The defense system of invertebrates to survive millions of years as an evolutionary important “living fossils” remained poorly understood. It is of considerable scientific interest to know the immune system of insects. Possibly it would help to understand the evolution of immunity and provide the basis of immune response in an invertebrates that survived for millions of years (Merrill and Benveniste (1996).

During the course of evolution invertebrates and vertebrates have maintained common signaling molecules such as neuropeptides which have been found in the blood circulation and act as immunomodulators. Neuropeptides are used as messengers to initiate and stimulate the innate immune response in invertebrates have been conserved during the course of evolution reflecting their vital importance.

Early invertebrates invested heavily in innate immunity and their basic blue print for pathogen recognition has been largely conserved in vertebrate organisms. Despite of it’s primitive origin, sensing pathogens through certain conserved structures, pathogen associated cellular or molecular patterns is an elegant strategy (Elmquist et al., 1997).

Research on immunity of insects can be divided into three periods. The first paralleled the early development of immunology in general and included a very active phase in the 1920’s. The second period began in 1958-1959 with
Briggs and Chadwick's *in vitro* demonstration of an inducible cell free immunity caused by antibacterial substances reviewed by Chadwick and Aston (1979). The third period started in 1980 with the isolation of the first pure antibacterial factors, latter named Cecropins. Interest in the infection of insects by parasites has been generated by the pathogenic potential of the parasite to the insect itself. Generally insects are well adapted to diverse environments and defend themselves against many parasites and microorganisms (Dunn, 1986). Antibacterial proteins have been identified in the gaint silkmoth *Hyalophora cecropia*. These proteins constituted an important part of humoral immune defense. A set of proteins induced by bacterial and protozoan infection has been isolated from pupal hemolymph, includes three groups of antibacterial proteins lysozymes, attacins, and cecropins (Hultmark, *et al.*, 1980). These proteins are also induced by wounding or by injection of components of the bacterial cell wall, such as lipopolysaccharides (LPS) and in few cases degradation products from peptidoglycans (Sun *et al.*, 1990).

Insect hemocytes contain a mammalian like proenkephalin molecule. Proenkephalin from invertebrates contain the antibacterial peptide enkelytin. The fact that invertebrate proenkephalin supports the hypothesis that these molecules first evolved in more primitive animals. Indeed, the presence of enkelytin with its strong antibacterial activity, strengthens the association of opioid peptides with insect immune-related activities. It is possible that immune signaling leads to enhanced proenkephalin proteolytic processing,
freeing both opioid peptides and enkelytin simultaneously. In this scenario these peptides would stimulate hemocyte chemotaxis and phagocytosis as well as secretion of cytokines and the simultaneously liberated enkelytin would attack bacteria immediately. Thus, it appears that many mammalian molecular and cellular survival strategies first appeared in invertebrate organisms that evolved at least 500 million years ago (Weigent and Blalock, 1997).

An insect defends itself against pathogens and parasites by several processes including phagocytosis, nodule formation and encapsulation. All these processes are multistage involving many signaling and effector molecules. The chitinous exoskeleton of insect is non-living matrix of carbohydrate and protein secreted from a monolayer of epidermal cells, which cover the entire surface of insect. The cuticle serves as a protective barrier between the internal tissues and the external environment. Pasteur, while studying silkworm (*Bombyx mori* L.) diseases, noted that the cuticle of healthy silkworms were often topically scratched, and as a result the cuticle darkened around the injured area. Cuticular melanization is thought to be an important defense reaction of insects because it is involved in wound healing and sequestration of pathogens.

In insects hemolymph proteins are the subject of many investigations as they are considered to be involved in immuno functions. In several species of insects, the developmental pattern of the synthesis of these hemolymph
proteins are attributed to the influence of hormones. Invertebrates, which lack adoptive immune systems have developed defense systems that responds to common antigens on the surface of potential pathogens. Hemolymph coagulation is one such defense system in innate immunity. Mitogen sensitive hemocytes which trigger the coagulation cascade has detect and respond to foreign materials. Hemocytes in the hemolymph of horseshoe crab play a major role in the defense system. When Gram negative bacteria invade the hemolymph, the hemocyte detects LPS molecule on their surface and then releases the contents of granules by rapid exocytosis. The released granular components include biosensors of the coagulation reaction. The invaders in the hemolymph are thus engulfed or immobilized by the clot. They are subsequently cell agglutinated and killed by various lectins and antibacterial substances that are also released from granules.

Insects and other arthropods in general do not possess immunoglobulins (Ig) found in higher animals, although proteins containing in immunoglobulin like domains have been identified in insects. During defense reactions, invariably the foreign organisms are found to be encapsulated and melanized. Therefore, melanin and the enzyme responsible for this biosynthesis of phenoloxidase are considered in integral part insect host defense reactions. Phenoloxidase is present throughout the body of insects including the open circulatory system of hemolymph (Iwama and Ashida, 1986).
Insects possess an innate immune system composed of cellular and humoral immune mechanisms that promptly respond to invasion by microorganisms and other parasites. The cellular immune mechanisms are include agglutination of hemocytes. The humoral immune response activated with in hours and generates a diverse set of broad spectrum anti-bacterial proteins and peptides such as cecropins, attacins, defensins and lysozymes (Yoshida et al., 1996).

Induction of antibacterial protein synthesis is a specific response to the presence of bacteria or bacterial cell wall components in the hemocoel. Bacterial peptidoglyca fragments are recognized by the fat body and elicit cecropin synthesis in silkworm larvae. Peptidoglyca fragments are released from invaded bacteria by the action of hemolymph lysozyme and to act as a signal molecule for activation of a series antibacterial cascade. *Lepidopteran* insects contain relatively high levels of lysozyme as a normal component of the hemolymph (Morishima et al., 1995).

