



American Journal of
**Biochemistry and
Molecular Biology**

ISSN 2150-4210



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An Overview of Insect Hemocyte Science and its Future Application in Applied and Biomedical Fields

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ABSTRACT

Insects represent almost all territories of the earth and account around 75% of total animal population. It is still ambiguous that being a cold blooded/poikilothermic organism, without acquired immune responses, how do they survive in all geographical regions of the world? Probably, it indicates its very strong innate immune responses i.e., cellular and humoral. Insect blood cells or hemocytes are the main component of cellular immune responses. They are biochemically very sensitive towards change and are the primary target of cellular defence system of insects and have proven their ability to mediate diverse immuno-defence processes against invading pathogens and other adverse conditions/stresses i.e., temperature, starvation, photoperiod, humidity etc. Therefore, in the present review, effort has been made to correlate various responses of hemocytes against the change, in order to propose hemocyte-based model in the form of catalogue to discern the health status and its future application in applied and biomedical sciences. Since hemocytes respond very instantly against change hence, it is expected that by using hemocyte catalogue as a pointer impact of several biotic and abiotic factors can be evaluated. Outcome of the present review will summarize the hemocytic response against adversaries/change in the form of catalogue for its potential use in applied and biomedical fields.

Key words: Insect, hemocyte, health, biomedical, stresses, cellular, immunity

INTRODUCTION

Hemocyte science is very vast, evergreen and interesting subject for scientific community. The ability to isolate and identify hemocytes is essential for studies in insect cellular immunity. It was the year 1758 when Schwammerdam for the first time described insect blood cells (hemocytes) as transport globules. Circulating hemocytes (sometimes called "blood cells") play vital role in defence mechanisms against microbes in the hemocoel. Although, in last 250 years, lot of research work (Schwammerdam, 1758; Cuenot, 1897; Kollmann, 1908; Wigglesworth, 1939; Paillot and Noel, 1928; Yeager, 1945; Jones, 1959, 1962; Rowley and Ratcliffe, 1981; Gupta, 1979, 1985a; Elizabeth *et al.*, 1994; Charalambidis *et al.*, 1996; Sorrentino *et al.*, 2002; Tiwari *et al.*, 2002; Pandey *et al.*, 2003a, b, 2008a, b, 2010; Pandey and Tiwari, 2004, 2011; Ceraul *et al.*, 2003; Pandey, 2004; Ling *et al.*, 2005; Figueiredo *et al.*, 2006; Gandhe *et al.*, 2007; Merchant *et al.*, 2008; Singh *et al.*, 2008) has been conducted on hemocytes but many of the

aspects still remained unexplained. Hemocytes are very vital components of the insect immune system and are biochemically very sensitive having multiple functions such as nodule formation, phagocytosis and encapsulation as defence mechanism; synthesis and transport of nutrients and hormones for proper growth and wound healing by way of connective tissue formation (Sorrentino *et al.*, 2002; Ceraul *et al.*, 2003; Ling *et al.*, 2005; Figueiredo *et al.*, 2006; Gandhe *et al.*, 2007; Merchant *et al.*, 2008; Singh *et al.*, 2008; Pandey *et al.*, 2008b, 2010). Although, the hemocyte are very responsive components of cellular immune responses of insects, the impact of diverse stresses on hemocytes immune responses and health status of insects and their applied and biomedical application parts are not studied/reviewed much. It was, therefore, thought desirable to explore this aspect of insect to link the haematological/immunological changes with the health measures.

WHY THIS STUDY?

Definition of the present study: It is known that insects are beneficial and harmful too. To get healthier result there is need to endorse 'beneficial insects and manage the harmful 'insect pests. Generally, to improve the productivity of beneficial insects and to control the harmful insects, a number of parameters are being used by the investigators to see the impact of feed, quality seed, resistant variety, consequence of chemical and plant based insecticides, disinfectants, drugs for health, feed supplements at different concentrations, effect of temperature, photoperiod and humidity, moth emergence pattern, seed crop preservation under different conditions, preservation of eggs in altered condition, disease incidence in outdoor preservation of cocoon, standardization of appropriate temperature, random identification of insect population and their emergence prediction etc. In almost all the cases, the effects are visible in late stage. The eventual result of the desired experimentation is being explained only with the survival which could have been affected because of other adversities/stresses of the environment or other unknown reasons which require explanation. It is reported that diverse kinds of stresses influence the insects at physiological level and are expressed through their poor survival and vigour. At present we do not have indicator for health. Our assessment on identification of stresses is based on morphological characteristics/phenotypic traits which is now and again contradictory and deceptive. In the absence of health catalogue, several important factors are not measurable and it is very difficult to say which factor has shaped what impact, whether it is positive or negative? To proceed in this direction, we need to have a catalogue-first-hand information on the physiologically and biochemically active and sensitive components of insects.

It is known that hemocytes are biochemically very active and very sensitive cellular components of insects and hence a catalogue on them will be very helpful in identifying and quantifying the stresses. Present review is written to overview information about hemocyte science in the form of a health catalogue in view of affecting factors i.e., drugs, disinfectants and insecticides etc. It will be useful to study the hemocyte immune responses of insects in benefits of human kind in the field of applied and biomedical sciences.

Rationale of the study: It was thought desirable to explore and link the haematological/immunological changes with the health measures. The effects of so many stresses on insect hemocytes at a time are being reviewed probably for the first time. The effects of

severance of dorsal aorta and nerves innervating the ovaries on hemocytes have been documented perhaps for the first time. The role of ventral nerve cord on moulting and hemocyte counts and hemocyte morphology has been studied only in few insect species. More such studies are needed to supplement the earlier findings. The present review article comprises the effect of certain stress-induced changes in hemocytes, which were not reviewed earlier. Present review is written to overview hemocyte in view of affecting factors and to know the insect health status at different stages of development. In addition, hemocyte-based-understandings will be helpful in assessing the impact of drugs, disinfectants and insecticides on insects in future.

Relevance to the current status: Insects display diverse immunological responses to foreign material, including microorganisms, metazoan parasites, xenogenic grafts and other adversaries. Much of the published work describing this immune system has been collected in a number of review articles (Lackie, 1988; Ratchiffe *et al.*, 1985; Gupta, 1986; Ribeiro and Brehelin, 2006). It consists of both cellular and humoral components which interact during an immune response in a manner comparable to the vertebrate system, although insects do not possess the specific remembrance component which is characteristic of vertebrate immunity. Few of the molecular or cellular mechanisms which regulate and support insect immunity have been defined. In terms of cellular immunity, a major obstruction to progress has been its descriptive morphology; both cellular categorisation and function. The varieties of insect species that have been studied, has led to extensive confusion. The literature contains scores of different names for insect blood cells describing several acronyms/morpho-types (Table 1). It is generally accepted that the main hemocytes involved in immunological responses are the PLs and GRs. Some basic issues regarding their relationship, heterogeneity and activity during immunological responses have been unanswered. Even scanty information is available about the molecular mechanisms which govern these responses.

