INTRODUCTION
Alkaline phosphatase

Alkaline Phosphatase (APase, EC 3.1.3.1), an orthophosphoric-monoester phosphohydrolase, is present in most species from bacteria to man (McComb et al 1979, Millan 2006). The enzyme was first isolated from ossifying bone and cartilage (Robinson 1923). The enzyme catalyzes the hydrolysis of phosphomonoesters, including Tyr/Ser/Thr-phosphates in phosphoproteins with release of inorganic phosphate and alcohol at an alkaline pH between pH 8.2 to 10.7 (Schwartz & Lipmann 1961, McComb et al 1979, Chan & Stinson 1986, Pezzi et al 1991, Sarroulihe et al 1992, Coleman 1992).

![Reaction mechanism of Alkaline phosphatase](image)

Reaction mechanism of Alkaline phosphatase: Scheme of the reaction path for APase (E) that takes into account both transphosphorylation \( (k_t) \) and hydrolysis \( (k_h) \). \( R_1OP \), phosphate monoester; \( R_1OP.E \), noncovalent enzyme complex; \( E-P \), phosphoenzyme complex; \( R_1OH \), alcohol product; \( R_2OH \), phosphate acceptor; \( R_2OP \), phosphorylated acceptor; \( Pi \), inorganic phosphate. (Taken from Coleman JE, Gettins P. Spiro TG, ed. In: Zinc enzymes, Vol. 5. pp. 153-218. New York, Wiley-Interscience, 1983).

The enzyme is inactivated under acidic conditions (Fishman & Ghosh 1967). The pH optimum is affected by the type and concentration of the substrate (Ross et al 1951, Fedde et al 1988). The catalytic activity of APases depends on a multimeric configuration of identical monomers. Each identical subunit possesses one active site, and contains two Zn\(^{2+} \) ions and one Mg\(^{2+} \) ion that stabilize the tertiary
structure. (Coleman & Calebowski 1979, Kim and Wyckoff 1991). APase is a metalloenzyme believed to be homodimeric in serum and membranes, but the tissue non-specific isoform may exist as a homodimeric and/or homotetrameric structure in membranes (Hawrylak & Stinson 1987). Unlike the bacterial enzymes, mammalian APases are all anchored to the plasma membrane via a glycophosphatidylinositol moiety (Udenfriend & Kodukula 1995).

**APase – Genomic localization**

In humans, three out of the four APase isoenzymes are tissue specific: placental (PLAPase); germ cell (GCAPase); and intestinal (IAPase). They are 90-98% homologous at the protein level with PLAPase and GCAPase differing only by 12 aminoacid substitutions. The genes encoding these enzymes are clustered on chromosome 2q37.1. The fourth isozyme, tissue non-specific APase (TNAPase) is ubiquitous particularly abundant in bone, liver and kidney and is only about 50% identical with the other three APases (Stigbrand 1984, Harris 1989, Millan 1992).

Post-translational modification and differential glycosylation of TNAPase gives rise to tissue-specific APases of liver, bone and kidney (Komoda & Sakagishi 1978). It was hypothesized that the physiological role of the sugar moieties could be in the protection of the enzyme from rapid removal from circulation through binding to the asialoglycoprotein receptors of the liver. The different isoenzymes of APase can be differentiated based on their structure, immunological properties and sensitivity to heat and different inhibitors.

The gene encoding TNAPase is localized on short arm of chromosome 1 in humans and on chromosome 4 in mice (Terao et al 1990, Greenberg et al 1990). The gene encoding TNAPase consists of 12 exons distributed over 50kb (Weiss et al 1988a).

Intestinal and placental APase genes are present closely on chromosome 2 in both mice as well as humans (Weiss et al 1988b, Henthorn et al 1988, Knoll et al 1989).
The tissue specificity is achieved by differential transcription and subsequent cotranslational and post-translational modifications controlled by two alternative exons (Terao 1990, Matsuura et al 1990, Studer et al 1991). The transcript of exon I-B is found in bone and kidney while the transcript of exon I- L is found in liver. The mechanism of tissue-specific regulation of TNAP is not yet known in detail.


Structure of Tissue Non specific Alkaline Phosphatase (TNAPase)

Recent determination of the human PLAPase structure provides a complete view of an APase from higher organisms (Le Du et al 2001). Based on the structural evidence and functional divergence of APases conservation in mammals has been proposed (Mornet et al 2001, Le Du & Millan 2002).

The molecular and structural data suggests that APase acquired specialized functions progressively during evolution. Five functional regions have been identified in TNAPase. The active site is the centre of the catalytic activity. The N-terminal arm is essential for stability and allosteric properties of the enzyme (Hoylaerts 2006).

