# 1. Introduction

The Immune system is a powerful barrier protecting the body of an organism from invading pathogens. The inherent ability or first line of defense towards an infecting agent is called innate immunity. It is characterized by broad specificity, lack of memory responses and a limited repertoire of molecules recognizing antigens. On the other hand, adaptive immunity refers to antigen-specific responses, which are long lasting and generated after complex interactions between various immune cells. Adaptive immunity can be conveniently classified into humoral and cell mediated immunity. The humoral component of the immune system is responsible for producing antigen-specific antibodies through B cells and the cell-mediated immunity involves the generation of effector cells, which are mainly CD4 and CD8 T cells. CD4 T cells are important for helper activity and CD8 T cells for cytotoxic activity. Cells of the immune system develop and interact in specific structures, which are called primary lymphoid organs (bone marrow and thymus) and secondary lymphoid organs (spleen, lymph nodes and payers patches).

In 1966 Claman et al., working with bone marrow and thymus cell mixtures, provided the first evidence that these two cell types co-operate with each other (Claman *et al.*, 1966). This was the time when markers for B and T cells were still not known. A year later, Miller and Mitchell found that it was bone marrow that produced precursors of antibody forming cells and that thymus derived cells are required to help the antibody forming cells to produce antibodies (Mitchell and Miller, 1968). This work was the gateway to the vast field of cognate cell-cell interactions in immunology. It was in 1969, that Roitt et al., introduced the term B and T cell for thymus derived and bone marrow derived cells, respectively (Roitt *et al.*, 1969). Eventually the term T cell-B cell collaboration or more specifically T cell help came into existence.

## 1.1. Chemokines and chemokine receptors

The chemokine receptors are members of a superfamily of seven transmembrane domains and coupled to pertussis toxin sensitive heterotrimeric G-proteins (Baggiolini *et al.*, 1997; Murphy, 1994; Rossi and Zlotnik, 2000). Chemokine receptors get activated and signal upon binding with specific chemokine ligands. There are about 50 different chemokines binding to one or more of 20 chemokine receptors (Table 1). Chemokines derive their name from chemotatic cytokines. Most chemokines contain at least 4 conserved cysteine residues that form two disulfide bonds, one between the first and the third and one between the second

and the fourth cysteine. Chemokines are systematically classified into four sub-families CC, CXC, XC and CX3C, based on the number of amino acids between the two cysteines (Murphy *et al.*, 2000; Rossi and Zlotnik, 2000; Rot and von Andrian, 2004). CC, CXC and CX3C chemokines have four cysteines, whereas C chemokines have only two cysteines corresponding to the second and fourth cysteine in the other groups. CC and CXC chemokines are the most common ones with CC being the largest sub-family. There is only one member belonging to each of C and CX3C chemokines, lymphotactin $\alpha/\beta$ and fractalkine, respectively. Chemokine receptors are classified according to the type of chemokine they bind like CC, CXC, XC and CX3C, followed by "R" for receptor. A number in each chemokine and chemokine receptor indicates the chronology of their discovery. Before introducing this nomenclature chemokines and their receptors were referred to by their common synonyms (Table 1).

| Receptor | Ligand | Expression |
|---|---|---|
| CXC family | | |
| CXCR1 | CXCL2, 3,5,6,7,8 | PMN, MC, Mo, Φ |
| CXCR2 | CXCL1, 2,3,5,6,7,8 | PMN, MC, Mo, Φ |
| CXCR3 | CXCL9, 10,11 | T (Th1>Th2), B, NK |
| CXCR4 | CXCL12 | Pro, T, B, PMN, Mo, Φ, DC |
| CXCR5 | CXCL13 | B cells, $T_{FH}$, memory T cells |
| CXCR6 | CXCL16 | memory T cells |
| CXCR7/RDC1 | CXCL12 | T, B, Mo, DC |
| CC family | | |
| CCR1 | CCL3, 5,7,14,15,16,23 | memory T |
| CCR2 | CCL2, 7,12,13 | Mo, DC, NK, Bas, PMN |
| CCR3 | CCL5, 7,8,13,15,24,26 | Eos, Bas, MC, T (Th2>Th1) |
| CCR4 | CCL17, 22 | T (Th2>Th1) |
| CCR5 | CCL3, 4,5 | Pro, Th1, Mo, Φ, DC |
| CCR6 | CCL20 | memory T, DC |
| CCR7 | CCL19, 21 | T, B, DC |
| CCR8 | CCL1, 4 | Th2 cells |
| CCR9 | CCL25 | $\alpha4\beta7^{+}$ T, DC, Φ, Thy |
| CCR10 | CCL27 | CLA T cells |
| CCR11 | CCL2, 8, 13 | n.a |
| C family | | |
| XCR1 | XCL1, 2 | T |
| CX3C family | | |
| $CX_3CR1$ | $CX_3CL1$ | PMN, Mo, NK, T |

Table 1: Chemokine receptors, their ligands and expression on various immune cells. T – T cell, B – B cell, DC – dendritic cell, NK – natural killer cell, Mo –

monocytes, Φ – macrophage, MC – mast cell, Eos – eosinophils, Bas – basophils, Th1 – T helper 1, Th2 – T helper 2, $T_{FH}$ – Follicular B helper T cells, Thy – thymocytes, Pro – progenitor cells, n.a – not available (from Oslon *et al*., 2002; Murphy *et al*., 2000).