Stresses developed due to several reasons affect the survival, growth and development by affecting the insect at physiological level and express through poor survival. In insects, hemocytes are found to show changes in their types, number and configuration under different stresses which finally affect the health and loss of insect. A hemocyte based catalogue for identification of affliction at early stage may help in taking preventative measures to save the stock and at later stage for

Table 1: Different types of hemocytes and their acronyms

Hemocytes	Acronyms
Prohemocytes	PRs
Plasmatocytes	PLs
Granulocytes	GRs
Spherulocytes	SPs
Adipohemocytes	ADs
Oenocytoids	OE
Vermicytes	VEs
Podocytes	POs
Coagulocytes	COs
Lamellocytes	LAs
Thrombocytes	THs
Spinocytes	SNs

judicious rejection of stock of economically important insects, on one hand, and to control the insect pests, on the other. A catalogue of hemocytes of insects as health indicator will help in simple recognition of adversaries and also to see the impact of drugs, disinfectants, pesticides etc.

Basic principle of the work: Our purpose is to review the impact of stresses on insects based on physiological status. This can be done through hemocyte catalogue/parameters. It is expected that various kinds of stresses manifest as change in hemocyte profile/catalogue (Pandey *et al.*, 2007, 2010; Pandey *et al.*, 2008a, b; Pandey and Tiwari, 2011). Further, the techniques to identify the stress at gene/DNA level are comparatively costlier. Hence the application of the present study is urgently needed. Insects are versatile so far as its adaptation to environment is concerned but adaptation has its limits and beyond this the survival and health conditions worsen quickly. This is particularly obvious in commercially beneficial insects. The mechanism of worsening in health state due to the influence of different stresses/adverse factors is not well understood and even their physiological indicators are not well recognized. Once the insect population is lost, stresses impacts are realized. This restriction has hindered the scientists in working out a counteractive measure against different stress conditions and to work out a threshold level of affecting factors. In this background we need to have hemocyte catalogue as marker. It has been observed that growth, development and environment of insect are closely related with their hemocyte parameters.

Generally, the counts of hemocytes; Total Hemocyte Count (THC) and Differential Hemocyte Count (DHC) are basically influenced by environmental conditions like humidity, temperature, photoperiod and disease stresses (Singh *et al.*, 2008; Pandey *et al.*, 2003b, 2010). Furthermore, the cellular (phagocytosis, encapsulation etc.) and humoral factors (release of enzymes/proteins) immune responses play a major and essential role in immune recognition of foreign materials and resistance to disease in insects. Haematological investigations provide diagnostic keys to various diseases and valuable indicators of the physiological status of the organisms. It is hypothesized that variation in hemocyte profile/catalogue and other histological/enzymatic changes consequent to stress conditions can be used as indicator for health and it can be utilized in biomedical field.

OVERVIEW OF HEMOCYTE

What is hemocyte?: The circulating cells in hemolymph are called hemocytes. Insects lack erythrocytes and hemocytes cannot be directly equated with vertebrate leucocytes. Some fraction of hemocytes remains sessile and attached to the surfaces of tissues and in some species, such cells may account for the majority of the hemocytes. Insects possess an open circulatory system which contains various types of hemocytes, the mesodermal cells which perform several physiological functions including protection from pathogens. Due to this reason, the study of hemocytes has become an important area of research. Hemocytes are not only good bio-indicator to determine the health of the insects but biochemically very responsive component also. Hemolymph contains several cell types (Table 1), collectively called hemocytes; but their entire functions are still unknown. The most abundant are Plasmatocytes (PLs) and Granulocytes (GRs)-these are phagocytic on bacteria and other foreign microorganisms; some encapsulate parasitoid eggs. Hemocytes and the (prophenol-) phenoloxidase system constitute the instantaneous innate immune system in insects. These components of insect immunity are present at

Table 2: Hemocyte categorisation in different insects

Investigators	Organism	Types of hemocyte reported
Yeager (1945)	<i>Prodenia eridania</i>	Proleucocytoids, small chromophilic cells derived from proleucocytoids, OEs, PLs, POs, VEs, cystocytes, spheroidocytes, eruptive unstable cells and degenerate cells
Jones (1959)	<i>P. eridania</i>	PRs, OEs, PLs, POs, VEs, GRs, cystocytes, spheroidocytes and SPs
Jones (1962)	Insects	PRs, PLs, GRs, ADs and OEs
Gupta (1979)	Insects	Haemocyte types, their structure, synonymies, interrelationships and taxonomic significance have been documented. PRs, PLs, GRs, ADs and OEs The VEs, POs, COs, LAs, THs and SNs were also documented
Arnold (1982)	85 species of the family noctuidae	Four functional cell types; PLs, GRs, SPs and OEs
Ashhurst (1982)	<i>Galleria mellonella</i>	On the basis of histochemical study, confirmed her earlier observation on the occurrence of PRs, PLs, SPs, ADs and OEs
Mishra (1999)	<i>Dysdercus koenigii</i>	PRs, PLs, GRs, ADs and OEs, presence of VEs at a specific developmental stage
Pandey <i>et al.</i> (2003a,b)	<i>Papilio demoleus</i>	PRs, PLs, GRs, SPs, ADs and OEs. POs and VEs as PLs variants
Pandey and Tiwari (2005)	<i>Danaus chrysippus</i>	PRs, PLs, GRs, SPs, ADs and OEs, presence of POs and VEs at specific developmental stage
Tiwari <i>et al.</i> (2006)	<i>D. koenigii</i>	PRs, PLs, GRs, ADs and OEs. The SPs was not found
Pandey <i>et al.</i> (2010)	<i>Antheraea mylitta</i>	PRs, PLs, GRs, SPs, ADs and OEs. The VEs and POs were found in late larval and pupal stage as PLs variant

any post-embryonic life stage devoid of preceding infection. Differences between individuals and species in these immune parameters can emulate differences in infection menace, life expectancy and biological function.

