RESULTS

PLASTIC ADHERENT CELLS FROM SPLEEN AND LYMPH NODES OF BAT

Spleen and lymph node cells from the bats were incubated in plastic petridishes under appropriate conditions to separate the group of cells adhering to the plastic substratum. It was found that some cells from spleen and lymph nodes adhered readily to the plastic and could be removed later by mechanical means. This plastic adherent cell population represented about 3.55% of the total spleen and lymph node cell population of lymphocytic and monocytic origin. This percentage was fairly constant from experiment to experiment even with varying numbers of cells from different bats (Figre 1).

NYLON WOOL ADHERENT AND NON ADHERENT CELLS

The plastic non adherent cells were further separated by nylon wool fibre column following two protocols. In the first porotocol, the nylon wool was not pretreated and in the second, the wool was pretreated with EDTA and $NaHCO_3$ following the suggestion by Henry (1980). In both the protocols, the results indicate two distinct populations of lymphoid cells — nylon wool adherent and non adherent populations. However, following the first protocol, the yield of adherent and non adherent cells was low, about 2.16% and 1.57% respectively of the initial cell population (Table 1). The pretreatment of nylon wool in

the second protocol led to higher yield of both the cell types, 8.77% adherent and 31.83% non adherent (Table 2), and in further experiments, this second protocol was used.

However, even after this increase in the efficacy of the pretreated nylon wool column, the cell recovery was about 41% in the present study, whereas 50-60% in case of mouse or human (Trizio and Cudkoicz, 1974).

The 3 cell types, plastic adherent, nylon wool adherent and nylon wool non adherent populations have been recovered in the ratio of 1:2:9 approximately (Table 3).

Neutral red positive cells were about 89.6% in the plastic adherent cell population, whereas the nylon wool adherent and non adherent cell populations showed about 12.3% and 6.8% contamination of the neutral red positive cells respectively (Table 4).

SEM ANALYSIS OF SURFACE TOPOGRAPHY OF THE PLASTIC ADHERENT CELLS

Cells from normal bats: The plastic adherent cells from normal bats under the scanning electron microscope revealed an irregular shape, with a diameter usually centered around 5 μm. The conspicuous feature of these cells was the presence of pseudopodial projections. Sometimes they were in the form of a

uropodium at the back of the cell, or as flattened lamellipodia, or finger shaped filopodia all over the cell (Plate I, Figs. 1 & 2). Usually more than one type of pseudopodia were found on a cell. There were certain distinct plastic adherent cells which were comparatively bigger in size, 6-8 µm in diameter and with characteristic bulbuous protrusions of 2-4 µm diameter (Plate II, Figs. 1 and 2). These projections were different from the usual lamellipodia or filopodial pseudopodia.

Cells from immunized bats: Plastic adherent cells from bats immunized with 25% SRBC for 10 days showed an apparent increase of cell size to about 8 µm. The pseudopodia seemed not as prominent as in the cells from normal bats (Plate III, Figs. 1 and 2).

SURFACE TOPOGRAPHY OF NYLON WOOL NON ADHERENT CELLS

Cells from normal bats: About 80% of the cells in this group had a diameter ranging from 6µm to 7 µm. The cells were very regular and round in shape. The cell surface was relatively smooth except a few surface ridges (Plate IV, Fig. 1). Pseudopodia of any type were absent in these cells, but occasionally some localized membrane rufflings could be seen (Plate IV, Fig. 2).

Cells from immunized bats: Immunization caused slight increase in cell size which ranged from 7 to 8 μm and more, and there were some prominent cell surface rufflings (Plate V, Fig. 1). Occasionally some large cells, about 10 μm in diameter, showed some surface 'blebs' (Plate V, Fig. 2).

