S U M M A R Y

Bats, classified under the order Chiroptera which originated almost at the beginning of mammalian evolution, are known for their role as carriers of a number of dreaded pathogens without being infected. Previous studies in our laboratory revealed a noticeably delayed onset and decay of immune responses in these animals, but little was known about the cells mediating immunity in bats. In the present work, the major categories of immunocompetent cells in the Indian fruit bat Pteropus giganteus have been characterized in detail by several criteria, viz. their adhessibility to different substrata, cell surface topography, surface antigenic markers, organization in lymphoid organs, and ultrastructural details.

Cell separation techniques based on physical adherence to plastic and nylon wool distinguished three major groups of immunocompetent cells with differential surface adhessibility — the plastic adherent cells, the nylon wool adherent cells and the nylon wool non adherent cells. Intake of the vital dye neutral red by the plastic adherent cells but not by other cell types indicated the phagocytic nature of these

cells. Subsequent immunofluorescence and cytotoxicity experiments revealed that the plastic adherent cells were negative for both surface Ig and Thy-1 type antigen; the nylon wool adherent cells were surface Ig positive but Thy-1 negative, while the reverse was true for the nylon wool non adherent cells.

The plastic adherent, nylon wool adherent and nylon wool non adherent cells appeared in a ratio of about 1:2:9, which is pretty close to the ratio of murine macrophages, B and T lymphocytes obtained by similar techniques; this indicates that delayed responses in bats are probably not caused by a deficiency in the type or quanta of the immunocompetent cells.

Next, scanning electron microscopic analysis delineated significant differences in surface topography among these three categories of cells. The plastic adherent cells were characterized by their possession of different types of pseudopodia, the nylon wool adherent cells showed small microvilli, while the nylon wool non adherent cells characteristically had a smooth cell surface. These features are in compliance with the macrophages, B and T lymphocytes respectively of many other mammals.

A small group of plastic adherent cells possessing bulbuous protrusions were distinguished by SEM from the usual pseudopodia bearing plastic adherent cells; these cells resembled the murine follicular dendritic cells. Such cells, because of their proven role in antigen retention and presentation to other lymphoid cells in mouse, rat, man etc. led us to propose that they might have important implications for the delay in decay of immune responses in bats.

Significant changes in surface topography of especially the nylon wool adherent cells were noted after in vivo stimulation with a model antigen like sheep erythrocytes. A notable increase in surface ruffling and the production of long filamentous spikes on the surface of nylon wool adherent cells possibly reflected an alteration in membrane fluidity which is usually associated with transitional changes in the lipid bilayer of the membrane during lymphocyte transformation.

Further characterization of the cell types was brought about by analyses of cell surface antigenic moieties. In course of this study, serum immunoglobulins of bat isolated by affinity chromatography, were fractionated into two major classes by gel filtration. The molecular weights of these two fractions as judged by their chromatographic elution pattern and their

relative electrophoretic mobility resembled those of human Ig M and Ig G. These two classes of Ig thus represent the Ig M and Ig G in this animal. Subsequent immunofluorescence microscopy using rabbit antisera to bat Ig M and Ig G fractions revealed the presence of surface Ig M or Ig G specifically on the nylon wool adherent cells, thereby confirming further their equivalence to the B lymphocytes of other mammals. Majority of the B cells possessed cell surface Ig M as in other higher vertebrates while presence of Ig G on some B cells distinguished them from reptilian or avian Ig Y bearing cells.

Analysis of the tissue distribution of the B lymphocytes revealed that bone marrow of bats contained 32-45% B cells, probably indicating that bone marrow is the primary lymphoid organ for generation of B lymphocytes. Spleen and lymph nodes contained 64-71% and 29-35% B cells respectively; such a reciprocal proportion is also true for mouse and man. Number of B cells in peripheral blood is above 70% which is strikingly higher than in normal mouse or man; such a higher proportion is encountered in human patients with certain immunodeficiency disorders. The exact significance of it for the immune responses in bat is yet to be resolved.

The existence of a T cell compartment in the bat immune system was confirmed by the demonstration of susceptibility of

specifically the nylon wool non adherent cells to rabbit anti-bat brain serum, thereby indicating sharing of a Thy-1 type cell surface antigen between these cells and brain cells like the T lymphocytes of other mammals. Anti-thymocyte serum could not be used in this experiment because of non availability of thymus in adult bats due to thymic involution as in man. Thus a clear dichotomy of the lymphocytic population along T and B lines in the bat was established.

In vivo administration of anti-bat brain serum in bats effectively depleted the T lymphocyte population from secondary lymphoid organs such that the ratio of plastic adherent : nylon wool adherent : nylon wool non adherent cells came down to about 1:2:4 after 72 hours of treatment, as compared to the normal ratio of 1:2:9. This experiment then led us to investigate the localization of the T lymphocytes in the secondary lymphoid organs of bat. Histological study of spleen and lymph nodes of anti-brain serum treated bats revealed T cell depletion chiefly in the periarteriolar lymphocytic sheath of splenic lymphoid follicles and in lymph node paracortex; these regions may therefore be marked as T dependent regions in bats as in mouse or man.

Besides the surface topographic and antigenic characterization, the internal architecture of the cells was studied by

transmission electron microscopy of the cells in secondary lymphoid organs. On the basis of size and shape of the cells, nucleocytoplasmic ratio, nuclear heterochromatinization, cytoplasmic organelles etc., four types of immunocompetent cells were identified; small lymphocytes had a heavily heterochromatinized nucleus surrounded by a thin rim of cytoplasm, while the larger lymphocytes possessed a less heterochromatinized nucleus and a higher content of mitochondria, Golgi vesicles, endoplasmic reticulum, ribosomes etc. Plasma cells had characteristically large, mainly euchromatic nucleus and a large amount of cytoplasm while macrophages showed an irregular shape, less heterochromatimized nucleus and cytoplasm containing vesicles resembling lysosomes and phagosomes.

After immunization, an increase in cell size, nuclear cytoplasmic ratio and ribosomal content and a decrease in nuclear heterochromatimization indicated the differentiated state of these cells under TEM.

The present investigation tried to characterize the immunocompetent cells of Pteropus giganteus, an evolutionarily old mammal, from several points of view, such as structural endowments, specific cell surface antigenic markers and their differential distribution. All these features are comparable

to those of the highly evolved recent mammals like primates. So it appears that the characteristic features of mammalian lymphocytes evolved almost at the time of origin of mammals and since then they remained as permanent fixtures in course of evolution, or one needs to envisage parallel evolution of immunocompetent cells in different orders of mammals to accommodate the idea of constant changes during evolution.

Furthermore, the similarities in types and ratio of the immunocompetent cells of bat with those of other mammals suggest that the reason for delayed immune responses in bats lies somewhere else than in the possibility of deficiency in types or quanta of immunocompetent cells. Recent revelations in our laboratory about lower density of antigen/mitogen receptors on lymphocyte surface (Paul and Chakravarty, 1989) and slower energy turnover in lymphocytes during activation (Paul, 1986) in bats as genomic deficiency or adaptation might hold the key for explaining peculiarities of delayed immune response in this interesting animal.