Hemocyte categories: Hollande (1911) following the works of earlier authors particularly those of Cuenot (1897) and Kollmann (1908) first attempted to classify hemocytes and categorised them into six types: proleucocytes, phagocytes, granular leucocytes, adipoleucocytes, oenocytoids and spherule cells. It was Wigglesworth (1939) who following Paillot and Noel (1928) and including each of Hollande's categories, divided phagocytes into two classes on the basis of their nuclear size. Yeager (1945) studied hemocytes of southern armyworm, *Prodenia eridania* and on the basis of their morphological features, classified them into nine types. Jones (1959, 1962), on the other hand, studied the blood cells *in vitro* with phase contrast microscope and compared his observations with earlier works on fixed and stained cells. Though, he adopted Hollande's classification but discarded the term leucocytes meaning white cells used in earlier paper in favour of the hemocytes meaning-blood cells. The physiological terms like phagocytes, amoebocytes, trophocytes reflecting cell functions were also avoided. Gupta (1979, 1985a) and Rowley and Ratcliffe (1981) attempted to synonymise them across the different orders and reduced them to six main types. The identification and classification of insect hemocytes based on their ultrastructural features (Ratcliffe *et al.*, 1985; Brehelin and Zachary, 1986) and immunochemical identification (Elizabeth *et al.*, 1994; Charalambidis *et al.*, 1996; Ceraul *et al.*, 2003; Ling *et al.*, 2005; Gandhe *et al.*, 2007; Merchant *et al.*, 2008) have also been made. Based on Jones (1962) classification which is still the most accepted one, the following main categories of hemocytes are recognized in insects. More or less similar categorisation has been also reported by other investigators (Table 2). Based on morphology, staining reaction and response towards stresses, six

types of hemocytes were identified in various insects (Tiwari *et al.*, 2002; Pandey *et al.*, 2003a, b; Pandey, 2004; Tiwari *et al.*, 2006; Pandey *et al.*, 2008a, b, 2010; Pandey and Tiwari, 2011). They are Prohemocytes (PRs), Plasmatocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs), Oenocytoids (OEs) and Adipocytes (ADs). Besides, Vermicytes (VEs) and Podocytes (POs) were occasionally observed in specific stage of insect life (Pandey, 2004; Tiwari *et al.*, 2006; Pandey *et al.*, 2010; Pandey and Tiwari, 2011). Categorisation of hemocyte schemes based on morphology or a combination of morphological and functional typescripts, has been developed for various insects (Gupta, 1985a, b; Brehelin and Zachary, 1986; Lackie, 1988; Gillespie *et al.*, 1997; Gardiner and Strand, 1999, 2000; Lanot *et al.*, 2001; Lavine and Strand, 2002; Irving *et al.*, 2005; Wertheim *et al.*, 2005).

Its history and some major break-through: After Schwammerdam (1758), Jones (1970) reported that these cells originate from the midventral strand of mesoderm in the embryo and differentiate further into distinctive functional types multiplying by mitosis or possibly from haemopoietic tissues. The important reviews dealing with various aspects of hemocytes (viz., categories, function, cellular responses etc.) are those of Wigglesworth (1959), Rizki (1962), Jones (1962), Gupta (1979, 1985a,b), Arnold (1982), Rowley and Ratcliffe (1981), Brehelin and Zachary (1986), Lackie (1988) and Ribeiro and Brehelin (2006). The origin and derivation of these cells have also been studied by a number of workers (Gupta and Sutherland, 1967; Gillespie *et al.*, 1997; Chapman, 2002; Tiwari *et al.*, 2002). Gotoh *et al.* (2004) found that oxygen consumption profiles of insect cells get affected due to temperature. Impact of high temperature on hemocyte cell cycle has also been studied (Kiuchi *et al.*, 2008). It is also found that temperature influence the physiological level and expressed through cellular and immunological change, tissue degeneration, poor survival and vigor (Neven, 2000; Contreras and Bradley, 2010; Lalouette *et al.*, 2011; Catalan *et al.*, 2011). It is reported that very high temperature slows down the growth, may lead to developmental malfunction, such as larval ecdysis, immune responses and adult emergence (Neven, 2000; Chapman, 2002; Pandey *et al.*, 2010; Arias *et al.*, 2011; Beckett, 2011). Impacts of temperature, starvation, hormone, microsurgeries and plant based insecticides on hemocytes have been studied in different insects by several investigators (Tiwari *et al.*, 2002; Pandey *et al.*, 2003a, b, 2008a, b, 2010; Pandey and Tiwari, 2004; Pandey, 2004).

The current state of hemocyte: Hemocytes have multiple functions viz., defence mechanism like nodule formation, phagocytosis, encapsulation and role in synthesis and transport of nutrients and hormones, wound healing and source of storage protein etc. During encapsulation response, hemocytes attach to the foreign target and also with one another eventually forming a smooth capsule comprising overlapping layers of cells. The morphology of capsules is broadly similar across taxa although variation exists among species in how rapidly a target is encapsulated, the hemocyte types suggested to participate in capsule formation and whether or not the capsule melanizes (Rowley and Ratcliffe, 1981; Lackie, 1988; Strand and Pech, 1995a, b). Effects of certain stresses on hemocyte profile (THC, DHC and cell contour) have been observed by several investigators (Pandey *et al.*, 2003a, b, 2010; Pandey and Tiwari, 2005; Tiwari *et al.*, 2006; Singh *et al.*, 2008). It is reported that cellular immune responses of different insects under various temperature regimes showed that the plasmatocytes and granulocytes are the cells which get affected with

all types of temperature treatment (Pandey, 2004; Pandey *et al.*, 2010). Biochemical/histochemical and ultra-structural aspects of synthesis, storage and secretion in hemocytes were studied by several workers (Crossley, 1979; Ashhurst, 1982). Hemocyte surface Phenoloxidase (PO) and immune response to Lipopolysaccharide (LPS) in *Ceratitidis capitata* was identified by Charalambidis *et al.* (1996).