SURFACE TOPOGRAPHY OF NYLON WOOL ADHERENT CELLS

Cells from normal bats: The nylon wool adherent cells revealed a characteristic cell surface morphology that was distinctively different from that of plastic adherent or nylon wool adherent cells. The surface of these cells showed the presence of some small microvilli like projections along with some pit like formations with a diameter of 0.7 to 1.2 μm (Plate VI, Figs. 1 and 2). During screening, 70 to 80% of the cells were found to be large in size, having a diameter ranging from 7 to 9 μm.

Cells from immunized bats: After immunization, the nylon wool adherent cells showed a marked difference in cell surface topography from that of any other cell types examined. Average diameter of the cells was found normally to have increased to about 10 μm. The characteristic microvilli as seen in normal cells were absent, rather the cell membrane was highly ruffled (Plate VII, Figs. 1 and 2). Such degree of surface ruffling was not found in any other cell types. In addition, some long,

filamentous membrane projections or 'spikes' were present on these cells. The spikes had a length of 2.5 to 2.8 μm, and a width of about 0.3 μm.

Occasionally, very large cells, more than 10 μm in diameter, were found which had extremely ruffled membrane; the spikes were not prominent in these cells (Plate VII, Fig. 3).

IMMUNOFLUORESCENCE STUDY OF SURFACE IMMUNOGLOBULIN BEARING CELLS

Separation of bat Ig M and IgG: Bat anti-BSA immunoglobulins (Ig) were first isolated by affinity chromatography of whole bat anti-BSA serum in BSA conjugated Sepharose 6B column. When this bat Ig was fractionated by gel filtration in Sephadex G-200, two protein peaks were observed at 280 nm. One of the proteins was eluted in the void volume, while the other protein was eluted later (Fig. 1). When purified human Ig G (Sigma, U.S.A.) was chromatographed in the same column, a single protein peak was observed almost at the same position of the second protein peak of bat Ig.

Polyacrylamide gel electrophoresis of the bat Ig isolated by affinity chromatography also revealed two major protein bands, of which one band showed a relative mobility close to that of

purified human Ig G (Plate VIII, Fig. 1). For practical purpose, these two fractions of bat Ig may be called Ig G and Ig M respectively.

Enumeration of surface Ig M and Ig G bearing lymphocytes : Surface Ig M and Ig G bearing cells in the plastic adherent, nylon wool adhrent and nylon wool non adherent cell populations, as well as in spleen, lymph node, bone marrow and peripheral blood were detected by indirect immunofluorescence microscopy using rabbit anti-bat Ig M and anti-bat Ig G as first antibody, and fluoresceinated goat anti-rabbit Ig as second antibody.

Three different types of fluorescent staining were observed using either anti-bat-Ig M or -Ig G antibody — (a) ring like fluorescence around the periphery of the cell (Plate Ix, Figs. 1, 2 and 3) which was often discontinuous (Plate X, Fig. 1) (b) fluorescence spots or patches (Plate XI, Fig. 1) and (c) in case of dead cells, a diffuse and dull fluorescence all over the cell body (Plate IX, Fig. 1) When Ig from normal rabbit (obtained by ammonium sulfate precipitation of normal rabbit serum) was used as the first antibody, no fluorescence was observed, which indicated the specificity of the anti-bat Ig M and anti-bat Ig G antibodies.

Enumeration of positively labelled cells showing fluorescent rings and patches revealed 51-58% surface Ig M bearing cells and 30-39% surface Ig G bearing cell, together 81.89% positive cells in the nylon wool adherent population (Table 7).

In the plastic adherent population, only 16-24% of the cells were positively stained, of which 7.5% to 14.5% showed the presence of surface Ig M and the rest showed surface Ig G (Table 5). Similarly in the nylon wool non adherent population, only 10-20% cells showed fluorescence of which 6-10% cells showed the presence of surface Ig M and 2-9% cells showed surface Ig G (Table 6).