The crown domain is a key factor of uncompetitive inhibition heat stability and allosteric behaviour (Bossi et al 1993, Hoylaerts et al 1997, Kozlenkov et al 2004). The crown domain may also be involved in the binding of TNAP to collagen.
Mammalian APases can be inhibited uncompetitively by a wide range of compounds, which include amino acids, tetramisole, theophylline and NADH. Inhibition by amino acids is stereospecific as L- isomers only display inhibitory activity. Similarly, only the L-isomer of tetramisole (Levamisole) is a good inhibitor of certain APases (Van Belle 1976).

L-Phenylalanine and L-Tryptophan, amino acids with hydrophobic side chains, inhibit mammalian IAPases and human PLAPases with Ki values of about 1mM (Ghosh & Fishman 1966). They are not active towards TNAPase. On the other hand, amino acids with positively charged side chain, such as L-lysine, L-arginine, and especially L-homoarginine, are active as inhibitors with similar Ki values towards TNAPase but not towards other isozymes (Fishman & Sie 1970). Levamisole is also a good selective inhibitor of TNAPases, with Ki values lower than 100µM (Jalanka & Lindberg 1975).

**B-Lymphocyte APase :**

APase activity has been shown to be expressed specifically by mitogen-stimulated B-lymphocytes (Greaves & Janossy 1972). It has also been used as a marker of B cell activation (Garcia-Rozas et al 1982, Burg & Feldbush 1989, Marquez et al 1989). Resting B-lymphocytes and resting and activated T lymphocytes do not express APase activity (Garcia-Rozas et al 1982).

Expression of Apase activity occurs in early G1 phase (around eight hrs) after mitogenic stimulation and continues till differentiation (120 hr) (Kasyapa & Ramanadham 1992).

It has also been shown that the enhancement of APase activity correlates with proliferation and differentiation. It precedes maximal immunoglobulin secretion (Ohno et al 1986, Burg & Feldbush 1989, Marquez et al 1989, Kasyapa & Ramanadham 1992, Souvannavong et al 1994).
APase has also been proposed to be involved in the Ig transport in B-lymphocytes as it was shown to be complexed and secreted out along with IgM. A role for APase in phosphorylation/ dephosphorylation reactions in early stages of signalling in mitogen activated B lymphocytes has also been suggested (Feldbush & Lafrenz 1991, Souvannavong et al. 1992).

It has also been shown that APase activity is not expressed in B-lymphocytes stimulated with incomplete mitogens like (Kasyapa 1996). Further studies have shown that APase activity is expressed in B-lymphocytes committed to proliferation. It has also been shown to be enhanced in antigen activated B-lymphocytes and the activity increases further in the antibody secreting cells (Padmaja & Ramanadham, 1998).

cAMP has been reported as a positive regulator of enhancement of APase activity in mitogen stimulated B-cells (Kasyapa & Ramanadham 1995). Treatment of mitogen stimulated cells with antibody to APase resulted in the inhibition of proliferation and APase activity. It has been shown that the level of APase mRNA increased in mitogen stimulated cells and probably plays a crucial role in cell cycle progression.

Normal B cells do not spontaneously show APase activity but cell activation induces APase expression (Burg & Feldbush 1989, Marquez et al. 1989, Souvannavong & Adam 1990). Importance of APase is indicated by the capacity of antibodies to various glycosyl Phosphatidylinositol anchored molecules to transduce activation signals (Low, 1989).

Several drugs like puromycin, actinomycin D, Colchicine enhance APase activity in culture (Moog 1964, Ikehera et al 1978, Moog & Wiemerslage 1981). Levamisole, an uncompetitive inhibitor of TNAPase has been shown to inhibit APase activity of liver, bone and kidney. 5-‘Bromodeoxyuridine, a thymidine analogue, dbtc AMP, an analogue of cAMP, Sodium butyrate and prednisolone an analogue of hydrocortisone are some of the potent inducers of APase activity.
(Koyama & Ono 1971, Hamilton et al 1979, Firestone & Heath 1981, Herz & Hawler 1983). APase substrates like phenyl phosphate have also been shown to enhance APase activity.

**Functions of Alkaline Phosphatase**

Although APase has been studied for many years, its role has remained largely enigmatic and is still under intensive investigation. Like many other GPI anchored proteins, APase has also been proposed to be involved in transmembrane signalling function. As the GPI anchored proteins are located on the outer leaflet of the bilayer of cell membrane, they are more mobile than other cell surface proteins and may be involved in cell-cell interaction, reception or transduction of extracellular stimuli. Low & Saltiel 1988 have shown that GPI anchor acts as an apical targeting signal in the polarized epithelial cell and in this way could act as an activation antigen in the immune system.