Exterior properties of the target also manipulate encapsulation by distressing the adhesive and spreading behaviour of hemocytes. With the possible exception of some Diptera (Gotz, 1986), encapsulation in most insects clearly depends on a co-operative response between hemocytes. What remains uncertain is whether this response is umpired by a functionally consistent population of hemocytes or through a communication among functionally dissimilar sub-populations of hemocytes. Electron microscopic investigation with *Galleria mellonella* and *Clitumnus extradentatus* indicates that the encapsulation is biphasic. It is established that Granular cells (GRs) contact a foreign target, disintegrate or de-granulate liberating material that endorses attachment of PLs. Subsequently multiple layers of PLs form the capsule (Schmit and Ratcliffe, 1977). But Brehelin *et al.* (1975) reported only a single class of cells, designated as granular hemocytes which mediate encapsulation in *Locusta migratoria* and *Melolontha melolontha*. Probably, variation reflects in part interspecific differences in the morphology of hemocytes. Though, it also imitates two major teething troubles that have stalled understanding how hemocytic immune responses in insects are harmonized? First, because hemocytes were primarily categorised on basis of their morphology, it is difficult to identify the cell types in multi-layered capsules or to know whether morphological classes of cells (variants/morphotypes) are actually comprised functionally specialized subclasses. In addition, bewilderment also arises when hemocytes are evaluated among species as cellular immune functions assigned to one morpho-type in one species or sometimes assigned to a different morphotype in another. Second, it is extremely complicated to manoeuvre encapsulation responses *in vivo*. Therefore, our understanding of how hemocytes act together in forming a capsule is mostly based on descriptive, microscopic studies. An understandable option would be to conduct harmonizing trial *in vitro*. But, insect hemocytes are often very unstable in culture with some cell types rapidly clumping or lysing upon removal from the haemocoel while others promptly extend upon contact with the surface. Thus, hemocytes deplete themselves from suspension and do not form capsule around the experimental target. Methods for overcoming aggregation of cells during bleeding include collecting hemocytes in low pH/Ca²⁺ buffers (Mead *et al.*, 1986) or collecting hemolymph from the pericardial sinus (Horohov and Dunn, 1982). Hemocyte attachment during culture has also been repressed either by trypsinizing hemocytes (Ratner and Vinson, 1983), or culturing hemocyte/cells in plasma proteins such as lipophorin (Coodin and Caveney, 1992) or hemolin (Ladendorff and Kanost, 1991) roller cultures (Ratcliffe and Rowley, 1975; Dunphy and Nolan, 1980). A number of transcription factors have also been found to influence differentiation of hemocytes in *Drosophila* (Lebestky *et al.*, 2000; Fossett *et al.*, 2001, 2003; Fossett and Schulz, 2001; Evans and Banerjee, 2003; Evans *et al.*, 2003; Meister and Lagueux, 2003; Ribeiro *et al.*, 2003). It is reported that, hemocyte transformation is common in *Drosophila*: PLs can convert into lamellocytes (Rizki, 1962) and crystal cell predecessor also transform into PLs upon mis-look of the fate determinant (Lebestky *et al.*, 2000). Recently Kelvin (2007), Kevin (2009) revealed that insects such as some moths and fruit flies, respond to microbial infection in the same way as mammals and so can be used to test the efficiency of new drugs thereby dropping the need for animal testing. Neutrophils which are a type of white blood

cell and part of the mammalian immune system and hemocytes which are cells that carry out a similar function in insects, react in the same way to infecting microbes. Both the insect and mammalian cells produce chemicals with a similar structure which move to the surface of the cells to kill the invading microbe. The immune cells then enclose the microbe and release enzymes to break it down.

Key points and challenges: In contrast to the situation in insects, our understanding of vertebrate haematology and the role of vertebrate blood cells in immunity has increased dramatically over the past thirty years. In large part, this has been a result of a move away from cellular morphology to the study of function of individual cellular molecules, acting as well-defined markers of cellular development, differentiation and activation. Hemocytes respond very instantly against change, it is expected that by using hemocyte catalogue as a pointer impact of several biotic and abiotic factors can be evaluated. Presently there is an urgent need to summarize the hemocytic response against adversaries/change in the form of catalogue for its potential use in applied and biomedical fields. Exploring the hemocyte immune response on insects, in benefits of mankind, is a big challenge for scientific community. Vertebrate leukocytes and their immune reactions, are defined in terms of a large number of diverse molecules on their surfaces, which are usually identified by specific monoclonal antibodies (mAbs). Similarly advances in the functional analysis of hemocyte will necessitate the recognition of specific molecules on hemocyte variants and sub-populations. Hemocytes play an essential role in defending invertebrates against pathogens and parasites that enter their haemocoel. A primary defense hemocyte response is encapsulation; a process in which cells attach to the foreign organism and endeavour to annihilate it. There is need to find out, whether encapsulation entails assistance among specific subpopulations of hemocytes. Only scanty information available about the types of hemocytes produced by other insects including numerous species of economic importance. Key challenges include the small size of many insects which makes collection and identification of hemocytes complex due to the inadequate volume of hemolymph and cells present in circulation. The hemocyte-type-insect produce and the names they are given also sometimes differ between taxa such that classification schemes and criteria used to recognize hemocytes in one group of insects, may not be fully appropriate to another.

KEY ISSUES IN HEMOCYTE SCIENCE

Hemocyte mystery behind vast endurance: Hemocyte science research signal shows that insects innate immune response is responsible for their vast survival and the blood cells “hemocytes” are the main constituent. Hence, it is assumed that innate immune system of insects is very strong. There is need to use the immunity of insect hemocyte as a model to explore the mystery. It is reported that a group of plasma proteins functions in defense against microbial infection. Lysozyme insect hemolymph degrades bacterial cell walls and antimicrobial peptides are synthesized in response to bacterial or fungal infection. Many of these peptides act by disrupting the integrity of bacterial cell membranes. It is reported that phenoloxidase, an enzyme present in hemocytes is synthesized as an inactive precursor, prophenoloxidase. In response to disease or injury, prophenoloxidase is activated and catalyzes the production of quinones that polymerize to form the pigment melanin, which helps to trap and kill invading organisms. The propensity of hemolymph to darken has been known for more than 100 years but this melanization has been only recently understood at molecular level.

Pioneers in hemocyte science: There are a number of pioneers in the field of hemocyte science. Schwammerdam (1758) was the first person who studied the insect blood cells (hemocytes) as transport globules. Hollande (1911) following the works of earlier authors particularly those of Cuenot (1897) and Kollmann (1908) first attempted to classify hemocytes and categorised the hemocytes. Then after, several scientists have attempted to classify the hemocytes based on different parameters (Wigglesworth, 1939; Paillot and Noel, 1928; Yeager, 1945; Jones, 1959, 1962; Rowley and Ratcliffe, 1981; Gupta, 1979, 1985a). The identification and classification of insect hemocytes based on their ultrastructural features (Ratcliffe *et al.*, 1985; Brehelin and Zachary, 1986) and immunochemical identification (Elizabeth *et al.*, 1994; Charalambidis *et al.*, 1996) have also been made. Based on Jones (1962) classification which is still the most accepted one, the following main categories of hemocytes are recognized in insects. They are prohemocyte, plasmatocyte, granulocyte, spherulocyte, adipohemocyte and oenocytoid. Impact of temperature, starvation, hormone, surgical operations, namely nerves, nerve cord and aorta severance, brain transplantation, hemolymph withdrawal and plant based insecticides on hemocytes have been studied in different insects by several investigators (Tiwari *et al.*, 2002, 2006; Pandey *et al.*, 2003a, b, 2008a, b, 2010; Pandey, 2004; Pandey and Tiwari, 2011).