Another classification is based on the biological function exerted by chemokines such as homeostatic, inflammatory or both (Loetscher *et al.*, 2000; Moser and Loetscher, 2001; Moser *et al.*, 2004; Sallusto *et al.*, 2000). Homeostatic chemokines, also known as lymphoid chemokines, are constitutively produced in bone marrow, thymus, secondary lymphoid organs and non-lymphoid tissues like skin and mucosa. These chemokines mediate trafficking of immune cells during hematopoiesis, control lymphocyte recirculation between into secondary lymphoid organs in the absence of infection. For example compartmentalization of secondary lymphoid organs into T cell zones and B cell follicles is based on the expression of homeostatic chemokines. Furthermore, homeostatic chemokines mediate fine-tuning of the micro-anatomical positioning of T and B cells at the boundary between T cell zone and B cell follicle (Muller *et al.*, 2002). Homeostatic chemokines are also expressed by endothelial cells of the lymphatics and high endothelial venules (HEVs) enabling mature dendritic cells (DCs), T cells and B cells to gain entry into secondary lymphoid organs.

During generation and progression of an inflammatory response inflammatory chemokines are induced to recruit effector leukocytes. Receptors for inflammatory chemokines include CCR1, CCR2, CCR5, CXCR3, CXCR6 and CX3CR1. Th1 and Th2 are the two helper subsets generated from naïve cells in CD4 compartment (discussed later). These cells are characterized by preferential expression of certain chemokine receptors. CCR1, CCR5 and CXCR3 are typically expressed on Th1 cells whereas CCR2 is expressed on both the subsets (Sallusto *et al.*, 1998a).

## 1.2. Entry of lymphocytes into lymphoid organs

Bone marrow and thymus are the primary lymphoid organs where lymphocyte development occurs. Lymphocytes differentiate from hematopoietic precursors in bone marrow. Pre-T lymphocytes that are formed in bone marrow emigrate into thymus, where they mature and depending on the affinity of the T cell antigen receptor (TCR) for self-major histocompatibility complex (MHC) antigens they are positively or negatively selected. Mature T cells express S1P1, which regulates the exit from thymus and enter into vascular compartment (Matloubian *et al.*, 2004). Newly formed naïve T cells recirculate between secondary lymphoid organs in search of cognate antigens. The function of lymphoid organs is to bring the

antigen presenting DCs, B cells and T cells in close contact to initiate immune responses. In order to enter lymphoid organs, lymphocytes have to cross the barrier of high endothelial venules (HEVs) and this process is termed as extravasation. It is divided into four steps: tethering, rolling, activation and arrest. All the steps are controlled by the expression of adhesion molecules and chemokine receptors on the surface of lymphocytes and their interaction with endothelial ligands. Endothelial cells of HEVs express chemokine CCL21, peripheral lymph node addressin (PNAd) and mucosal addressin cell adhesion molecule-1 (MAdCAM-1). On the other hand, T cells express CCR7, L-selectin and integrin $\alpha 4\beta 7$ which interacts with CCR7, PNAd and MAdCAM-1, respectively on endothelial surface (Berlin *et al.*, 1995; Berlin *et al.*, 1993; Bradley *et al.*, 1994; Luster *et al.*, 2005). As a result, T cells start to “tether” and “roll” on the surface of endothelial cells. At this point, chemokines play an important role by binding to chemokine receptors and “activating” integrins to initiate firm adhesion. Subsequently, “arrested” T cells enter the lymphoid organ by diapedesis.

CXCR4 may also contribute to T cell extravasation but its absence did not abrogate lymphocyte entry into lymphoid organs (Okada *et al.*, 2002). Similarly, B cell entry is mediated by CCR7 and CXCR4. CCR7 is expressed at a lower level on B cells as compared to Naïve T cells. An additional chemokine receptor CXCR5 expressed on B cells has subordinate role as compared to CXCR4 and CCR7 in B cell entry into secondary lymphoid organs (Okada *et al.*, 2002). Rather, CXCR5 direct B cell entry into B cell follicles where its ligand CXCL13 is abundantly expressed. Thus, CCR7 primarily mediates the process of T and B cell exit from blood to secondary lymphoid tissue.