When cells from different tissues were examined it was observed that bone marrow contained 32-45% surface Ig bearing cells, of which 20-24% had surface Ig M and 10-20% had surface Ig G (Table 8). Spleen had a high number of surface Ig bearing cells, about 64-74%. Of this, 40-41% cells were Ig M positive and 24-31% Ig G positive. Mesenteric lymph node on the other hand, showed a lesser number of surface Ig bearing cells — only 29-35% of which 19-23% were positive for Ig M and the rest positive for Ig G (Table 8). In the peripheral blood however, many cells bearing surface Ig were observed; of the 69-90% cells bearing surface Ig, 44-54% were Ig M bearing cells and 25-37% were Ig G bearing cells.

In spite of variation in the number of Ig M and Ig G positive cells in a particular purified cell population or a lymphoid organ, the number indicated a characteristic range for the cell population or the organ.

DIFFERENTIAL SUSCEPTIBILITY OF THE IMMUNOCOMPETENT CELL TYPES TO RABBIT ANTI-BAT BRAIN SERUM

The different immunocompetent cell populations isolated on the basis of adhessiveness to plastic substratum or nylon wool column were tested for their sharing of the brain cell antigen which is usually common with the thymus cell antigen in most mammals, as Thy-1 in mouse (Raff, 1971; Golub, 1971).

Cytotoxic ability of the rabbit anti-bat brain serum was found to be the highest in case of nylon wool non adherent cell population of bat, about 63% at serum dilution of 1:10 as shown in Figure 3. Percent cytotoxicity in the plastic adherent and nylon wool adherent cell populations was always below 10%. When the anti serum was pre-absorbed with nylon wool non adherent cells, the cytotoxic efficacy of the anti serum towards this cell type decreased drastically to almost background level, thereby indicating the specificity of the anti serum for these cells.

EFFECTS OF IN VIVO ADMINISTRATION OF RABBIT ANTI-BAT BRAIN SERUM ON THE IMMUNOCOMPETENT CELL TYPES

Rabbit anti bat brain serum diluted 1:10 with PBS was injected intravenously into each bat every 24 hours for 5 consecutive days following the method of Pitchappan and Muthukkaruppan (1977) to deplete the cells bearing the Thy-1 type antigen shared by brain cells and thymocytes. Spleen and lymph nodes were taken out of bats sacrificed at 24, 72 and 120 hrs after the schedule of 5 injections and the proportions of plastic adherent, nylon wool adherent and nylon wool non adherent cells were determined. The nylon wool non adherent cells were most affected by the antiserum treatment as indicated by the reduction of its proportion from 24 hrs onward and significantly at 72 hrs (Table 9); this is revealed by comparing the data with that in Table 3.

IN SITU LOCALIZATION OF THE AREA OF ANTI-BRAIN SERUM SENSITIVE CELLS IN SECONDARY LYMPHOID ORGANS

In spleen : A primary white pulp follicle of a normal animal was a compact mass of cells with deeply stained nuclei, surrounding a splenic arteriole in the form of a periarteriolar lymphocytic sheath. The white pulps were distributed in the splenic red parenchyma as typical in the primates, which were

previously described in detail by Chakraborty and Chakravarty (1984). The secondary follicles or germinal centres have a prominent circular zone of lightly stained large dividing cells surrounded by a jacket like mantle layer of small lymphocytes (Plate XII, Fig. 1).

After the schedule of 5 injections of anti-brain serum, the average number of lymphocytes per unit area of 0.001 mm^2 in the periarteriolar sheath region decreased 24 hrs onwards and significantly at 72 hrs (Fig. 4). The cells were loosely organized in the white pulps and some pycnotic cells were observed (Plate XII, Figs. 2 & 3).

In lymph node: In normal lymph nodes of bat roughly three areas could be delineated - (a) the outer cortex just below the collagenous capsule and harbouring the white pulp follicles, (b) the deep or paracortex containing cords of lymphocytes and (c) the innermost medulla, mainly consisting of the medullary sinus. The primary lymphoid follicles appeared as concentric masses of lymphocytes and with antigenic stimulation, they converted to germinal centres having a central, less dense zone of large lymphocytes surrounded by small lymphocytes (Plate XIII, Fig. 1).