Cellular and humoral immune responses: It is well understood that insect's immune system has both humoral and cellular components and shares many similarities with vertebrate immune system. The number and types of the host hemocytes are the two key factors required for a successful immune reaction. Changes in hemocyte morphology and differences in hemocyte counts during the immune response were observed by several workers (Sorrentino *et al.*, 2002; Ceraul *et al.*, 2003; Strand and Pech, 1995a, b; Ling *et al.*, 2005; Figueiredo *et al.*, 2006; Gandhe *et al.*, 2007; Merchant *et al.*, 2008; Singh *et al.*, 2008; Pandey *et al.*, 2008b, 2010). A summary of some recent research and development is given in Table 3.

Immune responses and molecular study: The innate immune system is one of the most important factors in the ability of insects to survive under adverse conditions. The innate immune system comprises hemocyte-mediated and soluble components and is initiated through recognition of pathogen-associated molecular patterns (Girardin *et al.*, 2002). In many invertebrates it is reported that the soluble constituent of the system includes anti-microbial peptides i.e., attacins (Gillespie *et al.*, 1997), lysozyme (Kopacek *et al.*, 1999), lectins (Chen *et al.*, 2001) defensin (Nakajima *et al.*, 2001, 2002; Johns *et al.*, 2001) and others (Strand and Pech, 1995a, b). In insects, the expression of defensin is induced in the fat body subsequent to bacterial injection (Gillespie *et al.*, 1997). In *Drosophila* microbial challenge induced the expression of defensin between 0 and 90 min post-infection (Lemaitre *et al.*, 1997). Though, this pattern of rapid induction is not common. In *Mytilus galloprovincialis*, defensin is constitutively expressed and the mature peptide is secreted from the GRs into the plasma 24 h post-injection of heat destroyed *Vibrio alginolyticus* (Mitta *et al.*, 1999). In *Ornithodoros moubata*, defensin is expressed in the midgut as early as 60 min post-feeding (Nakajima *et al.*, 2001). A defensin was also found in the hemolymph of *Dermacentor variabilis*, between 1 and 6 h post-injection of the Lyme disease spirochete, *B. burgdorferi* (Johns *et al.*, 2001). The sequence of the first 30 amino acids of the expected 38-40 amino acids was dogged and MALDI-TOF predicted a molecular weight of 4228.66 for the secreted peptide (Johns *et al.*, 2001). However, neither the full amino acid sequence for the mature peptide nor the pro-defensin was dogged.

Table 3: Overview of hemocytic immune responses

Investigators name	Organism	Findings
Christensen <i>et al.</i> (1989)	<i>Aedes aegypti</i>	Change in hemocyte population during the immune responses to infections
Stanley-Samuelson <i>et al.</i> (1991)	Insect	Insect immune response to bacterial infection is mediated by eicosanoids
Charalambidis <i>et al.</i> (1996)	<i>Ceratitis capitata</i>	Haemocyte surface phenoloxidase (PO) and immune response to lipopolysaccharide (LPS)
Cardens and Dankert (1997)	<i>Procambarus clarkii</i>	Understanding the activation of the proPO system could lead to the development of diagnostic tools to assess the animal health
Sorrentino <i>et al.</i> (2002)	<i>Drosophila</i>	Cellular Immune response to parasite infection in the <i>Drosophila</i> lymph gland is developmentally regulated
Hillyer <i>et al.</i> (2003)	<i>Armigera subalbatus</i>	Hemocyte mediated phagocytosis and melanisation in the mosquito following immune challenge by bacteria
Song <i>et al.</i> (2003)	Pacific white shrimp	Hemolymph parameters were examined to determine hematological changes in shrimp infected with Taura syndrome virus. They reported these parameters could be used as indicators to accurately assess the shrimp physiological status
Hoffmann (2003)	<i>Drosophila</i>	The immune response of <i>Drosophila</i>
Stanley and Miller (2006)	Insect	Eicosanoid actions in insect cellular immune functions
Figueiredoa <i>et al.</i> (2006)	<i>Rhodnius prolixus</i>	Cellular immune response in <i>Rhodnius prolixus</i> have been studied and showed role of ecdysone in hemocyte phagocytosis
Pandey <i>et al.</i> (2008b)	<i>Danais chrysippus</i>	Reduction in hemocyte mediated immune response was found after neem-based pesticide treatment
Alaux <i>et al.</i> (2010)	<i>Apis mellifera</i>	THC and PO activity were analyzed as parameters of individual immunity
Kevin (2009)	Insect	Hemocytes and mammalian neutrophils functions similarly engulfing and killing pathogens. Insects can be used for screening pathogens as well as candidate drugs that help to control them
Lorgeril <i>et al.</i> (2010)	Shrimp	Immune gene expression as a tool for health monitoring. AMP expression analysis could be used to monitor health status
Wang <i>et al.</i> (2010)	<i>B. mori</i>	Hemocytes can be used to study the mechanism of hemocyte immune responses among other physiological functions of hemocytes
Pandey <i>et al.</i> (2010)	<i>Antheraea mylitta</i>	Effect of temperature on hemocytic immune responses of tropical tasar silkworm GRs and PLs were found very responsive
Catalan <i>et al.</i> (2011)	Insect	Interplay between thermal and immune ecology was studied and effect of environmental temperature on insect immune response and energetic costs after an immune challenge have studied

The science behind hemocyte immunity: Insect immunity has gained importance in the past decade as a model to study mammalian innate immunity due to several matching between them. Insect immune system possesses only an innate immune arm that constitutes both humoral and cellular responses. It is reported that, humoral response is characterized by a rapid launch of the phenoloxidase cascade and synthesis of an array of antimicrobial proteins in the fat body within hours upon micro-organism infection that are then secreted in hemolymph. Cellular immune response is mediated by hemocytes and is an instant response triggered within minutes of microbial introduction. Hemocytic defense mechanisms constitute nodulation, encapsulation and phagocytosis. In insects, nodulation is the very vital defense mechanism against microbes and other adversaries. It is mediated by hemocyte aggregate formation around bacteria and fungi. Similarly, encapsulation refers to hemocyte aggregation around larger pathogens like parasitoids and nematodes. Though knowledge on antimicrobial peptide synthesis and phenoloxidase pathways accrues over the years, the cellular responses involved in phagocytosis and nodulation are not understood well. Recently, two transmembrane proteins namely Eater and Nimrod containing

multiple epidermal growth factor-like repeats were recognized on the surface of *Drosophila* hemocytes. These proteins have been found as a major mediator of bacterial phagocytosis. Though, knowledge on players involved in nodulation is not reported so far. Noduler was shown to bind several microorganisms and their ligands. Binding to microbial ligands is a principle exhibited by insect microbe's recognition receptors that bind pathogen-associated molecular patterns. Recently it is reported that *in vivo* RNA interference (RNAi)³ induced knock-down of Noduler especially affected the nodulation response thus suggesting the involvement of this protein in immunity. Comparable proteins have been reported earlier from other lepidopterans but their role in immunity was not well understood till date.