APase has little preference for a particular substrate and will hydrolyze all the phosphomonoesters but not diesters. Catalysis includes phosphorylation of a serine residue at the active site followed by the delivery of the phosphoryl group to either water (phospho hydrolysis) or to an organic acceptor alcohol (phosphor tranferase) (Herraez et al 1980, Sarrouilhe et al 1992). However, phosphoester cleavage is faster if the transfer of phosphate is to an acceptor rather than to water.

APase has been shown to be involved in cell adhesion (Hui & Tenenbaum 1993), proliferation differentiation (Fedarko et al 1990, Owen et al 1990, Andracchi & Korte 1991), and a protein tyrosine phosphatase in some tissues (Swarup et al 1981, Lau et al 1985). In liver plasma membranes, a 18KDa phosphoprotein has been demonstrated as a substrate for dephosphorylation by APase (Chan & Stinson 1986).
It has also been shown that APase is involved in the regulation of phosphate transport in intestine, kidney and in calcium transport in the intestinal and kidney epithelial cells.

Functions of Tissue Non-specific Alkaline Phosphatase

TNAPase cleaves the extracellular substrates - inorganic pyrophosphate (PPI), Pyridoxal-5’-phosphate (PLP) and phosphoethanolamine (PEA) (Millan 2006). Function of TNAPase in bone and dental mineralisation is likely to involve hydrolysis of PPI, to maintain a proper concentration of this inhibitor to ensure normal bone mineralization and collagen and calcium binding (Whyte 1989, Hoylaerts et al 1997, Whyte 2001, Mornet et al 2001, Hessle et al 2002).

Significant physiological function of APase in mammals is provided by studies of human hypophosphatasia where a deficiency in the TNAPase is caused by deactivating mutations in its gene (Weiss et al 1986, Henthorn et al 1992, Mornet 2000). This is associated with defective bone mineralisation in the form of rickets and osteomalacia (Whyte 2001).

Pyridoxal phosphate (PLP) is the phosphorylated form of pyridoxine. TNAPase hydrolyses PLP, and the unphosphorylated pyridoxal crosses the blood brain barrier to be regenerated as PLP in the cell (Whyte 2001). Consequently, in patients with hypophosphatasia, inability to cleave PLP has been shown to result in a localized vitamin B6 deficiency in the central nervous system causing seizures (Waymire et al 1995). TNAPase could be also involved in the intra neuronal balance between pyridoxal and PLP. In addition, studies of TNAPase activity in primate brain proposes an important role for this enzyme in neurotransmission (Fonta et al 2004, Fonta et al 2005)

Functions of Intestinal Alkaline Phosphatase (IAPase)

It is expressed in the small intestine of many species. Lymph and serum levels of IAPase increase after a fatty meal (Glickman et al 1970, Mc Comb et al 1979). IAPase is found associated with the brush border of the intestinal epithelium and

**Functions of Placental Alkaline phosphatase (PLAPase)**

PLAPase has been proposed to be involved in the transfer of maternal IgG to the foetus during gestation. It acts as a Fc receptor and has been shown to be involved in the internalization of IgG in HepG2 cell line. (Makiya & Stigbrand 1992 a and 1992 b, Stefaner et al 1997).

Other studies indicate a role of PLAP in regulation of cell division (Telfer & Green 1993, She et al 2000a). PLAPase stimulates DNA synthesis and cell proliferation in synergism with insulin, zinc and calcium and that it also acts as a survival factor in combination with ATP in serum-starved mouse embryonic and human fetal fibroblast cultures (She et al 2000b). Since PLAP is synthesised in the placenta, its effects on the growth and survival of fetal cells strongly suggest that it may have a key role regulating the growth of the fetus.

**Functions of Germ Cell Alkaline Phosphatase (GCAPase)**

It had been postulated that germ cell APase (GCAP) may be able to interact with extracellular matrix proteins and therefore serves as the cell guidance molecule during the migration of germ cells (Millan 1990). Ligands involved in directing the cell migration via APase binding might be phosphoprotiens representing its natural substrates. The enzyme activity expression has been shown to be stage specific during embryonic development.

**Alkaline Phosphatase in Diseases**

An tumor marker would be one that is produced solely by the tumour and is secreted in measurable amounts in body fluids. (Jacobs & Haskell 1991). While at present no such ideal marker exists, amongst the APase isozymes, PLAPase and
GCAPase come closest to the definition given above and therefore have been evaluated extensively in various malignant conditions. TNAPase due to its ubiquitous nature fails to serve as a marker of primary malignancy and its increased level is often taken as a confirmatory finding.

Human APases are abundantly expressed in tumor cells, and serum levels of APase isozymes are often used as tumor markers (Millan & Fishman 1995). Many different isozyme patterns have been reported in malignancies and renal diseases (De Broe & Van Hoof 1991). APase activity provides the clinician valuable information for diagnosis and follow up of patients during treatment.

