INTRODUCTION

Higher vertebrates mount mainly two types of immune reactions, humoral and cell mediated responses to eliminate invading agents or pathogens bearing non-self antigen. During the last decade immunologists worked out the cellular components of the immune system of an organism responsible for two types of immune reactions. Essential conclusion is that B or bursa/bone marrow derived lymphocytes synthesize antibody molecules (Chession et al., 1966; Porter, 1973; Williamson, 1973; Greaves et al., 1973; Melchers and Andersson, 1974) and T lymphocytes, derived from thymus organ perform varieties of cell mediated reactions, such as killer cell activity against foreign cells (Cerottini et al., 1970; Cerottini and Brunner, 1974; Bevan and Cohn, 1975; Falkoff and Dutton, 1977; Degiovanni et al., 1979), delayed Hypersensitivity (Cooper and Ada, 1972), graft rejections (Miller and Mitchell, 1967; Cerottini and Brunner, 1974), suppression of the specific helper activity (Moller, 1975a,b; Herzenberg et al., 1976) etc. Besides these reactions T cells are also known to provide helper factor(s) to B cells in antibody synthesis for certain types of antigen (Mitchell and Miller, 1968; Raff, 1970; Katz and Benacerraf, 1972; Gershon, 1974; Baum and Pilarski, 1978; Schreier et al., 1980). Recently T cell mediated immune reactions have also been implica-

ted with the surveillance mechanisms in the higher organisms (Timonen et al., 1979; Droller et al., 1979; Timonen, 1979). Macrophages, another important component of reticulo-endothelial system are also known to help in the immune reactions and for their phagocytic ability towards foreign agents and cell debris (Gordon, 1977; Beller et al., 1978; Kavai et al., 1979).

Besides source and function, these cells are nowadays characterized by cell surface markers as each of the type of cells bear characteristic cell surface constituent molecules (Raff, 1971). Depending on the cell surface markers, several subsets of T cell population have been characterized; Cantor and Boyse (1975a,b) have elegantly characterized null, helper and killer types of T cells on the basis of their specific cell surface markers or Lyt antigens. in mouse

Differentiation of the immunocompetent lymphocytes is a long drawn and elaborate process; it has been shown that the stem cells originate in the yolk sac of the embryo and migrate to bursa or bone marrow and thymus (Harris and Ford, 1964; Moore and Owen, 1965, 1967a,b; Mickelm et al., 1966; Ford et al., 1966; Moore and Metcalf, 1970) via fetal liver in case of mouse (Umiel et al., 1968; Umiel, 1973). The different cellular components

like bone marrow and thymus derived lymphoid cells and adherent cells responsible for immune response in the mouse attain immunologic maturation at different stages of ontogeny (Tyan and Cole, 1963; Ball, 1963; Mosier, 1967; Auerbach, 1967; Argyris, 1968; Bortin and Saltzstein, 1969; Ritter, 1971; Chiscon and Golub, 1972; Spear and Edelman, 1974; Mosier and Johnson, 1975; Landahl, 1976).

Even different subsets of the T cells assume functional status at different stages in ontogeny of the mouse. The evidences in this regard had accumulated even before the concept of cell surface markers for the different subsets came into existence. Claman et al. (1966) detected the thymus cell helper function in an antibody response with thymocytes from 6 day old mice by using the technique of reconstitution of irradiated animals with different types of lymphoid cells. Chiscon and Golub (1972) have shown that the helper function of mouse thymus cells remained less than 10% of adult level until birth, reaching adult level within 48 hr of birth. Chakravarty and his co-workers have shown that even 16 day old embryonic murine thymus cells are capable of providing helper function for humoral response when stimulated for 24 hr in vitro with Concanavalin A (Con A); by the 18th day of gestation, this collaborating response of thymus cells was virtually

identical with that of adult thymocytes (Chakravarty et al., 1975; Chakravarty, 1977). Chakravarty (1977) has also shown that maturation of thymus cell function in graft-versus-host (GVH) response occurs after birth in case of mouse. Wu observed that while the spleen and thymus of a mouse was immunocompetent at birth as measured by MLC, and could stimulate allogeneic cells, it was not yet capable of CML activity; this arose only after a few days (Wu et al., 1975; Wu, 1978).

