INTRODUCTION

Bats are an evolutionarily old group of flying mammals under the order Chiroptera and interesting from several points of view. The ability of echolocation by ultrasonic sound waves, hibernation and aestivation in extreme weather conditions, ability of the females to store spermatozoa for long times in their genital tracts are only a few of the several peculiar characteristics of these animals (Young, 1962; Wimsatt, 1977). It was also known since the early fifties that bats harbour several dreadful pathogenic bacteria (Klite, 1965; Arata et al, 1968; Constantine et al, 1970), viruses like rabbies (Soave, 1966; Smith et al, 1967; Fischman and Ward, 1968; Correa-Giron et al, 1970), Japanese B encephalitis (Sulkin et al, 1963, 1966a, 1966b, 1970a, 1970b; Miura et al, 1970; Allen et al, 1970), St. Louis encephalitis and many other arthropod borne arboviruses (Ito and Saito, 1952; Sulkin, 1962; Banerjee et al, 1984); epidemiological association between bats and mycotic agents like Histoplasma capsulatum etc. was also shown (Klite and Young, 1965). However, most of these studies chiefly emphasized on the carrier role of bats. Although the bats carry such pathogens, they do not usually manifest any apparent symptoms of the diseases (Queiroz-Lima, 1934; Pawan, 1936; Burns et al, 1956; Smith et al, 1967; Sulkin

and Allen, 1970). This fact alone makes these animals an interesting subject for immunological analyses. However, a systematic approach towards the problem of immunity in bats was lacking.

Reports on the immune system of bats started to come when different groups of workers (Heck, 1965; Sulkin et al, 1966a,b; Leonard et al, 1968; Hatten et al, 1970) made investigations on the production of humoral immunity in bats against experimentally inoculated JBE or ϕX 174 viruses. Although no clear evidence of the presence of complement fixing antibodies in bats was obtained, naturally occurring antibodies detected by haemagglutination inhibition test to group B arboviruses in bats was reported (Whitney, 1963; Williams et al, 1964; Stanley and Choo, 1964; Pavri and Singh, 1965). One important finding by Leonard and his co-workers (1968) was that these animals have both 19S and 7S types of immunoglobulins, but the immune response was quantitatively deficient as compared to guinea pigs. McMurray and co-workers (1978, 1979, 1981, 1982) reported both humoral and cell mediated immune responses in bats in terms of blast transformations and other parameters (McMurray et al, 1978, 1979, 1982; Greer and McMurray, 1981).

A systematic approach to analyse the immune system and immune responses in bats was attempted by Chakraborty and

Chakravarty (1983) who demonstrated that bats, while being ranked as a group of evolutionarily old mammals, possess a well defined immune system almost similar to that of the primates. A study of the ontogency of the primary lymphoid organ thymus revealed a remarkable age dependent involution just like in human (Chakraborty and Chakravarty, 1984c). Analyses of the secondary lymphoid organs like spleen and lymph nodes of the Indian fruit bat Pteropus giganteus showed the presence of highly organized white pulps in spleen and lymph nodes of normal animals and of well differentiated germinal centres in immunized animals (Chakraborty and Chakravarty, 1984a). The organization of the lymphoid cells in these secondary lymphoid organs resemble very much the situation in the higher primates.

In spite of their possession of well evolved and well organized lymphoid organs, bats show a significant delay in the onset and reaching the peak of immune response (Chakraborty and Chakravarty, 1984b). Antibody mediated immune response as measured by plaque forming cell (PFC) assay was found to persist for a longer period and both the B-mercaptoethanol (BME) sensitive primary and BME resistant secondary responses were observed with a single antigenic challenge unlike the other animals which usually require a booster challenge for

the production of secondary response. Cell mediated immunity in these animals was also expressed at a lesser degree (Chakraborty, 1982), reaching a peak with a similar delay as in case of humoral response.

The understanding of the mechanism of the delayed onset and decay of immune responses of bats is yet at minimum. Cell types, their interactions among themselves and with ubiquitous factors in plasma may likely play crucial roles. Thus, a critical characterization of the different types of immunocompetent cells and their interactions in bats needs to be initiated.

The immunocompetent cells of higher vertebrates are the B and T lymphocytes and other accessory cells like macrophages and follicular dendritic cells. These cells can be categorized on the basis of their differential adhessibility to plastic substratum and nylon wool fibres. The macrophages have been shown to possess a high surface adhessibility to glass, plastic and several other substrata including Sephadex and Latex beads. (Sjoberg et al, 1972; Ly and Mishell, 1974; Lee et al, 1976; Steinman et al, 1979). The follicular dendritic cells are also known to possess similar adhessive property (Steinman et al, 1979). Thus in the present study attempts were made to isolate these cell types on the basis of adhession to plastic surface.