## 1.3. CCR7 and CXCR5 compartmentalize secondary lymphoid organs in homeostasis

When lymphocytes enter secondary lymphoid organs they differentially migrate to cortical and paracortical areas under the influence of chemokines and chemokine receptors (Fig 1). T cells are retained around the arteriole whereas B cells move further out in forming B cell follicles. Stromal cells within the T cell zone of secondary lymphoid organ constitutively express CCL19 and CCL21, which are known ligands of CCR7 (Cyster, 1999a; Luther *et al.*, 2000). Mature dendritic cells, which migrate into T cell areas, also secrete CCL19 (Ngo *et al.*, 1998). These cells also express CCR7 and localize to T cell area to be able to prime naïve T cells (Steinman *et al.*, 1997). Thus, during homeostasis, naïve T cells expressing CCR7 are retained in T cell areas. CCR7-deficient mice showed reduced numbers of naïve T cells in lymph nodes, severely delayed antibody response due to disturbed

morphology of secondary lymphoid organs and activated dendritic cells failed to migrate into draining lymph nodes (Forster et al., 1999). The mutation, paucity of lymph node T cell (*plt*) involves a spontaneous mutation resulting in loss of CCL19 and CCL21 in secondary lymphoid organs. In *plt*/*plt* mice a second gene for CCL21 is active, which is expressed in non-lymphoid organs and lymphatic endothelium. These mice exhibit a defective migration of T cell and DCs in secondary lymphoid organs as observed in CCR7-deficient mice (Nakano *et al.*, 1998; Nakano *et al.*, 1997).

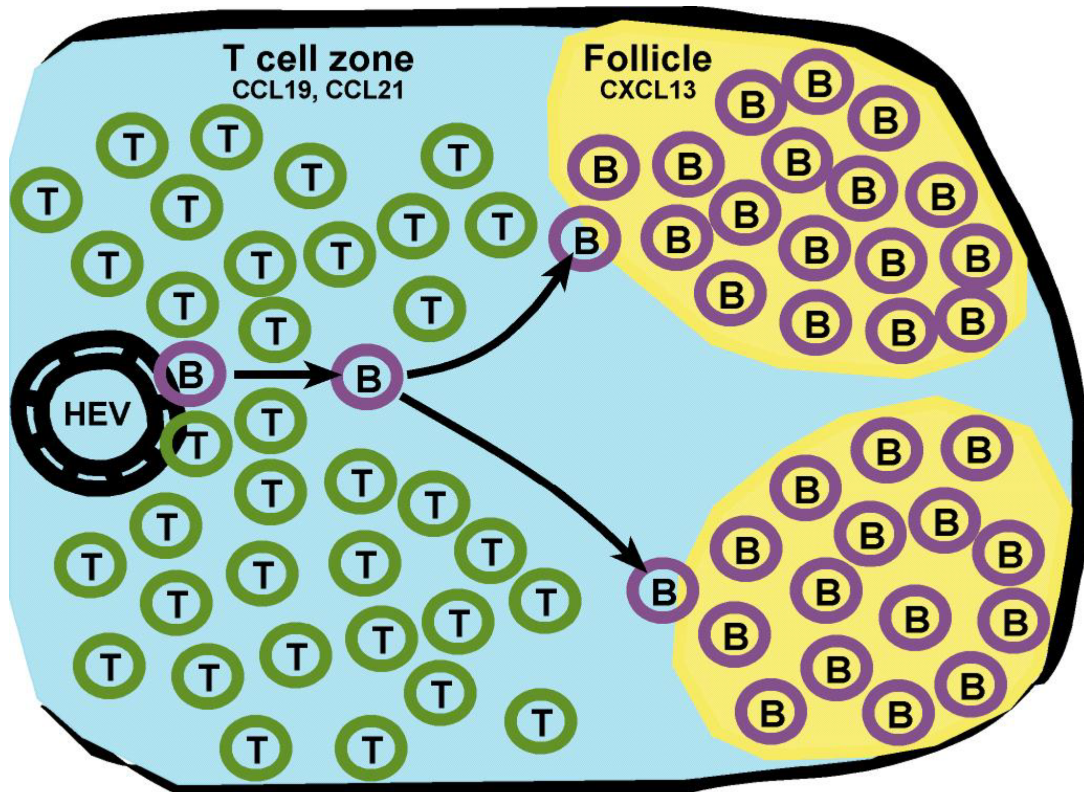


**Fig. 1: Compartmentalization of secondary lymphoid organ during homeostasis.** B cells and T cells undergo transendothelial extravasation from high endothelial venules (HEVs) and enter the lymphoid organs. T cells (green) expressing CCR7 are retained in the T cell zone as stromal cells express CCL19 and CCL21 (blue) whereas dendritic cells express CCL19 (Blue). On the other hand, expression of CXCR5 on B cells (Violet) renders them to respond to CXCL13 (yellow) and migrate to B cell follicles.