HEMOCYTE AND STRESSES

Temperature: Exposure to high temperature affects the health of living organisms and it depends upon the exposure period and the magnitude of the temperature. Since insects are poikilothermic organism, therefore, marked variation in temperature influences the physiological level and is expressed through cellular and immunological changes, tissue degeneration, poor survival and vigor (Neven, 2000; Contreras and Bradley, 2010; Lalouette *et al.*, 2011; Catalan *et al.*, 2011). Gotoh *et al.* (2004) found that oxygen consumption profiles of insect cells get affected due to temperature. The hemocyte counts in relation with temperature have been studied by a large number of researchers. Some of them found decrease in THC at low temperature (Tauber and Yeager, 1935; Shukla, 1995; Tiwari and Shukla, 2000; Chaubey, 2002) while others found an increase at high temperature (Tauber and Yeager, 1935; Rosenberger and Jones, 1960; Shukla, 1995; Chaubey, 2002). Jones (1967a) found an increase in THC during larval development of *G. mellonella* in both heat-fixed and unfixed larvae and the counts were significantly higher in heat-fixed insects. A similar increase in hemocyte counts has been noticed when *Prodenia* larvae were exposed to dry conditions at 25°C (Rosenberger and Jones, 1960). They have suggested that this increase in cell count is possibly due to loss of body fluid as a result of desiccation. Mishra (1999) also, stated that the increase in THC in *Dysdercus koenigii* following high temperature is due to a change in the blood volume after desiccation and his view thus conforms with those of Rosenberger and Jones (1960). He further stated that the increase in number is possibly because of increase in Mitotic Index (MI) of these cells. An increase in the mitotic rate of *Blaberus* hemocytes exposed to 37°C was also reported by Tauber (1935). The increase in THC in *P. demoleus* larvae after heating has been attributed to increased MI following increase in synthesis and release of Prothoracicotropic hormone (PTTH) from the brain and so of ecdysone from Prothoracic glands (PTG) (Tiwari and Shukla, 2000) as these physical factors are realized directly into the Neurosecretory cells (NSCs) of the brain. The role of ecdysone or PTG in production, multiplication and differentiation of hemocytes has been shown earlier by a number of workers (Pathak, 1983; Rao *et al.*, 1984; Tiwari and Shukla, 2000). Pandey (2004) also found similar results. The comparative reduction in THC following chilling has also been reported earlier by Tiwari and Shukla (2000) in *Papilio demoleus*. The decline in THC may be attributed to the clumping of the cells induced by chilling and thus rendering the hemocytes unavailable from circulating hemolymph for counting.

Pandey (2004) showed that the PLs are the only cell type which always remained lower at both the extremes of temperature in *Danais chrysippus*. While the count of PRs declines in 24 h old chilled larvae, it rises in 96 h old chilled larvae whereas their count peaks up in 24 h old heated larvae with a decline in 96 h. This shows that the PRs undergo mitotic divisions during early larval

period and establish their stem-cell nature. The stem-cell nature of PRs has been reported earlier by a number of workers.

Starvation: As regards the effect of starvation in THC amongst insects, a number of reports are available with different findings. A decrease in hemocyte number has been reported after starvation (Nittono, 1960; Shapiro, 1966; Sujatha and Dutta-Gupta, 1991; Tiwari *et al.*, 1999a; Tiwari and Shukla, 2000). On the other hand, Sujatha and Dutta-Gupta (1991) in *Corcyra cephalonica* and Tiwari *et al.* (1999a) and Pandey and Tiwari (2004) in *Papilio demoleus* reported an increase in THC following resumed feeding. The variation in hemocyte population is well known under different developmental and physiological conditions (Lackie, 1988). According to some reports, there is an enhancement in THC following starvation (Arvy *et al.*, 1948; Rosenberger and Jones, 1960) but according to others, there is a decline (Jones and Tauber, 1952; Nittono, 1960; Shapiro, 1966; More and Sonawane, 1987; Sujatha and Dutta-Gupta, 1991; Tiwari and Shukla, 2000). Likewise, different explanations have been given to these variations in hemocyte population. While Arvy *et al.* (1948) and Rosenberger and Jones (1960) attributed the increase in THC to the change in blood volume, More and Sonawane (1987) suggested the decline in THC due to the degeneration of the cells.

After prolonged starvation, Jones and Tauber (1952) in last instar *Tenebrio* larvae reported increase in the number of GRs but a decrease in the number of PLs. Reduction in the number of fusiform hemocytes was observed in starved *Bombyx* larvae (Ovanesyan, 1951) while Nittono (1960) reported that number of SPs increased as the starvation period decreased. In starved IV instar *Anagasta* larvae, Arnold (1952) noted a significant increase in the number of ADs and the degenerating cells but a decrease in the fusiform PLs. Shapiro (1966) found decrease in the number of PLs in *Galleria* larvae. More and Sonawane (1987) have reported a decrease in PLs and increase in GRs population in adult *Periplaneta americana*. In female adult, *D. koenigii*, Mishra (1999) reported decrease in PRs and PLs but increase in GRs, ADs and OEs. Chaubey, (2002) and Pandey *et al.* (2003a) found decline in the number of PRs, PLs and GRs but rise in the count of SPs, ADs and OEs. It is reported that very high stresses slow down the growth which may lead to developmental malfunction, such as larval ecdysis, immune responses and adult emergence (Neven, 2000; Chapman, 2002; Pandey *et al.*, 2010; Arias *et al.*, 2011; Beckett, 2011).

Repeated hemolymph withdrawals: About four decades after first report of Jones (1967b) on DHC without any gross effect on hemocyte picture in *R. prolixus* following repeated hemolymph withdrawals, Pandey *et al.* (2003a) investigated its effect in lemon-butterfly, *P. demoleus* and found a reduction in THC along with much variation in relative percentage of various cell types. Pandey (2004) found that the repeated withdrawals of blood have not only caused considerable variation in DHC but also showed a marked reduction in THC of *D. chryssipus*. They further reported that increase in PRs count is due to increase in their Mitotic Indices (MI) which play an important role in maintaining abundance of circulating hemocytes. A number of similar observations on increased mitotic activity under certain stress conditions (e.g., bleeding, injury etc.) have been made in other insect spp. (Gupta, 1985a). The high MI in PRs is, therefore, not surprising in view of their nature as stem-cells that give rise to certain other hemocyte types as has been reported earlier (Arnold, 1970; Lai-Fook, 1973; Gupta, 1985a). Pandey (2004) also found the difference in the increase of PRs count after one or two withdrawals a day in

D. cingulatus. It seems to depend upon the total volume of hemolymph withdrawn, more the withdrawal lesser the increase and vice-versa.