The major defense mechanisms in the hemocoel are either cellular or humoral effected by immune proteins, lectins and prophenoloxidase cascade. Recognition of microorganisms as non-self is apparently involved when the insect defense mechanisms are set in motion. In fact, clonally selected recognition molecules like immunoglobulins in higher vertebrates have not evolved in insects, but molecules for non-clonal pattern recognition play a
central role in discrimination of self from non-self. Thus a number of molecules with affinity to a particular structures of bacterial or fungal cell walls have been reported from insect hemolymph and they are thought to be potential recognition molecules for foreignness. Hemolin lectins with various ligand specificities and proteins with affinity to lipopolysaccharide and peptidoglycan have been suggested as recognition molecules (Ashida and Yamazaki, 1990).

In all insects, an immediate result of septic injury is the induction of two proteolytic cascades that lead to clotting and melanization and it is believed that these reactions play a major role in defense.

Quinones which can react with proteins and with thiol and amino groups of other compounds can be bactericidal (Teshima et al., 1986). When quinones are present in excess the black pigment melanin is formed. The melanin deposition is often associated with insect’s cellular immune response (Nappi, 1973). The melanin deposition around wounding is commonly associated with the cellular and humoral defense of lepidopteran insects (Nappi, 1973; Pye, 1974).

The phenoloxidase in insect hemolymph occurs as a proenzyme, prophenoloxidase which can be activated by an activator present in hemolymph and cuticle (Ashida et al., 1982). Active phenoloxidase is deleterious as it can catalyze the oxidative polymerization of phenols and catechols, but in doing so, it can also polymerize proteins and other macromolecules, posing a potential
threat to the host. Hence phenoloxidase is preserved as an inactive proenzyme form prophenoloxidase and is specifically activated proteolytically when it needed. Prophenoloxidase from larval hemolymph of the silkworm is activated by alpha-chemotrypsin and the reaction is inhibited by specific inhibitors of the enzyme which is proteolytic in nature (Ohinishi et al., 1970). Prophenoloxidase has been purified extensively from hemolymph of the silkworm larvae and basic properties of protein has been well characterised (Dohke, 1973).

*Lepidopteran* insects synthesize various antibacterial proteins which exhibit a broad spectrum of activity against Gram negative, Gram positive and even to microsporidian spores (protozoans). Many immune proteins are synthesized within fat body and it is functionally equivalent to mammalian liver and regularly secreted into the hemolymph but some of them are also synthesized by hemolymph (Ijiima et al., 1993). The cecropins were identified as highly potent antibacterial peptides in immune hemolymph from the silkworm. The cecropin has also been found in pig intestine (Lee et al., 1989) which implies that cecropins are wide spread in the animal kingdom.

Insects upon injury, the immediate onset of two proteolytic cascades leading to localized blood clotting and to melanization, the latter process involving production of cytotoxic molecules; the phagocytosis of bacteria and the encapsulation of larger parasites by blood cells; they induced to synthesize
by the fat body of a battery of potent antimicrobial peptides which are secreted into the hemolymph where they act synergistically to kill the invading microorganisms.

Many invertebrates have specialized cells that function as itinerant trouble-shooters within the body, acting to engulf or lyse off foreign material and repair wounds. The cells are variously known as amoebocytes, hemocytes, coelomocytes, and so on, depending on the animals in which they occur. If the foreign particle is small, it is engulfed by phagocytosis; but if it is larger than about 10 μm, it is usually encapsulated. Arthropods can wall off the foreign object also by deposition of melanin around it, either from the cells of the capsule or by precipitation from the hemolymph (Cheng et al., 1977).

Lysozymes are released from the hemocytes of molluscs during phagocytosis and encapsulation (Kassim and Richards, 1978) and bactericidal substances occur in the body fluids of a variety of invertebrates (Lackie, 1980). Substances that can agglutinate vertebrate erythrocytes in vitro have been found in many invertebrates, although the function of these lectin-like molecules in vivo is unclear. Bacterial infection in some insects stimulates production of antibacterial proteins (Sun et al., 1990).

Generally, invertebrates do not able to acquire a specific immune response. They will either respond at first exposure to a foreign material or not at all; repeated exposure does not elicit immunity (Van der Knaap and Loker,
In the cellular defense mechanism, hemocytes are able to recognize isografts (self) and allografts (non-self) (Lackie, 1986).

Encapsulaton is a common response to foreign invaders larger than the hemocytes involved in the immune response and involves enclosing the object in several layers of cells (Vinson, 1990). The cells aggregate around the entrapped microorganisms to form a compact capsule or nodule which may or may not undergo melanization. The degenerated granular cells and plasmatocytes are responsible for nodule formation and encapsulation.

Melanization through the action of phenoloxidase is an important aspect of insect defense against microorganisms and eukaryotic parasites (Gotz, 1986; Nappi and Vass, 1993). The phenoloxidase cascade results in the production of melanin and associated toxic metabolic by-products, which may kill encapsulated parasites because of potential toxicity of the ultimate products. Phenoloxidase activation is highly localized and increased phenoloxidase activity associated with melanogenic encapsulation of eukaryotic parasites.

Humphreyes and Rainherz (1994) have proposed a model in which hemocyte mediated recognition is self operative principle directing invertebrate immune reactions. In this model invertebrate pre immunocytes are positively selected through the expression of a self recognition receptor that is specific for a polymorphic histocompatibility antigen expressed on the surface of self cells.