On the other hand stromal cells in B cell follicles and follicular dendritic cells (FDCs) express CXCL13, which is the only known ligand for CXCR5 (Ansel *et al.*, 2000; Forster *et al.*, 1996). CXCR5 deficiency in mice results in lack of functional germinal center formation. B cells from CXCR5-deficient mice when adoptively transferred to wild type recipients fail to migrate into B cell follicles but are trapped inside the T cell areas (Forster *et al.*, 1996). In addition, CXCR5 mice also showed

lack of inguinal lymph nodes and impaired development of payer's patches. Mice lacking CXCL13 expression had similar defects in peripheral lymph nodes and payers patches (Ansel *et al.*, 2000). Recirculating mature B cells express CXCR5, which is used to rapidly pass through T cell areas and gain entry into B cell follicles where CXCL13 is abundantly expressed.

A Combined deficiency of CXCR5 and CCR7 in mice results in the lack of all peripheral, besides mesenteric lymph nodes, a severely disrupted morphology in spleen and an absence of B cell follicles (Ohl *et al.*, 2003). Taken together, the compartmental homing of T and B cells and dendritic cells is highly dependent on the expression and cooperation of the chemokine receptors CXCR5 and CCR7.

## 1.4. Activation of T cells in secondary lymphoid organs

During the onset of an infection, immature DCs capture antigen in peripheral tissues and process it to become mature DCs. The antigen is degraded into small peptides and presented on the cell surface bound to MHC. Subsequently DCs up regulate CCR7, which render these cells responsive to CCL19 and CCL21, allowing migration to draining lymph nodes (Chan *et al.*, 1999; Kellermann *et al.*, 1999). Unlike lymphocytes, which enter lymph node via HEVs, DCs use afferent lymphatic to enter secondary lymphoid organs (Gunn, 2003). The important role of CCR7 is reflected in CCR7-deficient DCs that failed to migrate into the draining lymph nodes as compared to wild type DCs (MartIn-Fontecha *et al.*, 2003). DCs in secondary lymphoid organs express co-stimulatory and adhesion molecules that make them highly effective antigen presenting cells. In addition, DCs are an additional source of CCL19 apart from T cell zone stromal cells (Cyster, 1999b). Secretion of CCR7 ligands by DCs is likely to render more efficient attraction of T cells for antigen presentation.

Within the T cell areas, naïve CD4 T cells encounter mature dendritic cells in search of antigens. T cells that recognize foreign peptide:MHC complexes on the surface of the DCs eventually get activated (Lanzavecchia and Sallusto, 2000). According to the "Two-signal model" proposed by Bretscher and Cohn way back in 1970, two signals are required for activation of T cells (Bretscher and Cohn, 1970). The first signal originates from the ligation of the TCR complex along with the CD4 or CD8 co-receptor and the second signal is mediated through the interaction of CD80 and CD86 on dendritic cells and CD28 on T cells (Lenschow *et al.*, 1996). Co-stimulatory signals are essential for T cells to sustain the immune response. Following activation T cells upregulate cytokine expression and undergo clonal expansion and differentiation. An important cytokine driving the proliferation of T

cells is IL-2, which is upregulated immediately after activation (Larsson *et al.*, 1980; Smith *et al.*, 1980). At this point, CD4 T cells transiently up regulate CXCR5 (Ansel *et al.*, 1999; Schaerli *et al.*, 2001) and display reduced responsiveness towards CCR7 ligands (Hardtke *et al.*, 2005). CD4 T cells that have downregulated CCR7 migrate towards B cell follicles in order to make cognate interactions with B cells. This migration process has been shown to be dependent on the balanced expression of CXCR5 and CCR7 on CD4 T cells (Hardtke *et al.*, 2005). Hence, CD4 T cells that express CXCR5 and localize to B cell follicles to provide B cell help are designated as Follicular B helper T cells ($T_{FH}$) (Breitfeld *et al.*, 2000; Schaerli *et al.*, 2000). $T_{FH}$ cells upregulate various cell surface molecules like ICOS and CD40L, which were one of the first few to be identified (Breitfeld *et al.*, 2000).

B cells also take up antigen and present it as peptide:MHC class II complex on their surface. However, for B cells to be able to differentiate efficiently into antibody secreting plasma cells, they have to come in contact with activated T helper cells having the same antigen specificity. Antigen loaded B cells upregulate CCR7 expression and respond to its ligands expressed in T cell area of the lymphoid organ (Reif *et al.*, 2002). As a result, B and T cells migrate towards each other to interact at the border between T cell area and B cell follicle to form B cell-T cell conjugates (Okada *et al.*, 2005).