The different mechanisms that have been suggested for the enhanced APase expression in tumor cells are:

1. Functional involvement of APase isozymes in tumorigenesis,
2. Representing one crucial factor in a multifactorial etiology.
3. A close linkage of APase gene with disease susceptibility.
4. Simultaneous deregulation with disease susceptibility gene.
5. Result of random chromosomal aberrations.

APase activity has been reported to be enhanced in cancer patients (Gordon 1993). Various tumor cell lines like teratocarcinomas and osteosarcomas also show elevated APase activity (Hamilton et al 1979, Benham et al 1981a). Hemopoietic tumor cell lines have been shown to express high APase activity (Neumann et al 1976). Especially in B lymphoid cell lines, APase activity has been reported to be high (Culvenor et al 1981). Thus expression of APase is considered as one of the important identifiable markers of malignancy (Ruddon 1987, Millan & Fishman 1995). Deficiency of TNAPase is associated with hypophosphatasia which manifests as a rare form of rickets and osteomalacia (Henthorn et al 1992, Whyte 1995). Additionally, hypophosphatasia abnormalities in the metabolism of pyridoxal-5’-phosphate (PLP), ‘putative’ natural substrate of TNAPase, leads to epileptic seizures, apnea and perinatal death (Waymire et al 1995, Narisawa et al 2001). Elevated plasma TNAPase levels have been reported in osteosarcomas,
Paget’s disease and osteoblastic bone metastases (Deftos et al 1991, Farley et al 1991, Demers et al 1995). Plasma TNAPase level (particularly heat labile bone derived fraction) has long been recognized as an indicator of osteoblastic activity (Leunget al 1993). Osteosarcomas display high serum TNAPase levels and these levels have been shown to be higher in metastatic disease than in patients with localized disease (Bacci et al 1993). Leukocyte TNAPase has been shown to serve as a useful marker in cases of advanced lung cancer (Walach & Gur 1993). GCAPase is a useful immunohistochemical marker of carcinoma-in-situ of the testis and IAPase is a marker of hepatocellular carcinoma (Higashino et al 1975, Wahren et al 1979, Jeppsson et al 1984, Roelofs et al 1999). While APases are homodimeric molecules, there is re-expression in cancer cells of more than one APase isozyme in human cancer cell lines and cancer sera (Higashino et al 1972, Higashino et al 1977). The human postnatal intestine also contains heterodimers of IAP and PLAP (Behrens et al 1983). Ovarian cancer cells often express both PLAPase and GCAPase (Smans et al 1999) and cell lines derived from these tumors have been shown to express PLAPase/GCAPase heterodimers (Watanabe et al 1989, Hendrix et al 1990). Increased PLAPase activity has frequently been found in serum samples from ovarian cancer patients and testicular cancer patients (Vergote et al 1987, De Broe & Pollet 1988, Lange et al 1982). PLAPase is a marker of cancer of the ovary, testis, lung, and the gastrointestinal tract (Nathanson & Fishman 1971, Jacoby & Bagshawe 1971, Loose et al 1984).

Immune response and B lymphocyte activation

Innate and acquired immune responses form the defense mechanisms for survival of living organisms against a wide variety of pathogens. T lymphocytes carry out cell mediated immunity while B-cell differentiation to antibody secreting cells forms the basis of the humoral adaptive immune system. The resting B lymphocyte is small, quiescent, non dividing cell with low metabolic activity and have been shown to be in G0 phase of the cell cycle. (Monroe & Cambier 1983a,b&c). They express cell surface IgM and IgD that recognise and help in specific binding of antigen leading to activation. From primary lymphoid organs mature lymphocytes circulate through blood and upon encounter with foreign antigen in the secondary
lymphoid organs, B cells are activated in either a T cell independent (TI) or T-cell dependent (TD) manner. The B cell undergoes several morphological and metabolic changes after mitogenic activation (Anderson et al 1972). TD-Ag responses often involve soluble factors that aid in activation and clonal expansion of antigen specific B cells ( De Franco 1987 ).

In response to TD-Ag, activated B cells enter the primary follicles of secondary lymphoid organs including spleen, lymphnodes, Peyer’s patches or tonsils where they undergo rapid proliferation and form germinal centers (GC). In GC, Plasma Cells and memory B cells are generated, that drive a potent immune response upon re-exposure to same antigen.

B cell antigen receptor

The B cell antigen receptor is involved in antigen recognition and signal transduction necessary for B cell activation ( De Franco 1987 & 1993, Pleiman et al 1994 ). It endocytoses the antigen which is processed and presented to the helper T cells ( Myers 1991).