After the antiserum treatment, there was a significant reduction in the number of lymphocytes per unit area (0.001 mm^2) in the paracortical area, particularly at 72 hrs and 120 hrs (Fig. 5), leaving some empty spaces (Plate XIII, Fig. 3). The cell loss was more severe than that in the spleen.

TRANSMISSION ELECTRON MICROSCOPY OF THE IMMUNOCOMPETENT CELLS IN SPLEEN AND LYMPH NODES

Normal Spleen: Under the transmission electron microscope, normal spleen tissue of bat revealed a compact organization of the lymphoid cells (Plate XIV, Fig. 1). The ultrastructural organization of the cells was more or less similar to those found in mouse and human. The cells were usually spherical or cuboidal in shape. Under the TEM, the average diameter of the cells of different size was found to range from 5 to 7.0 µm. Cells differing in cytoplasmic content, nuclear-cytoplasmic ratio, nuclear heterochromatinization etc. probably represented different types of immunocompetent cells. Four distinct categories of cells could be identified — small lymphocytes, large lymphocytes, plasma cells and macrophages.

To begin with, a typical lymphoid cell may be described.

Typical lymphocyte - The plasma membrane was of usual thickness; microvillous projections as seen in the SEM were not as prominent in the sections. Cytoplasmic granulation varied, possibly depending on the abundance of organelles. Number of mitochondria varied, structurally they were spherical or elongated sac like, and resembled typical mitochondria of murine or human cells (Plate XVI, Fig. 2). Short flattened cisternae of endoplasmic reticulum were seen (Plate XV, Fig. 1). Free ribosomal particles could also be observed.

Although a full fledged Golgi apparatus was not seen in the micrographs, some small vesicular structures present in the sections possibly indicated part of the Golgi apparatus (Plate XVI, Fig. 1). Some membrane bound vesicles, about 0.15 μm in diameter and sometimes surrounded by an electron dense coat were seen with opening to the exterior (Plate XVI, Fig. 2), probably in course of endo or exocytosis. The average dimensions of the cell organelles observed in course of the TEM study were as follows:

Mitochondrial diameter	: 0.25 μm to 0.50 μm
Outer mitochondrial chamber (bound by outer and inner membrane)	: about 820 ^{o}A
Ribosome diameter	: about 140 ^{o}A
Endo or exocytotic vesicle diameter	: about 0.15 μm

Nuclear membrane thickness	: 120°A to 160°A
Perinuclear space	: about 300°A
Nuclear pores	: about 180°A
Nucleolus	: 1.80 to 2.10 µm
Nucleolar granules	: about 150°A.

The nuclear morphology was typical with a perinuclear space below the nuclear membrane and with nuclear pores (Plate XVIII, Fig. 1). Usually the nucleus was round, ovoid or polygonal in shape. The nuclear material could be easily distinguished into the lightly stained granular euchromatin and the darkly stained heterochromatin. The latter was usually present as broad uneven patches mainly along the nuclear margin and also as small patches inside the nuclear mass. Nucleolus of about 2 µm diameter was observed with a central lightly stained region containing some dark granular material which were possibly indicative of ribonucleoprotein synthesis (Plate XVIII, Fig. 2).

Small lymphocyte - These cells represented the majority of the lymphocytes in normal spleen and were around 5 µm in size, and showed a thin rim of cytoplasm containing very few organelles except a few free ribosome (Plate XIV, Fig. 1). The nucleus of these cells was not always round in outline and was slightly notched at some places and contained a fair

amount of heterochromatin. Some of these features resembled those of small T lymphocytes in mouse.

Large lymphocyte - These cells were larger in size, usually about 6.5 µm to about 8 µm in diameter (Plate XVI, Fig. 1), and had more cytoplasmic content. Number of mitochondria was variable. Occasional short profiles of endoplasmic reticulum, and some small vesicles, probably indicative of a Golgi complex, were observed. Free ribosomes were scattered in the cytoplasm. Nucleus was usually polygonal in shape and the marginal heterochromatic patches were less heavy than in the smaller lymphocytes.