## 1.5. Germinal centers – product of $T_{FH}$ cell - B cell interaction

After receiving cognate help from CD4 T cells, B cell are known as “germinal center founder B cells” which migrate into the neighboring follicles and initiate germinal center formation by rapid expansion. Resting B cells are displaced towards the outer edge of the follicle by the accumulating mass of centroblasts creating a “mantle”. This part of the germinal center with tightly packed centroblasts is called “dark zone”. Centroblasts that have either lost the capacity to divide are known as centrocytes; these migrate out of the “dark zone” to form the other half of the germinal center known as “light zone” (Cozine *et al.*, 2005). Chemokine and chemokine receptors play an important role in the formation of germinal centers. Centroblasts express higher levels of CXCR4 and are drawn towards CXCL12 expressed by stromal cells in dark zone, whereas the light zone is organized by CXCL13 secreting follicular dendritic cells (FDC) and $T_{FH}$ cells (Allen *et al.*, 2004). Dark and light zones are further characterized based on the functional events occurring in dividing centroblasts and non-dividing centrocytes. In the germinal center dark zone, isotype switching and somatic hypermutation takes place, where as light zone is the site of affinity maturation.

Germinal center founder B cells synthesize IgM and IgD, however, during the course of an immune response they switch to another class IgG, IgA or IgE to increase the functional diversity of immunoglobulin molecules without affecting their antigen binding specificity. Isotype switching or class switch recombination (CSR) is the hallmark of T cell dependent immune responses that requires immunoglobulin gene rearrangement. This involves the replacement of the $\mu$ (for IgM) constant region with one of the downstream $\gamma$ (for IgG), $\alpha$ (for IgA) or $\varepsilon$ (for IgE) constant regions. The constant region of the heavy chain determines the isotype of the antibody. The variable region (VDJ) specific for the antigen will be retained along with the newly formed heavy chain. This process occurs using different flanking recombination sequences and various enzymes and is highly dependent on T cell help. B cells secreting antibodies of one isotype and specificity are known as plasma cell.

Somatic hypermutation (SHM) is a process leading to point mutations in the variable regions of heavy and light chain genes to substitute new amino acids. This enables B cells to generate post germinal center clones with high affinity for the antigen. Nonetheless, both low affinity and high affinity clones formed in dark zone are now pushed into the light zone where they are selected based on the affinity to the specific antigen. High affinity germinal center B cells receive stimuli from $T_{FH}$ cells and follicular dendritic cells and give rise to memory B cells and plasma cells. On the other hand low affinity B cells do not receive survival signals and as a result undergo apoptosis and are ingested by tangible body macrophages.

## 1.6. Molecular interaction of activated T and B cell in lymphoid organs

When activated B and CD4 T cells are drawn in close proximity to each other a number of cognate receptor-ligand pairs are formed to initiate contact-mediated signals between T and B cells. Since activated CD4 T cells have to mediate antigen specific signals acquired from dendritic cells to B cells, a number of cell surface receptors are differentially regulated for a productive humoral immune response. Most notable are the co-stimulatory molecules belonging to CD28 and TNFR families, due to their profound impact on the outcome of immune responses. Each of the groups will be summarized in the context of B cell help. ICOS will be dealt with more elaborately because of its importance for the present study.

## 1.7. The CD28 family

Currently there are five known members of this family expressed on T cells (Table 2). Two of them, CD28 (CD28 antigen) and ICOS (inducible co-stimulator) exert positive co-stimulation; the other two, CTLA-4 (cytotoxic T-lymphocyte associated protein 4) and PD-1 (programmed cell death 1) have negative co-stimulatory effects. The role of BTLA (B and T lymphocyte-attenuator protein) is still not defined. In order to dampen an immune response, positive co-stimulatory signals generated during the T cell activation are countered by negative signals. CD28 is constitutively expressed on resting T cells and its expression is enhanced on upon activation (Lenschow *et al.*, 1996). All others CD28 family members are expressed upon activation at different time points. Members of CD28 family act as receptors for ligands from the B7 family, which are expressed on various cell types including dendritic cells and B cells. CD28 and CTLA-4 share the same ligands, CD80 and CD86, whereas ICOS, PD-1 and BTLA-4 possess their own ligands. ICOS binds to ICOSL, also known as B7h or B7RP-1, PD-1 has two ligands PD-L1 (B7-H1) and PD-L2 (B7-DC) and BTLA has one ligand B7x (B7-H4) (Greenwald *et al.*, 2005; Rudd and Schneider, 2003).