Interaction of antigen with B cell antigen receptor

It is a multiplolypeptide chain composed of monomeric IgM and IgD molecules non-covalently associated with two accessory molecules Igα, Igβ and Igγ linked through disulfide linkages (Campbell & Cambier 1990, Chen et al 1990, Friedrich et al 1993, Park house 1990).

The binding of antigen to the immature B cell receptor leads to either clonal deletion or clonal anergy (Burnet 1959, Hartley et al 1991, Goodnow 1992), while binding to mature B cell leads to activation and clonal proliferation. Nature of antigen, differentiation state of the B cell and nature of additional signals by helper T cells have influence on activation pathway followed by B cell. Multivalent antigen complexed with carbohydrate induces strong activation leading to cell proliferation (Mosier & Subbarao 1982). Paucivalent and proteinaceous antigen requires additional signals from activated T cells to induce activation (Monroe & Cambier 1983c, Noelle et al 1983). Thus signal derived from ligand binding and second signal provided during the G1 phase of the cell cycle mostly by the T cell
derived soluble factors induce further proliferation and differentiation of B cell (Brestcher 1975, Anderson et al 1979 & 1980).

**Signal transduction through B cell antigen receptor**

B cell receptor engagement to cognate antigen results in the activation of the SRC-family protein tyrosine kinase LYN and the cytoplasmic tyrosine kinase SYK (spleen tyrosine kinase), both of which phosphorylate target sequences, such as tyrosine residues in immunoreceptor tyrosine based activation motifs (ITAMs) in the cytoplasmic tails of Igα and Igβ (Sanchez 1993, Flaswinkel & Reth 1994, ). Subsequently, the SYK-mediated phosphorylation of ITAMs of BCR complexes amplifies the signal and initiates a positive feedback loop. Ultimately SYK has a central role in the activation of pathways that regulate the B-cell proliferation and differentiation.

With regard to proliferation, it has been shown that constitutively active SYK induces constitutive phosphorylation and activation of the lipid-modifying kinase phosphoinositide 3 kinase (PI3K), which regulates diverse biological processes, including cell growth, survival, proliferation, migration and metabolism.( Deane & Fruman 2004, Kanie 2004, Streubel 2006).

The signaling pathways and regulation mechanisms may vary in different species. For example, the toll-like receptor (TLR)-4 agonist lipopolysaccharide (LPS) alone efficiently activates murine naïve B cells but not human naïve B cells (Lanzavecchia 2006) because of the absence of TLR4 expression in the latter (Muzio 2000).

**B cell co-receptors in signal transduction**

Downstream of the BCR, SYK and SRC family protein tyrosine kinases induce phosphorylation of the co-receptor protein CD19 and/or the adaptor protein B-cell PI3K adaptor (BCAP), resulting in the recruitment and activation of PI3K (Deane & Fruman 2004, Aiba et al 2008). Subsequently, PI3K phosphorylates its
substrate phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2), thereby generating the second messenger, phosphatidylinositol-3,4,5-triphosphate (PtdIns (3,4,5)P3) (Okkenhaug & Vanhaesebroeck 2003)B, which recruits signaling molecules that have a pleckstrin-homology domain to the plasma membrane (Vanhaesebroeck & Alessi 2000). These molecules include the serine / threonine kinase protein kinase B (PKB) and 3-phosphoinositide-dependent protein kinase 1 (PDK1). PKB is dominant mediator for controlling cellular proliferation downstream of PI3K and its activating kinase (PDK1). (Manning & Cantley 2007).

Because SYK activates PI3K downstream of the BCR, tight regulation of BCR-induced signaling is important to avoid abnormal PKB activity, which can result in uncontrolled cellular expansion and malignant transformation.

**Role of cAMP and cGMP during B lymphocyte activation**

cAMP and cGMP, cyclic nucleotides which act as second messengers are produced and participate in signal transduction during B lymphocyte activation (Kammer 1988, Sutherland 1972). It has been shown that cAMP acts synergistically with LPS in activating the B lymphocyte proliferative response activity (Kasyapa & Ramanadham 1995).

**Transcription factors that regulate B cell activation and differentiation**

The ensuing differentiation of B cells during the GC reaction is regulated by a complex network of transcription factors. PC differentiation is most likely initiated by the downregulation of PAX-5, the ‘identity’ gene of Bcells. B-cell lymphoma (BCL)-6, is upregulated and suppresses apoptosis and promotes proliferation. B lymphocyte induced maturation protein (BLIMP)-1, a zinc finger-containing transcriptional repressor, induced in B cells and PCs. The X-box binding protein 1 (XBP-1) is upregulated in PCs and induces secretory phenotype of the PCs. Interferon regulatory factor (IRF)-4 is involved in PC differentiation. (Shapiro-Shelef & Calame 2005, Tarlinton 2008). Importantly, B cell malignancies are often associated with aberrant expression of GC transcription factors.
Lymphokines in B lymphocyte activation

Lymphokines are a group of cytokines that regulate the activation, differentiation, maturation, migration of lymphocytes.

