD4+ T CELLS:** The CD4+ Th cells differentiate through a number of intermediate stages before maturation. The first stage of a naive Th cells is Th0 which finally differentiates into a inflammatory T cells (Th1) cell or helper T cell (Th2). The Th1 cells are characterised by secretion of lymphokines like IL2, IFN-γ and lymphotoxin, while on the other hand, the Th2 is characterised by secretion of IL4, IL5 and IL10 (Mossman & Coffman 1987b). The Th1 cells help to activate macrophages while the Th2 cells activates B cells. Following the interaction of Th cells with antigen a number of genes are expressed. They can be classified as immediate genes (within 30 minutes) like c-Fos, c-Myc, NF-AT and NF-κB. The early genes includes IL-2, IL2R, IL-3, IL-6 and IFN-γ which are expressed within 1-2 hours of interaction between MHC-peptide and TcR. The last category of genes are known as late genes which includes HLA-DR, VLA-1, VLA-2, VLA-3, VLA-4 and VLA-5. These genes require more than two days for their expression (Crabtree 1989).

The activation of T-cells also leads to the expression of CD40L. Interaction between CD40L on activated T cells and CD40 on B cells results in class switching from IgM to other isotypes e.g. IgE (Zhang et. al.1991., GasCan et. al. 1991).

**ACTIVATION OF CD8+ T CELLS:** The naive CD8+ T cell passes through different developmental stages in response to tumor associated antigens, allogenic
Maturation factors (?)

Any cell (some expressing B7), MHC Class I and Class II, respectively

\( \gamma \), T cell receptor (TCR)

\( \uparrow \), IL-2

\( \uparrow \), IL-2 receptor

\( \blacksquare \), peptide antigen

B7 CD28

Fig-A: Schematic diagram showing cellular interactions in the generation of mature CTL (Giedeon Berke in Fundamentals of Immunology by William Paul. Raven Press. Third Ed. Page 967).
stimulation (for example allotransplantation, intracellular pathogens or virally infected syngenic cells etc). Given below is the postulated mechanism for development of CD8+ CTL.

The development of quiescent pre CTL precursor to its mature stage involves complex series of events (Fig-A). The stages of development of quiescent pre CTLs are as follows:

The quiescent pre CTLs have the following characteristics (i) they do not proliferate (ii) they do not contain perforin and granazymes (iii) they do not show cytotoxicity (iv) they are CD45RA+, CD45RO (v) they do not express IL-2 receptor.

These quiescent pre CTLs upon interacting with stimulating cell develop into activated pre CTLs, which show following characteristics: (i) the activated pre CTLs do not proliferate (ii) they exhibit low levels of cytotoxicity (iii) they are CD45RA+, CD45RO* (iv) express low levels of perforins and granazymes (v) they express IL-2 receptors.

The activated pre CTLs upon reaching the site of inflammation, interact with the target cell and as a result of the interaction between them they develop into CTL blasts showing following characteristics (i) they proliferate, (ii) show enhanced cytotoxicity as compared to activated pre CTLs (iii) they are CD45 RA; CD45RO* (iv) they express high levels of perforin and granazymes (v) the expression of IL-2 receptor increases.
These CTL blasts then develop into mature CTLs which are capable of lysing the target in an antigen specific manner. The mature CTLs show following the characteristics: (i) they do not proliferate (ii) show enhanced cytotoxicity (iii) are CD45RA', CD45RO+ (iv) express low levels of granazymes and perforin (v) In comparison to CTL blasts the expression of IL2 receptor is down regulated (Berke 1994).

The mechanism by which the cytotoxic T cells destroy its target is by secreting granules. These secretory granules contain at least two different classes of proteins, perforin and granazymes, collectively called cytotoxins. Perforin generates transmembrane pores in cell membrane of target cell (Mossman and Tschopp 1985., Podack et. al. 1985., Young et. al. 1986b, 1986f). The granazymes containing proteases or fragmentins are serine proteases in nature. They cause DNA fragmentation and protein proteolysis. However, granazymes are not directly responsible for DNA fragmentation and probably act by inducing cells endogenous apoptotic program (Fig B & C) (Russel et. al. 1980., Martz et. al. 1989).