Ventral nerve cord sectioning: The role of Ventral Nerve Cord (VNC) in metamorphosis has been experimentally investigated in various lepidopterous insects by several workers (Kopec, 1922; Bounhiol, 1938; Yamashita *et al.*, 1975; Mala and Sehnal, 1978; Tiwari *et al.*, 1989, 1999b; Shukla *et al.*, 1993). The effect of its severance on the population of hemocytes which are known to be implicated in insect moulting has been also studied in the lemon-butterfly, *P. demoleus* (Tiwari *et al.*, 1997; Tiwari and Shukla, 2000). These authors have reported that implantation of brain reversed the effect of nerve cord severance. The role of hormones/hormonal analogues on hemocyte count in VNC severed larvae has also been investigated. Recently, Pandey (2004) studied the effect of VNC sectioning at different levels on THC in *D. chrysippus*. He revealed that the sectioning between first and second abdominal ganglion (AG₁-AG₂) elicited a gradual decrease in THC reaching its lowest count. The effect of VNC sectioning on relative percentage of different hemocyte types has also been studied (Pandey, 2004). Pandey (2004) found an increase in PR-percentage in experimental group reaching its maximum to 30.9 per cent in the larvae having their VNC severed between AG₁-AG₂. The PLs-percentage revealed a decreasing trend. The number of GRs also rose in experimental insects while SPs and ADs both declined. OEs exhibited a very little fluctuation in experimental larvae. VEs were not traced in experimental group since their moulting was delayed or prepupae were not formed. Impact of nerve cord severance at the level of first and second abdominal ganglia affected the contour of different cell types (Pandey, 2004). He found PLs with reduced cytoplasmic prolongations, called pseudopods and the GRs were observed with three or four large granules towards their periphery. Vacuolization of the cells and breaking of the subcellular organelles as well as nucleus took place and ultimately these cellular contents came out of the cells by breaking the cell membrane.

Aortectomy/severance of ovariolar nerves: The neurohaemal role of aorta has been reported in most of the heteropteran insects studied so far (Tiwari and Srivastava, 1975; Tiwari *et al.*, 1975; Raabe, 1989). Srivastava and Tiwari (1978) by severance of aorta inhibited egg maturation in *D. koenigii*. A similar effect was found by transecting the nerves supplying the ovaries in the same insect species by Srivastava *et al.* (1991). The effects of aortectomy and severance of ovariolar nerves have also been studied on the number and relative percentage of hemocytes and their role in the transfer of nutrient material and in the initiation of hormone secretion in prothoracic glands have been investigated (Wigglesworth, 1972; Takeda, 1977). Pandey and Tiwari (2007) found the impact of severance of aorta on normal pattern of THC in *D. cingulatus* during the first egg laying cycle. Immediately after surgery, the total number of hemocytes was more or less same to those recorded in control bugs but after 6 days, it was reduced to about 50% in aortectomised females. Similar to aortectomy, the normal pattern of THC changed following severance of ovariolar nerves and the total count was reduced to 50% after 6 days of surgery (Pandey, 2004).

Feeding, brain implantation and hormone application: Pandey (2004) revealed that while resumption of feeding, brain implantation and 20-HE injection caused an increase in THC, the topical application of methoprene resulted in a further decline in 48 h starved larvae in comparison to the control insects. He further observed that the maximum increase in hemocyte percentage was brought about following 20-HE injection. Pandey (2004) found that depending upon the body weight, the experimental larvae showed varied response to these treatments. While brain

implantation and 20-HE injection caused 20 to 23% increase in THC, respectively in the larvae whose VNC was severed before AG₂, a 17 and 19% rise was found in those larvae whose VNC was severed between AG₂-AG₃. The effect of sectioning between AG₅-AG₆ on THC following these two treatments was little pronounced. The methoprene application caused a decline depending upon the level at which the VNC was sectioned (Pandey, 2004).

Certain botanicals and neem-based insecticides: The hemocytic responses to biological agents as well as toxins have been studied in many insects and the resultant phenomena are phagocytosis, encapsulation and disintegration or distortion of cell contour (Saxena and Tikku, 1990; Sharma *et al.*, 2003; Pandey and Tiwari, 2011). Effect of some botanicals on THC (Saxena and Tikku, 1990; Chaubey, 2002) and DHC (Saxena and Tikku, 1990; Sharma *et al.*, 2003) have been also reported. Pandey *et al.* (2007) found feeding of V instar larvae of *D. chrysippus* for 24 h on fresh *Calotropis* leaves dipped in 50% crude leaf extract of *A. conyzoides* caused about 53% reduction in THC. They found that the hemocyte profile also differed in treated larvae. While PRs, PLs and GRs decreased, SPs, ADs and OEs increased in number in both the groups of larvae after 24 and 48 h of treatment. The maximum decrease of 23-25% was shown by GRs while ADs exhibited an increase between 35-48%, which was reduced to 11 from 47% in case of OEs after 48 h of treatment.