IL-2 secreted by activated T cells causes proliferation and activation of T and B cells. It is also involved in the cycling of B cells and enhances antibody production.

IL-4 stimulates division and differentiation in B cells.

IL-5 and IL-6 act as B lymphocyte differentiation factors.

IL-7 and IL-11 have a role in antigen independent phase of B cell differentiation in bone marrow (Callard 1990).

IL-21 is the most potent cytokine in activation and differentiation of human B cells is secreted by helper T cells (Spolski & Leonard 2008, Ettinger et al 2008). It is also required for B-cell activation, proliferation, PC differentiation and Ab production (Bryant et al 2007, Kuchen 2007).

Phosphorylation has been recognized as a key mechanism by which the cytokine receptors induce a variety of intracellular events. The phosphorylated substrates assemble into a complex that translocates to the nucleus to activate the transcription of specific genes (Keegan et al 1994).

Kinases and phosphatases involved in B lymphocyte activation

Tyrosine phosphorylation catalyzed by protein tyrosine kinases is the major event that occurs during B lymphocyte activation.
Protein tyrosine kinases, Several serine threonine kinases, cAMP –dependent kinases, mitogen activated kinases (MAP kinases), S6 kinase are some of the kinases that are activated and regulate the transcription of various genes down stream to the phosphorylation event during B lymphocyte activation (Gold et al 1990, Campbell & Cambier 1990, Lane et al 1990, Brunswick et al 1991, Burkhardt et al 1991, Yamanishi et al 1991, Campbell & Sefton 1992, Leprince et al 1992, Li et al 1992, DeFranco 1992). Protein phosphatases are also expressed during lymphocyte activation to maintain the balance between the phosphorylation and dephosphorylation reactions.

Two types of protein phosphatases are known to operate in the cell:
1. Serine-threonine phosphatase ex: Calcineurin.
2. Tyrosine phosphatase ex: CD45.

Among the various phosphatases that are expressed upon lymphocyte activation, alkaline phosphatase is expressed specifically in activated B lymphocytes.

Multiple Myeloma

Multiple myeloma is a B cell malignancy accounting for 1.0% of all cancer deaths globally (Parkin et al 2005) and 10% of hematologic malignancies ( Kyle & Rajkumar 2004, Rajkumar & Kyle 2005). It is characterized by the monoclonal proliferation of malignant plasma cells with in the bone marrow. The disease occurs in older individuals above the age of 80 yr (Kyle et al 1994, Kyle & Rajkumar 2004, Jemal et al 2005, Parkin et al 2005).

Disease characteristics

Multiple myeloma is a B-cell malignancy with terminally differentiated plasma cell phenotype. The characteristic findings in MM are lytic bone disease with bone pain, renal insufficiency, anemia, fatigue, hypercalcemia, and immunodeficiency with recurrent infections ( Kyle et al 2003). The myeloma cells produce an identical immunoglobulin or immunoglobulin fragment known as the monoclonal protein (M-Protein) . The myeloma proteins when produced as light chains are
excreted in the urine and are called as “Bence-Jones proteins”. The isotype of M protein is usually IgG or IgA, occasionally IgM, IgD or IgE. The M-Protein in the serum and as light chains in the serum and / or urine is the diagnostic hall mark of this malignancy.

Renal failure may develop both acutely and chronically. It is commonly due to hypercalcemia. It may also be due to tubular damage from excretion of Bence Jones proteins, which can manifest as the Fanconi syndrome (type II renal tubular acidosis). Other causes include glomerular deposition of amyloid, hyperuricemia, recurrent infections (pyelonephritis), and local infiltration of tumor cells.

The myeloma cells also generally express CD138 (syndecan-1), CD38 and other heterogenous immunophenotypic markers and secrete the cytokine IL6 as an autocrine growth factor.

**Gene mutations and cytogenetic abnormalities in the aetiology of multiple myeloma**

Little is known about the etiology of multiple myeloma. Both environmental and genetic factors may play a role in the development of this cancer (Riedel & Pottern 1992). Exposure to ionizing radiation has been linked with multiple myeloma. The strongest associations have been noted for Japanese atomic bomb survivors, radiologists, and radium dial workers (Matanoski et al 1975, Stebbings et al 1984, Shimizu et al 1990). Nuclear power plant workers were reported to have an increased risk for developing multiple myeloma (Darby et al 1988, Gilbert et al 1989).