Usually immunocompetent lymphocytes can only differentiate into functional or effector cells after being activated with antigen. In case of T cells, it has been shown that alloantigen with specific markers defined by LD locus is responsible for triggering of immunocompetent T cells for mixed leukocyte reactions and ultimately causing differentiation of killer or cytotoxic cells against antigen defined by SD loci (Bach et al., 1971).

The precise role of the antigen in activating the lymphocytes is not yet understood. However, several studies have shown that antigenic stimulus can be substituted by the mitogenic and polyclonal stimulus of lectins which can activate both B and T cells for antibody secretion and cell mediated responses respectively (Coutintio et al., 1975; Waterfield et al., 1975; Charmot

et al., 1975; Heininger et al., 1976). Pokeweed mitogen (PWM) and lipopolysaccharide (LPS) have been used for activating B cells in vitro (Andersson et al., 1972; Parkhouse et al., 1972; Piguet and Vassalli, 1972; Shortman et al., 1973; Weber, 1973). For activating the T cells in vitro, a number of workers have used mitogenic chemicals like Con A (Stobo and Paul, 1973; Waterfield et al., 1975; Heininger et al., 1976; Chakravarty and Clark, 1977) and phytohaemagglutinin (PHA) (Stobo and Paul, 1973; Haskovee and Angelisova, 1975) and bacterial suspension (Bomford, 1980b). Of course, a variety of mitogens for the proliferation of lymphocytes have been discovered (Farnes et al., 1964; Reisfeld et al., 1967; Stobo and Paul, 1971; Trowbridge, 1973; Tokuyama, 1973; Andersson et al., 1973; Peavy et al., 1973) since the initial observation with PHA was reported (Rigas and Osgood, 1955; Beckman, 1962; Barkhan and Ballas, 1963; Holland and Holland, 1965; Barker, 1969; Blomgren and Svedmyr, 1971; Owen et al., 1971). It has been shown that thymocytes develop maximal responsiveness to different mitogens, such as PHA, Con A, PWM, at different stages of ontogeny starting from the embryonic state (Howe and Manziello, 1972; Stobo and Paul, 1972; Mosier, 1974). Most of the experiments for activating the T cells with polyclonal agents like PHA and Con A have been done in vitro (Winkelstein and Craddolock, 1967; Stobo, 1972; Greaves and Janossy, 1972; Shortman et al., 1973; Lee et al., 1973;

Oliver et al., 1977). Extensive works have been done with murine T cells treated with Con A (Sjoberg et al., 1973; Waterfield et al., 1975; Heininger et al., 1976; Bevan et al., 1976; Tartof and Fitch, 1977; Bonavida, 1977; Chakravarty and Clark, 1977; Pilarski et al., 1977; Chakravarty, 1978; Lomnitzer and Rabson, 1979), and this system has been considered as a model to analyse the basic mechanisms involved in the activation of lymphocytes after binding with mitogen or antigen.

In course of activation of lymphocytes several events starting from binding of the mitogen have been observed (Peters and Hausen, 1971a; Peters and Hausen, 1971b; Mendelsohn et al., 1971; Cross and Ord, 1971; Quastel and Kaplan, 1971; Dent, 1971; Allwood et al., 1971). It is considered that most of these events are in common with the process of activation of lymphocytes initiated with specific antigen. It has been shown that Con A activated T cells closely resemble the blast cells produced by specific antigen and can express antigen-specific, receptor-mediated cytotoxicity in absence of Con A during the cytotoxic assay (Clark, 1975). Con A generated memory T cells, have been found qualitatively similar to those generated with specific antigenic stimulus (Chakravarty and Clark, 1977). Con A as a potent mitogen for murine T cells,