Julius and his co-workers (1973) showed that murine T and B cells differ in their surface adhessiveness to nylon wool fibres and that this differential surface adhessibility can be used successfully to separate the T lymphocytes from the more adhessive B lymphocytes. Since then, several workers have used columns of nylon wool fibres to separate the T and B lymphocytes of mouse, human and other mammals (Handwerger et al, 1974; Trizio and Cudkowicz, 1974; Tada, 1977, Tada et al, 1978). Similar procedures may also be applied in case of the immunocompetent cells of bat to see whether they can be categorized according to their differential cell surface adhessiveness.

The immunocompetent cell types not only differ in their adhessibility and function, but are also distinguishable by means of their characteristic cell surface morphology. This is why several workers in the past have used the scanning electron microscope (SEM) to study the different immunocompetent cell types (Lin et al, 1972; Polliack et al, 1973a,b; Lin and Wallach, 1974; Alexander and Wetzel, 1975; Alexander et al, 1976; Newell et al, 1976; Roath et al, 1978). Studies with murine and human lymphocytes from normal peripheral blood and tissues as well as from abnormal tissues including tumours and other cell lines (Wilson and Nossal, 1972; Holt et al, 1972;

Polliack and De Harven, 1975; Polliack et al, 1975, 1981; Kwock et al, 1976; Polliack et al, 1981) revealed detailed and characteristic surface morphology of the immunocompetent cells. Polliack and his associates reported the presence of cell surface microvilli on the B lymphocytes while the T lymphocytes were shown to possess a comparatively smooth cell surface (Lin et al, 1972; Polliack et al, 1973a, 1973b, 1975, 1976; Lin and Wallach, 1974; Polliack and DeHarven, 1975). Even some neoplastic cells of B lymphocyte origin revealed the presence of surface microvilli, while some leukemic cells of T lymphocyte origin demonstrated a smooth cell surface (Polliack and DeHarven, 1975; Polliack et al, 1981, 1983). This was also supported by immunofluorescence and other microscopic techniques (Sciorra and Eckert, 1974; Fagraeus et al, 1974; DeHarven et al, 1975; Reyes et al, 1975; Vila and Taub, 1975; Padnos, 1976; Mason et al, 1977; Renau-Piqueras, 1978; Renau-Piqueras and Knecht, 1979; Polliack and Gamliel, 1983). Wetzel and co-workers (Wetzel et al, 1974; Alexander and Wetzel, 1975). Barber and Burkholder (1975) Kwock and co-workers (1976) and Newell and associates (1976) stressed the importance of type of fixation, temperature and other parameters for the study of cell surface morphology. Kwock and associates (1976) also reported that some alterations in the surface topography of lymphocytes may be caused by the use of nylon wool columns.

The macrophages have been well characterized by their possession of irregular surface morphology and different types of pesudopodial projections (Albrecht et al, 1972 & 1978; Besis, 1973; Brynes et al, 1976). There is another type of accessory cells, the follicular dendritic cells, which are known to play major roles in trapping and modification of antigens and possibly retention of antigens for long time before presenting them to the lymphocytes (Nossal et al, 1968a, 1968b; Steinman et al, 1973, 1974a, 1974b, 1975, 1978; Klaus et al, 1980; Van Rooijen, 1980).

Thus the scanning electron microscopic study would help to characterize and distinguish the different immunocompetent cell types of bats.

Besides the cell surface characteristics, immunoreactive cells in higher vertebrates also differ by several cell surface antigenic markers. B lymphocytes have characteristic immunoglobulin (Ig) markers on their cell surface (Raff et al, 1970, 1971; Wilson et al, 1971; Rabellino et al, 1971; Padnos, 1976). T lymphocytes lack this marker, but possess other specific cell surface antigens such as θ or Thy 1, and Ly in mouse, CD in human etc (Reif and Allen, 1964; Shaw, 1987; Roitt, 1988). Similarly, macrophages of monocytic origin possess characteristic

Mac-1 or-2 surface markers in mouse (Klaus et al, 1983). Thus, characterization of different types of immunocompetent cells of bat on the basis of cell surface markers have been locked into in the present investigation. B lymphocytes in peripheral blood and secondary lymphoid organs of man and mouse bear predominantly IgM or IgG and also IgD molecules (Papamichail et al, 1971; Pernis et al, 1971; Cooper and Lawton, 1972; Fröland and Natvig, 1972; Teale et al, 1980; Lafrenz et al, 1986). As because both IgM and IgG mediated immune responses have been demonstrated in the bats (Leonard et al, 1968; Chakraborty, 1983a), the IgM and IgG bearing cells in bats equivalent to B lymphocytes have been investigated in the present study with fluorochrome conjugated antibodies to bat IgM and IgG.