Numerous epidemiologic studies have reported a link between multiple myeloma and work involving pesticides, agricultural exhausts, chemicals, dusts, or a combination of these exposures(Riedel & Pottern 1992). Other non-specific occupational exposures that have been associated with myeloma include metals, rubber, wood, leather, paint, and petroleum (Riedel & Pottern 1992). Workplace exposure to benzene, a chemical used in many manufacturing processes, may play a role in the development of multiple myeloma (Decoufle et al 1983, Rinsky et al 1987).
Prolonged stimulation of the immune system by repeated infections, allergic conditions, or autoimmune disease may also increase the risk of myeloma.


While alterations of proto-oncogene loci, including mutations, amplifications, chromosomal translocations, or deletions have been found associated with several types of human tumors, no such association has been firmly established for MM. Ras gene mutations were found to occur in 47% of the patients. p53 is a tumor suppressor gene that functions as transcriptional regulator influencing cellular responses to DNA damage. Alteration or deletion of p53 gene represent an important late event in MM tumor progression. and were observed in 20% of the patients. (Antonino et al 1993, Owen et al 1997, Wei Xiong et al 2008).

The role of cytogenetic abnormalities on disease outcome has not been studied in detail. The most common and unfavourable cytogenetic abnormalities detected are:

1. del(13q), t(4;14),
2. del(17p13).
3. t (6;14)(p25;q32)
4. Loss of Chromosome 13
5. t(11;14)(q13;32)

Chromosome rearrangement of 14q32.33 occurs with variable partner sites, including 11q13.3, 8q24.1, 18q21.3, and 6p21.1 (Kazuhiro et al 1997). A significant number of myelomas (50% to 70%) also carry translocations targeting the switch regions of the IgH genes located at chromosome 14q32. (Bergsagel et
These aberrant rearrangements juxtapose oncogenes into the proximity of the powerful IgH enhancers, driving abnormal expression of the translocated oncogenes. These characteristics together with the known biased usage for certain V and D gene segments establish a molecular archaeology for myeloma in which positive and negative selection processes shape the Ig repertoire prior to the acquisition of the malignant phenotype. (Rettig et al 1996, Gonzalez et al 2005)

**Treatment**
A number of therapeutic tools have been used in the management of multiple myeloma for the last 40 years. Approximately 5% of patients were found to achieve complete remission with conventional therapy

**Chemotherapy**
Melphalan, Prednisone, cyclophosphamide, Vincristine, Carmustine, Adriamycin, Doxorubicin, Dexamethasone.

**High-Dose chemotherapy and stem cell Transplantation**
High dose chemotherapy has also been used in conjunction with stem cell transplantation. High dose chemotherapy though more effective in killing myeloma cells than conventional chemotherapy also destroys normal blood forming cells in the bone marrow

**Radiation therapy**
This mode of treatment uses high energy radiation to induce cytosolic and cytotoxic effects. The treatment is localised and it affects only the cells in the treated area and has been found to be effective in patients with single plasmacytoma.
Supportive therapies

Supportive therapies address the symptoms and complications of the disease. Supportive therapies commonly used in multiple myeloma include bisphosphonates, growth factors, antibiotics and intravenous immunoglobulin. Bisphosphonates work by inhibiting the activity of the bone destroying osteoclasts. Erythropoietin and Colony stimulating factors are also used to enhance the production of RBC and WBC.

Recently immunomodulatory agents like thalidomide and lenalidomide and protease inhibitor like bortezomib were introduced and investigated for their efficacy in the management of multiple myeloma. Deep vein thrombosis with thalidomide and myelosuppression and contraindication in patients with creatinine levels greater than 2.5 mg/dL in patients under the regimen of lenalidomide; and peripheral neuropathy, transient thrombocytopenia and gastrointestinal disorders in patient under treatment of bortezomib were encountered. Eventual relapse in autologous stem cell treated patients and absence of clear cut evidence in case of allogenic stem cell transplantation suggest search for new therapeutic agents.
## Multiple Myeloma Cell lines Used for the study

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<td>------------</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgE; lambda light chain</td>
</tr>
<tr>
<td>Biosafety level</td>
<td>1</td>
</tr>
<tr>
<td>Propagation</td>
<td>The base medium for this cell line is ATCC-formulated RPMI-1640 medium supplemented with fetal bovine serum to a final concentration of 10%</td>
</tr>
<tr>
<td>Temperature</td>
<td>37.0°C</td>
</tr>
<tr>
<td>Atmosphere</td>
<td>air, 95%; carbon dioxide (CO₂), 5%</td>
</tr>
<tr>
<td>Growth properties</td>
<td>suspension</td>
</tr>
<tr>
<td>Organism</td>
<td><em>Homo sapiens</em> (human)</td>
</tr>
<tr>
<td>Morphology</td>
<td>lymphoblast</td>
</tr>
<tr>
<td>Source: Organ</td>
<td>peripheral blood</td>
</tr>
<tr>
<td>Disease</td>
<td>plasmacytoma; myeloma</td>
</tr>
<tr>
<td>Cell Type</td>
<td>B lymphocyte</td>
</tr>
<tr>
<td>Cellular Products</td>
<td>Immunoglobulin; monoclonal antibody; interleukin-6</td>
</tr>
</tbody>
</table>
Levamisole