| Receptor | Ligand | Expression | Function |
|---|---|---|---|
| CD28 family | | | |
| CD28 | CD80 (B7.1), CD86 (B7.2) | T cell, constitutive | activation |
| ICOS | ICOS-L (B7h-2, B7h) | T cell, activated | activation |
| CTLA-4 (CD512) | CD80 (B7.1), CD86 (B7.2) | T cell, activated | inhibition |
| PD-1 | PD-L1 (B7-H1), PDL-2 (B7-DC) | T cell, activated | inhibition |
| | | B cell, activated | CC arrest |
| BTLA | B7-H3 | T cell | activation? |

Table 2: CD28 family, their ligands, expression and function on T and B cells. ICOS – inducible co-stimulator, CTLA-4 – cytotoxic T-lymphocyte associated protein 4, PD-1 – programmed cell death 1, BTLA – B and T lymphocyte-attenuator protein, CC – cell cycle (from Peter-Warnatz, 2005)

CD28 co-stimulation is important for T cell survival, as cells receiving TCR signal in the absence of CD28 become anergic and undergo apoptosis. When T cells receive both signals from mature dendritic cells, they get activated, differentiate and upregulate CTLA-4 and ICOS. CD28 and ICOS-deficient mice have profound defects in T cell dependent B cell immune responses as they share downstream signaling pathways necessary for providing survival signals (Rudd and Schneider, 2003). In line with this finding, Riley et al., have found that CD28 or ICOS ligation

in combination of TCR stimulation, modulates similar set of genes (Riley *et al.*, 2002). Activated T cells do not require CD28 signaling which indicates that secondary responses are not dependent on the expression of CD28, rather its function is substituted by ICOS (Ogawa *et al.*, 2001).

## 1.8. Role of ICOS in regulating activated T and B cells

ICOS and CD28 have a consensus motif in their cytoplasmic domain, Tyr-Met-Asn-Met in CD28 and Tyr-Met-Phe-Met in ICOS (Rudd and Schneider, 2003). This motif binds to SH2 domains of the p85 subunit of phosphatidylinositol 3-kinase (PI3K). ICOS costimulation leads to greater PI3K activity compared to stimulation via CD28 alone (Parry *et al.*, 2003). Engagement of PI3K leads to activation of MAP-kinases and NF-κB resulting in increased transcription of IL-10, IL-4 and IFN-γ and prevention of apoptosis (Arimura *et al.*, 2002). However, in contrast to CD28, ICOS lack the ability to bind the adaptor protein Grb2 that is required for the initiation of IL-2 production via NFAT/AP-1 (Harada *et al.*, 2003).

ICOS-deficient mice have normal B and T cell populations indicating that ICOS-ICOSL signaling is not important during lymphocyte development (Dong *et al.*, 2001b; McAdam *et al.*, 2001). T cell-independent responses are normal but T cell-dependent responses were impaired with reduced germinal center formation during secondary responses and profound defects in immunoglobulin isotype class switching. Cytokine production including IL-2, IL-4, IL-10 and IL-13 are also diminished in $ICOS^{-/-}$ mice. A similar phenotype was observed in case of ICOSL-deficient mice (Mak *et al.*, 2003; Wong *et al.*, 2003). Presumably, germinal center formation by B cells essentially requires CD4 T cell help, which is abrogated in the absence of ICOS-ICOSL ligation. Recent investigations reveal the essential requirement of ICOS signaling in humans. Common variable immunodeficiency (CVID) is a primary immunodeficiency disorder characterized by hypergamma-globulinemia. It is caused by various genetic defects and ICOS-deficiency was reported in 4 patients with adult-onset CVID (Grimbacher *et al.*, 2003). These patients had inherited homozygous deletion of ICOS. T cell subset distribution and their phenotype were normal but in contrast to $ICOS^{-/-}$ mice, where B cell development was normal, these patients had reduced B cell numbers, lack of memory B cells and low serum immunoglobulin levels. This indicates that in humans expression of ICOS on T cell is essential for providing B cell help. These findings also suggest that ICOS deficiency in mice and humans had variable consequences. ICOS signaling is regulated in activated T cells upon interaction with its cognate ligand ICOS-L. Apart from being constitutively expressed on B cells, ICOS-L mRNA is also detected in non lymphoid tissues like kidney, liver,

heart and brain (Carreno and Collins, 2002). IFN-γ has been shown to induce the expression of ICOS-L on monocytes (Aicher *et al.*, 2000).

### 1.8.1. ICOS in autoimmunity and infections

ICOS-L expression is thought to contribute not only to regulating immune responses but also contribute to autoimmune responses in case of aberrant expression. Hence the role of ICOS was studied in several murine models with autoimmune diseases like collagen-induced arthritis (CIA), experimental allergic encephalomyelitis (EAE), systemic lupus erythematosus (SLE).