Next, the immunocompetent cell types of bat bearing Thy-1 type of surface antigen were characterized with anti-Thy-1 type serum. Raising of anti bat thymocyte serum is difficult because of total involution of thymus in adult bats (Chakraborty and Chakravarty, 1984), but it is known that Thy-1 is often shared by brain cells in different vertebrates (Reif and Allen, 1964; Acton et al, 1974; Trowbridge et al, 1975; Mansour and Cooper, 1975; Williams et al, 1976) and it has also been shown that the Thy-1 molecule has been highly conserved during evolution (Cambell et al, 1981; Cotmore et al, 1981;

Mackenzie et al, 1981; Williams and Gagnon, 1982; Mansour et al, 1985, 1987; Shalev et al, 1985). Golub (1971, 1972) raised anti-mouse brain serum in rabbit and employed it successfully to identify the mouse thymocytes. Later, anti-brain Thy-1 serum have been used by several workers to detect T lymphocytes of many vertebrates (Claggart et al, 1973; Acton et al, 1974; Morris et al, 1975; Cotmore et al, 1981). Chakraborty and Chakravarty (1983a) took advantage of this fact by absorbing the rabbit anti-bat lymphocyte serum with bat brain homogenate and showed a differential susceptibility of the lymphocytic cell population of bat to this pre-absorbed anti serum, and suggested a possible dichotomy of lymphocytes in the line of B and T cells. In the present investigation, Thy-1 type antigen bearing lymphocytes of bat have been identified by their susceptibility to anti-bat brain serum, and then localization of these cell types in secondary lymphoid organs have been studied after repeated injections of the serum in the bat and making histological preparations of spleen and lymph nodes from the anti serum treated bats. Such studies for anatomic compartmentalization of B and T Cells and their differentiation in lymphoid organs were attempted in mouse, rat and lizard (Parrot and De Souza, 1966, 1969, 1971; Howard et al, 1972; Bhan et al, 1975; Gutman and Weisman, 1972; Pitchappan and Muthukkaruppan, 1977b; Barclay, 1981; Van Ewijk et al, 1981,

Rouse et al, 1982). In man, recent immunofluorescence and histological observations of primary and secondary lymphoid organs from normal subjects as well as patients with T or B cell deficiency have yielded important information regarding the T dependent and B dependent regions in these organs (Lamelin et al, 1978; Seymour et al, 1980; Bhan et al, 1980; Poppema et al, 1981).

For characterization of mouse and human immunocompetent cells, transmission electron microscopy (TEM) has been used since early 1960's (Zucker-Franklin, 1963, 1969; Inman and Cooper, 1963; Movat and Fernando, 1964, 1965; McFarland and Heilman, 1965; Harris et al, 1966; Hummeler et al, 1966; Heiniger, 1967; McFarland, 1969; Tanaka and Goodman, 1972; Besis, 1973). Analysis of cellular and nuclear volume from serial ultrasections revealed a significant difference between thymic and lymph node lymphocytes of mice (Heiniger, 1967). Later, the use of specific antibodies coupled to ferritin, horse raddish peroxidase and such other electron dense markers resolved two distinct populations B and T cells (De Petris et al, 1963; De Petris and Karlsbad, 1965; Storb et al, 1969; Reyes and Bach, 1971; Murphy et al, 1972). Using this technique, Matter and his associates (1972) distinguished different stages of differentiation of B and T cells depending on cell size and cellular content of organelles.

Cohn and his co-workers (Cohn and Benson, 1965; Cohn, Hirsch and Fedorko, 1966) and others (Sutton, 1967; Chapman et al, 1967) studied the structure and differentiation of monocytes and macrophages under the TEM. Further study of the structure and fate of several subcellular organelles in the macrophages were also made (Cohn, 1968; Nicholas et al, 1971; Allison et al, 1971). Allison and his co-workers (1971) studied in detail the formation of pseudopodia, the striking and characteristic feature of macrophages. To our knowledge there is as yet no report on the fine structure of the immunocompetent cells of bat and so, attempts were made in the present investigation to study the fine structure of these cells by transmission electron miscroscopy.

Summarily, the present study attempts first to isolate the different types of immunocompetent cells from the secondary lymphoid organs of the Indian fruit bat, Pteropus giganteus on the basis of their differential cell surface adhessibility to plastic and nylon wool. The subsets of immunoreactive cells thus isolated are further analyzed by scanning electron microscopy to reveal the cell surface topography. Then the different cell populations are characterized with reference to cell surface markers such as IgM, IgG and Thy-1. Anatomical compartmentalization of B and T cell type, is studied by rabbit anti Thy-1 type serum in vivo. Finally, transmission electron microscopy is used to reveal ultrastructural characteristics and organization of the immunocompetent cells in situ.