Levamisole has been introduced in 1960 as an antihelmithic drug (Thienpont et al 1966, Rollo 1990.) It is the levo enantiomer of tetramisole and since then it has found many uses in the clinical medicine (Renoux & Renoux 1971). It is still being used in conjunction with 5-Fluorouracil (5-FU) as an adjuvant to treat colon cancer relapse following surgical resection (Amery & Gough 1981, Amery et al 1997, Moertel 1995, Kerr et al 2000). Levamisole and its derivatives have also been used as potent inhibitors of alkaline phosphatase, thymidylate synthase and tyrosine phosphatase (Vanbelle 1972 & 1976, Bhargava & Men 1977). It is also a good selective inhibitor of TNAPs, with Ki values lower than 100 uM (Jalanka and Lindberg 1975) in vitro. Mammalian APase activity of Liver/bone/kidney type has been demonstrated to be inhibited uncompetetetively by Levamisole (Borgers 1973, Goldstein et al 1980).

Levamisole at 2mM concentration has been shown to inhibit the proliferation of cultured bone cells with concomitant inhibition in bone APase activity (Marie et al 1986). Levamisole avidly binds to the putative “substrate-APase” complex to mediate the inhibition. Levamisole has been shown to act as immunosupressor at high doses (Artwohl et al 2000). Levamisole is currently used as an adjuvant therapy in the treatment of human cancer (Reid et al 1998). Also, levamisole was found to bring significant clinical improvement in patients with chronic infections and inflammatory diseases such as Herpes and Rheumatoid arthritis (Symoens & Rosenthal 1979). Further, it has been demonstrated that levamisole exerts anti-metastatic effects, particularly when it is used as an adjuvant to conventional anti-neoplastic therapy (Amery 1977, Amery & Gough 1981). In vitro, levamisole has been demonstrated to potentiate anti-proliferative effect of 5-Fluorouracil in tumor cell lines (Kovach et al 1992, John et al 1992 and de Waard, et al 1998).

Chemical structure and metabolism of Levamisole

Levamisole is a synthetic drug having the structure as L-2,3,4,5-tetrahydro-6-phenyl-imidazo [2,1-b] thiazole. Levamisole has been shown to be decomposed non-enzymatically into three degradation products (Kimberly et al 1991). The
three products were purified and their structures were determined. The structures of the products are
A) 3-(2-mercapto ethyl)-5-phenyl imidazolidine-2one or dl-2-oxy-3-(2-mercaptoethyl)-5-phenyl
B) 6-phenyl-2,3-dihydroimidazo(2,1-b)thiazole
C) bis(3-(2-oxo-5-phenyl imidazolidine-1-ethyl) disulfide.
The decomposition of levamisole in to the above products depends on temperature and pH. Upon oral administration, levamisole is well absorbed from the gastrointestinal tract and is extensively metabolised by the liver.

**Levamisole as an anti-helminthic drug**

Levamisole probably owes its anti-helminthic property to specific binding to nicotinic-acetylcholine receptors of muscles of nematodes. As a result of this interaction, Levamisole muscular hypercontractions which leads to paralysis and elimination of the worms (Lewis et al 1987a & 1987b). Levamisole is used in human medicine as an antihelminthic in a single dose form at 2,5 mg / Kg. Levamisole has been used in humans for the following indications: antihelminthic, rheumatoid arthritis, inflammatory disease, infectious disease and cancer therapy. The most important side effects are hematologic, including reversible leukopenia, agranulocytosis and thrombocytopenia.

**Levamisole induced apoptosis**

LMS affects antitumor cytotoxicity by inducing apoptosis in some tumor cells. Levamisole has been shown to induce apoptosis in adult human endothelial cells of veins and uterine capillaries (Artwohl et al 2000). Levamisole increased intergrin-dependent matrix adhesion and reduced the expression of survival factors such as clusterin, endothelin-1, bcL-2, endothelial NO-synthase and retinoblastoma protein (pRb). It also caused growth arrest and increased death signals such as p21 and bak. Levamisole induced apoptosis and regulation of pRb expression was inhibited by glutathione and N-acetyl cysteine suggesting that reactive oxygen species plays a role in this process (Cruchand et al 1979).
Direct toxic effects of levamisole alone on cancer cell lines have been found only with suprapharmacologic concentrations of the drug (Grem & Allegra 1989). Levamisole was found to be bound to plasma proteins.