Besides these cells, there were some cells that could be characterized as plasma cells and macrophages which were more in number after immunization. So they are described in detail later.

Spleen after immunization: The cells in the spleen from bats immunized with 25% SRBC for 10 days were not as compactly organized as in the normal spleen. The cells were usually 7 µm to 9 µm in diameter and were cuboidal or elongated in appearance (Plate XIX, Fig. 1). Most of these cells looked like the large lymphocytes seen in the normal spleen. Although some filamentous projections were observed by SEM on the nylon wool adherent

cells isolated from immunized bats, their presence was not revealed markedly in the tissue state of organization under the TEM.

Cytoplasm of the cells showed the presence of several mitochondria of usual shape and size, many free ribosomes and small vesicles as in the normal spleen cells (Plate XXI, Fig. 1).

Nucleus in these cells was large and often deeply invaginated (Plate XXI, Fig. 1). The average area wise ratio of nucleus to cytoplasm as calculated from planimetric measurements from the micrographs was about 0.7 which is slightly higher than the average of 0.6 obtained from normal small and large spleen cells. This condition was also reflected in the photograph of isolated nylon wool adherent cells from immunized bats (Plate XXII, Fig. 1). Nuclear heterochromatin in most of the cells was less in amount. Thin patches of heterochromatin were mostly distributed along nuclear margin and a few small heterochromatic patches were seen inside the nucleus. Occasionally a nucleolus was seen.

Plasma cells - Some of the nylon wool adherent large cells from immunized bats had a significantly higher cytoplasmic content almost equal to the nuclear amount, euchromatic nucleus and often with som

vesicles near to the plasma membrane. Although idealized ergastoplasmic reticulum was not prominent, but in all likelihood the cells represented the plasma cells (Plate XXII, Fig. 1). This type of cells were only occasionally seen in the normal spleen (Plate XVIII, Fig. 1).

Macrophage - Certain cells in the spleen from immunized bats showed an irregular outline (Plate XX, Fig. 1). Some of them were as big as 9 µm. A few vesicular structures of different sizes and containing granular or homogeneously osmophillic material were observed in these cells and resembled the lysosomal bodies seen in murine or human cells. Few microfilaments were also noticed. Possibly these cells represented the macrophages in bats.

Normal lymph node : The ultrastructural details of the lymphoid cells in lymph nodes of normal bats did not vary much from that of normal spleen cells. The cells, with a diameter usually ranging from 5 µm to 6 µm were roughly spherical or cuboidal in shape (Plate XXIII, Fig. 1 & 2).

Cytoplasm of the cells was light and granular, and contained some mitochondria. Sometimes, small membrane bound vesicles were seen, some of which were coated by electron dense

material. Some free ribosomes were observed. Full fledged Golgi apparatus or endoplasmic reticulum were not seen in the sections.

The centrally placed nucleus was varying in shape and in many cells, notched at some places. Nuclear heterochromatin was, as usual, chiefly distributed in thick patches along nuclear margin. Occasionally a nucleolus about 2 μm in diameter was seen.

Lymph nodes from immunized bats: Cells in the immunized lymph nodes were loosely organized with some intercellular space in between them. The cells ranged from 5 μm to 8 μm in diameter with a predominance of large lymphocytes and could be ranked as medium or large lymphocytes. Small lymphocytes and plasma cells were occasionally seen (Plate XXIV, Fig. 1). In a few regions, some surface irregularities were noted, otherwise the plasma membranes were simple in outline.

The cytoplasm of the cells was dark and granular in appearance. Number of mitochondria was comparatively more than in the normal lymph node cells (Plate XXIV, Fig. 1). Ribosomal particles and few vesicles were also observed (Plate XXV, Fig. 1).

The nucleus in these cells was usually large and often showed several deep invaginations (Plate XXIV, Fig. 1). Nuclear heterochromatin content was not changed much from the normal lymph node cells.