It has been seen that cytotoxic action of CD8+ T cells is dependent on calcium (perforin requires calcium) but even in absence of calcium lysis of target cell takes place. It means that a second pathway for target lysis operates. This second pathway is Fas (APO1) dependent, this molecule expressed on target cells and belongs to the TNF receptor family. Its counterpart or co-receptor is the Fas ligand (FasL) on the surface of the cytotoxic T lymphocyte (Fig). This interaction leads to intracellular signal transduction culminating in the apoptosis of the target cell. (Podack 1995., Kagi et. al
Target Death

(LFA-1, etc.)

TCR Engaged

'Off' signals to de-activate adhesion

Activation of Signals for activation of coreceptors

Lethal 'Hit' Delivered

As far as activation of T cells are concerned as mentioned earlier, they, initially interact with antigen in association with either MHC class I or class II. This interaction initiates signal transduction events which result in activation of transcription factors like NF-AT and NF-κB. These transcription factors bind to their respective enhancer regions of the IL-2 gene and leads to the transcription of IL2 mRNA. This is known as the first signal. However this alone is insufficient for the production of IL-2. It has been seen that for the optimum activation the T cells require other molecules like B7 on APC and its co-receptor CD28 on T cells. This ligation has two effect firstly, it has a stabilising effect on IL-2 mRNA since cytokine mRNAs are very unstable, due to an ‘instability sequence’ in their 3’ untranslated region. This stabilisation leads to increase in IL-2 synthesis by 20-30 fold. Secondly, it has a synergistic effect on IL2 mRNA transcription by increasing it by around 3 folds (Lindsten et. al. 1989) Costimulation also results in prolonged cell survival via the upregulation of Bcl-x (Boise et. al. 1995), a member of the Bcl-2 family of anti apoptotic genes (Boise et. al. 1993). This additional and essential requirement is known as the accessory signal or costimulatory signal.

Subsequently it has been found that there are two types of B7. The first one to be identified was B7.1 and the second is B7.2. The overall structure of B7.1 and B7.2 has been found to be similar. It has been found that B7 has another receptor apart from CD28, known as CTLA-4. These two are differentially expressed on different stages of T cells. The CD28 is expressed both on resting as well as activated T cells. It is
characterized by low avidity, high abundance and constitutive expression. On the other hand CTLA-4 (CD86) is characterized by high avidity, low abundance and inducible expression (Linsley and Ledbetter., 1993).

It has been seen that CTLA-4 cooperates with CD28 to upregulate T cell activation or it may antagonise CD28 and may help in down regulation of T cell response. (Lenschow et al. 1996)

**IMPORTANT OF COSTIMULATION:** In the absence of costimulation the T cells are destined to anergy i.e. deletion of mature T cells. It has been seen that in case of intracellular pathogens like *M. tuberculosis* and *L. donovani*, B7 is down regulated on the surface of the macrophages. The T cells interacting with these infected macrophages become anergised and are finally eliminated by apoptosis (Gobardhan Das, Personal communication). It has been shown that B7 is required for efficient induction, recruitment, and effector function of anti tumor CD8+ T cell responses. Transfection of B7/BB1 can successfully induce rejection of a MHC class II positive melanoma (Chen et al. 1992 & Townsend et al. 1993). Abrogation of B7/CD28 interaction by means of CTLA-4Ig have been shown to have significant effect on the progression of several autoimmune diseases like relapsing experimental autoimmune encephalitis (Perrin et al. 1995; Cross et al. 1995), diabetes in nonobese diabetic (NOD) mouse (Lenschow et al 1996) and other autoimmune models like murine lupus (Finck et al. 1994) and glomerulonephritis (Nishikawa et al. 1994). The treatment resulted in blocked of clinical disorder.
OBJECTIVE OF THESIS: The aim of this thesis is to examine the role of costimulation in the maturation of CD8+ pre-CTL to its mature stage. This mature CTL is capable of acquiring effector molecules to destroy the cells which are infected with intracellular pathogens or viruses or cancerous cells.
REFERENCES:


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