CIA is an approved model to study rheumatoid arthritis, which is considered to be a T cell dependent disease, resulting in cartilage destruction and synovial hyperplasia. EAE resembles multiple sclerosis in humans, in which mice immunized with myelin basic protein (MBP) develop an inflammatory reaction in the brain that cause a progressive paralysis and death. This reaction is Th1 mediated which secrete cytokines to activate macrophages. Activated macrophages in turn destroy oligodendrocytes. SLE in humans is a chronic systemic autoimmune disease characterized by antinuclear antibodies, arthritis, skin involvement, nephritis, cerebritis, pleuritis, pericarditis, hemolytic anaemia, clotting and thrombocytopenia.

ICOS deficient-mice were shown to be resistant to CIA (Nurieva, 2005), whereas ICOS-deficient mice or blocking of ICOS-ICOS-L interaction showed enhanced susceptibility to EAE (Dong *et al.*, 2001a; Sporici and Perrin, 2001). However, when ICOS-ICOS-L was blocked, progression of EAE showed different response at priming and effector phase (Rottman *et al.*, 2001). Blocking of ICOS signal in priming phase exacerbated the clinical symptoms whereas blocking at priming phase abrogated clinical symptoms. In patients with SLE, ICOS expression was increased on T cells but B cells on the other hand downregulated ICOS-L, which was attributed to be the consequence of recent B-T cell interaction (Hutloff *et al.*, 2004). In Inflammatory bowel disease, higher expression of ICOS was also observed on activated CD4 T cells from intestinal lamina propria mononuclear cells (Sato *et al.*, 2004). Kopf et al., have shown that both Th1 and Th2 responses for vesicular stomatitis virus and nematode *Nippostrongylus brasiliensis*, respectively, were reduced in the absence of CD28. Interestingly, when ICOS signaling was blocked in these mice, Th1 and Th2 responses were completely abrogated (Kopf *et al.*, 2000).

## 1.9. TNF/TNFR family

Tumor necrosis factor family members act as T cell co-stimulators and also play a key role in the induction and maintenance of germinal centers. Most notable are the up-regulation of CD40L and OX40 on T cells (Breitfeld *et al.*, 2000; Flynn *et al.*, 1998; Walker *et al.*, 1999). B cells express corresponding molecules - CD40 is the receptor for CD40L and OX40L binds to OX40.

The significance of CD40L on the outcome of T cell-dependent humoral immune responses has been well documented (van Kooten and Banchereau, 2000). Deficiency of either CD40 or its ligand in mice leads to a lack of germinal center formation apart from defects in B cell proliferation, immunoglobulin production and isotype switching. Activation of T cells leads to overlapping expression of members of the CD28 and TNF/TNFR families. Triggering via TCR and CD28 costimulation leads to expression of OX40 along with ICOS. Furthermore costimulation through OX40 induces CXCR5 expression. Hence, expression of ICOS precedes the induction of CXCR5, which is worth notable at this juncture. B cells express receptors for BAFF (B lymphocyte stimulator) and APRIL (a proliferation inducing ligand). BAFF binds to BAFF-R, BCMA and TACI and APRIL binds to BCMA and TACI. Involvement of BAFF and APRIL as T cell co stimulators is still not clear. Other receptor-ligand pairs like 4-1BB-1BBL, Trance-Rank, Fas-FasL are also known to be involved in T-B cell interaction.

## 1.10. CD4 T cell subsets in peripheral blood

Mossmann et al. first reported that naïve CD4 T cells differentiate into two distinct helper subsets namely, T helper 1 (Th1) and T helper 2 (Th2) cells based on the pattern of cytokine production (Mosmann *et al.*, 1986). The generation of Th1 cells is primarily driven by IL-12 that signals via STAT4 to produce cytokines such as IFNγ and IL-2 to favors a strong cellular response. On the other hand, IL-4 signals via STAT6 to skew differentiation towards the Th2 phenotype, which is characterized by secretion of cytokines, like IL-4, IL-6, IL-10 and IL-13. Th1 and Th2 lineage decision is dictated by the nature of the antigen and cytokine milieu. Th1 and Th2 differentiation is controlled by distinct transcription factors, T-bet and GATA3, respectively. This classification was soon adopted widely because of two characteristic features. First, cytokine secreted by each cell type, IL-12 for Th1 and IL-4 for Th2, served as their own growth factors in the form of a feed-forward mechanism. The second feature was that the cytokines IL-12 and IL-4 were inhibitory to Th2 and Th1, respectively. Hence, these two mechanisms efficiently modulated the outcome of Th1 1 or Th 2 immune responses.

Since Th2 cells are considered to be important for B cell maturation it was previously thought that these cells are also involved in B cell help (Abbas *et al.*, 1996; Mosmann *et al.*, 1986; Randolph *et al.*, 1999). Th2 cells are shown to promote strong humoral responses with class switching to IgG1 and IgE whereas Th1 cells induce class switching to IgG2a, but Th1 cells are inefficient in providing B cell help (Stevens *et al.*, 1988). Hence the existence of a separate subset for B cell help was not conceived until recently when it was shown that the existence of a non-Th1/Th2 subset is responsible for B cell help (Breitfeld *et al.*, 2000; Schaerli *et al.*, 2000).