Biotic factors: Insects express robust innate immune reactions to microbial and parasitic infections. Two broad categories of immune reactions are recognized, humoral and hemocytic. The intracellular signal transduction systems acting in humoral immune reactions have been extensively characterized (Hoffmann, 2003; Lemaitre and Hoffmann, 2007). Hemocytic immune reactions are characterized by direct interactions between host hemocytes and foreign invaders. Specific cellular defence reactions include phagocytosis, micro-aggregation, nodulation and in the case of larger invaders, encapsulation (Lavine and Strand, 2002; Stanley and Miller, 2006). It is reported that, hemocytic immune reactions are well described but less information is available on the biochemical signalling systems responsible for mediating and coordinating them (Gillespie *et al.*, 1997; Stanley, 2006). Eicosanoids mediate insect cellular immune reactions (Stanley-Samuelson *et al.*, 1991; Miller *et al.*, 1994). Experiments with a broad sampling of insect taxa and developmental stages support the idea that eicosanoids mediate cellular immune reactions in all insect species that express cellular immunity (Bedick *et al.*, 2001). Likewise, experiments with various species of protozoans, fungi, bacteria, parasitoids and viruses point out that eicosanoids act in immune defence against a phylogenetically broad variety of challengers (Stanley, 2006; Stanley and Miller, 2006; Durmus *et al.*, 2007; Stanley and Shapiro, 2007). It is reported that eicosanoids are crucial intermediaries of phagocytosis, micro-aggregation, cell spreading and nodulation responses (Stanley and Miller, 2006). It also mediates whole-animal behavioural fever reactions to infections in *Schistocerca gregaria* and likely in other insect species too (Bunday *et al.*, 2003). Insect micro-aggregation, nodulation and wound reactions necessarily engage migration to direct hemocytes toward developing micro-aggregates and nodules as well as toward wound spots. Relocation is a property of prokaryotic and eukaryotic, including insect cells (Baker *et al.*, 2006; Jin and Hereld, 2006). It is expected that insect hemocytes are able to notice and wander toward a source of N-formyl-met-leu-phe (fMLP), the major chemotactic peptide from *E. coli* (Marasco *et al.*, 1984) and that pharmaceutical modulation of eicosanoid biosynthesis restrain hemocyte migration.

FUTURE APPLICATION OF HEMOCYTE SCIENCE

Use in insect applied sciences: It is known that insects are both beneficial and harmful. To get healthier result there is need to endorse 'beneficial insects and manage the harmful insect pests. Generally, to improve the productivity of beneficial insect and to control the harmful insect, a number of parameters are being used by the investigators to see the impact of various experiments. In almost all the cases, the effects are visible in late stage. The eventual result of the desired experimentation is being explained only with the survival which could have been affected because of other adversities/stresses of the environment or other unknown reasons which require explanation. Our assessment on identification of stresses is based on morphological characteristics/phenotypic traits which is now and again contradictory and deceptive. To proceed in this direction, we need to have a catalogue-first-hand information on the physiologically and biochemically active and sensitive components of insects. It is reported that hemocytes are biochemically very active and very sensitive cellular components of insects and hence a catalogue on them will be very helpful in identifying and quantifying the stresses. It will be useful to study the hemocyte immune responses of insects in benefits of human kind in the field of applied and biomedical sciences. Proper understanding of insect hemocytes will lead to help the betterment of applied sciences of insects and related industry. For example in recent past, due to global warming, day time temperature (May-June) shoots up and it goes up to 35 to 45°C or even more. It causes adverse impact on the survival of tasar silkworm pupae and invites heavy loss to the tasar silk industry. If hemocyte based indicator will be standardised, the suitable temperature in reference to proper storage can be evaluated.

Pest control: Synthetic chemical insecticides have played important and beneficial roles in the control of insect pests and the reduction of insect borne diseases for nearly 50 years. Their use will remain necessary for many more years. Nonetheless, it also poses real hazards. Some leave unwanted residues in food, water and the environment. Low doses of several insecticides are toxic to humans and other animals and some are suspected to be carcinogens. Hence, many researchers, farmers and homeowners are seeking less hazardous alternatives to conventional synthetic insecticides. Presently it focuses on microbial insecticides; botanical insecticides and insecticidal soaps; attractants and traps; and beneficial insects and mites (natural enemies of pests). Proper understanding of hemocyte in the form of catalogue may lead the effective control of pests in assuring healthy crops and enhancing agricultural productivity and other application.

Use in medical and pharmaceuticals sciences: A detailed understanding of insect immune system presumes significance for scheming novel disease control strategies against insect-vector borne diseases. Towards this goal, several large-scale genome and transcriptome projects have been initiated in insects in the past decade. Noduler was found to be evolutionarily conserved among insects and it would be worthwhile to analyze its role in other insects. Recently numerous analogues have been observed between insect and mammalian innate immune pathways. However, nodulation like response is not reminiscent of any of the vertebrate innate immune mechanisms. It is reported that several mammalian extra cellular matrix proteins of unidentified function show resemblance with Noduler. Thus, it will be interesting to explore their role in vertebrate immune pathways in view of the insect hemocytes. Insects possess a complex and efficient system of biological defence against pathogens and parasites. An essential component of immunity is a mechanism for surveillance by which an organism can detect foreigners or the

presence of nonself molecules. The recognition of nonself stimulates defence responses with the recruitment of hemocytes and with humoral response. The hemocytes of insects play a very important role in general metabolism and in defending against the foreign bodies as these are the main immune competent cells of insect cellular immunity. Present catalogue will help studying the role of hemocyte in drugs and vaccines.

Can hemocyte help induce mammalian immune-tolerance?: In the recent past a strong resemblance has been found between the insect immune system and the mammalian innate immune mechanisms signifying their common base. It is reported that, insects have no adaptive immunity like vertebrates. Thus, they depend on the innate immunity to defend themselves against adverseries and infectious organisms. The humoral immune components of insects play a crucial role in prokaryotic and eukaryotic parasite killing, hemocytes, which are cells circulating in the hemolymph, are also responsible for defending insects against pathogens and other adversities/stresses. These defence reactions are mediated by phagocytosis, encapsulation, wound repair and coagulation. In response to pathogenic infection, insects can express highly efficient defence mechanism using both cellular and humoral immunity. Hemocyte capsule for immunity is one major area of research. Hence there is need to work out the possibility to use hemocyte for induction of mammalian immune-tolerance.

Hemocyte as indicator for change: Literature says that, our awareness of insect hemocytes and their functions in general is quite extensive. As a new trend, workers are now trying to use the hemolymph as a medium for controlling insect pests because the changes occurring in the hemolymph are expected to get transferred to other portions of the body rapidly. Any change in THC of particular insect directly or indirectly affects the insect negatively. Insect hemocytes represent a suitable cell type to analyze differentiation and their differential profile varies against stresses and other changes. Hence, hemocyte can be used as an indicator for change.

Insect-science in future: Insects are evolutionarily successful organisms and account around 75% of total animal population. It is still ambiguous that being a cold blooded/poikilothermic organism, without acquired immune responses, how do they survive in all geographical regions of the world? An efficient immune system is one of the attributes for this evolutionary success. The increased availability of genome sequences has provided the basis for comprehensive understanding of organisms at the molecular level. Molecular mechanisms involved in hemocytes immune responses should be studied. It is expected that variation in hemocytes consequent to stresses /adversities can be used as indicator for health and it can be potentially utilized in applied and biomedical sciences. Hence these areas should be explored and utilised for human welfare.

ACKNOWLEDGMENTS

RKT is thankful to Vice-chancellor Nehru Gram Bharati University (NGBU) for constant support and to Dr. P.S. Mishra, for extending his assistance in exalting the language.

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