The Th1/Th2 paradigm is the benchmark to understand the two arms of immune system, humoral and cell mediated immunity. But to understand the migration pattern of naïve, memory and effector T cells, a second classification was inevitable. In addition, the relationship of Th1/Th2 effector cells to memory compartment seems to be disconnected. Hence a classification based on the expression of chemokine receptors was put forward which elucidates migration and homing aspects in relation to their memory and effector properties.

In peripheral blood naïve T cells are characterized by expression of CD45RA whereas antigen experienced T cells switch to another isoforms of CD45, CD45RO. Based on the expression of chemokine receptor CCR7, peripheral blood $CD4^+CD45RA^+$ T cells can be conveniently subdivided into $CD4^+CD45RA^-CCR7^+$ central memory T ($T_{CM}$) cells and $CD4^+CD45RA^-CCR7^-$ effector memory T ($T_{EM}$) cells (Sallusto *et al.*, 1999). $T_{CM}$ cells have classical memory features: recirculate through lymph nodes as they retain CCR7 expression and lack immediate effector function. In contrast, $T_{EM}$ cells resemble more like classical effector cells: enriched in non-lymphoid organs, with immediate effector function. Based on the length of telomerase, $T_{CM}$ cells are considered to have divided less as compared to $T_{EM}$ cells. $T_{CM}$ cells preferably produce IL-2 whereas $T_{EM}$ cells have capacity to secrete cytokines like IFN$\gamma$, IL-4, IL-5 and IL-13 (Geginat *et al.*, 2001; Sallusto *et al.*, 1999). $T_{EM}$ cells but not $T_{CM}$ cells, downregulate CD62L (L-Selectin) which is one of the key players to initiate rolling of T cells on the surface of endothelial cells for extravasation into secondary lymphoid organs. The cytokine pattern of $T_{EM}$ cells suggests that it might comprise Th1 and Th2 cells. They also express receptors for inflammatory chemokines such as CCR3, CCR5 and CXCR3, which allow them to enter sites of inflammation in the peripheral tissues (Sallusto *et al.*, 1998b). Central memory T cells have the capacity to differentiate into pre-Th1 and pre-Th2 cells that were identified based on the expression of the chemokine receptors CXCR3 and CCR4 (Rivino *et al.*, 2004).

The lineage relationship among the peripheral blood CD4 T cell subsets is still a matter of debate. It is not clear if the memory cells are generated before or after the effector phase. In humans, stimulation of naïve CD4 T cells gives rise to $T_{CM}$ and $T_{EM}$ cells whereas upon restimulation $T_{CM}$ cells gives rise to terminally differentiated $T_{EM}$ cells. Based on these findings it is hypothesized that CD4 T cells follow a linear differentiation pathway. Naïve T cells upon antigen encounter probably differentiate into $T_{CM}$ cells, which further give rise to $T_{EM}$ cells (Sallusto *et al.*, 1999). In contrast, others have proposed that the generation of effector phase precedes memory formation (Dutton *et al.*, 1998; Kaech *et al.*, 2002; Murali-Krishna *et al.*, 1998). Using an acute model of lymphocytic choriomeningitis virus and intracellular bacterium *Listeria monocytogenes* in mice Wherry et. al., have observed that upon antigen clearance, $T_{EM}$ cells differentiated directly into $T_{CM}$ cells in CD8 compartment (Wherry *et al.*, 2003). Another study in mice reported that effector generation is not required for memory formation in CD8 T cells indicating a non-linear pathway (Manjunath *et al.*, 2001). Hence, the lineage relationship of memory and effector cells in CD4 and CD8 T cell compartments in mice and humans might differ depending on the nature and strength of antigen which still needs to be investigated in detail.

An additional memory subset with similar phenotype as $T_{CM}$, but expressing CXCR5 is identified. This constituted about 10% - 14% of CD4 T cells in peripheral blood of humans. This subset is named as central memory 1 ($T_{CM1}$) (Muller *et al.*, 2002). Recent finding reveal that $T_{CM1}$ cells lacking CXCR3 and CCR4 are nonpolarized and require TCR and cytokine stimulation to differentiate into Th1 and Th2 cells (Rivino *et al.*, 2004). The origin and fate of $T_{CM1}$ cells is still not clear. It was proposed that these cells might arise from naïve cells after antigen encounter in secondary lymphoid organs. Since these cells have not downregulated CCR7 but start to express CXCR5 they might exit into circulation as $T_{CM1}$ (Muller and Lipp, 2003).