is capable of stimulating the cells polyclonally for blast transformation *in vitro* and ultimately causing these blasts to become killer cells to allogeneic target cells, bearing non-self antigen. This act of killing is mediated by the specific receptors of the effector cells for the MHC products on the target cells (Heininger *et al.*, 1976; Chakravarty and Clark, 1977; Green *et al.*, 1978; Chakravarty, 1980; Kimura and Ersson, 1981; Berke *et al.*, 1981). Except a few cases of utilizing Con A, PHA or a few other adjuvants in *in vivo*, there is no detailed account of the analysis of the process of activation of T cells and their differentiation into killer cells *in vivo*. This study will be interesting from several points of view, including raising multiple clones of killer cells *in vivo* against target cells bearing unknown antigen or multiple varieties of antigen which cannot be used for stimulating the lymphocytes *in vivo*. Possibility of such situations will be discussed later.

Analysis of the T cell activation *in vivo* by using different types of mitogen and assaying the immune responses of the *in vivo* activated cells are the main interest of the present investigation. This attempt might lay the foundation for physiological use of mitogens besides their usage for *in vitro* study of activation of lymphocytes. Before going into details of the

objective of the study, it would possibly be better to relate the substances to be used for *in vivo* stimulation of the T cells in this investigation. Three different substances like Con A, PHA and killed Mycobacteria in Freund's complete adjuvant have been used in this study.

Con A, a tetrameric protein structure, is the best ~~best~~ characterised of the plant lectins extracted from *Canavalia ensiformis*. It was Sumner (1919) who first discovered this by extracting from the jack bean in a crystalline state which bind specifically to α-D-glucopyranosides and α-D-mannopyranosides, and to polysaccharides or glycoproteins containing such residues (Sumner and Howell, 1936). Some of these compounds on the cell membrane act as receptors for Con A (Goldstein *et al.*, 1965; Yariv *et al.*, 1968).

Most of the early studies on lymphocyte activation were carried out using either very impure preparations of PHA (PHA-M) or a partially purified fraction (PHA-P) prepared by the method of Rigas and Osgood (1955) from the plant *Phaseolus vulgaris* (red kidney bean). PHA is tetramer and composed of subunits with molecular weights variously estimated at between 29,000 and 36,000 (Allan and Crumpton, 1971; Oh and Conard, 1972; Weber *et al.*,

1972; Yachnin and Svenson, 1972). Two different types of subunits were found, one of which had all the mitogenic activity, while the other was responsible for erythroagglutination. Crude PHA contain some carbohydrate residues, mainly mannose and glucosamine (Weber, 1969; Allen et al., 1969). They also contain Ca^{++} and rather smaller amounts of Mn^{++} (Galbraith and Goldstein, 1970). The sequence of amino acid residues at the N-terminal ends of both the erythroagglutinating and mitogenic subunits has been determined by the Edman degradation method (Miller et al., 1973). The two subunits are different at six of the seven amino acids at the N terminal end, but their sequences from residue 8 to residue 24, the last amino acid determined, are identical.

It is likely that many common bacteria produce lymphostimulatory substances. The mode of action of such immunological adjuvant is of theoretical as well as practical importance. It has long been recognized that certain microorganisms or bacterial extracts increase the host's resistance to a variety of unrelated bacterial or viral infections by stimulating reticuloendothelial system (RES) and also stimulate immunity against tumours (Howard et al., 1959; Old et al., 1959; Shilo, 1959; Halpern et al., 1966; Berman et al., 1967; Zbar et al., 1970). Uchitel and Khasman (1964 and 1965) observed plasmocytosis in lymphoid organs and activation

of the metabolic processes with several bacterial adjuvants. The phagocytic activity of the RES was considered as an index of the immune response (Biozzi et al., 1953) and it was demonstrated that adjuvants greatly enhance phagocytosis.

Adjuvants cannot only increase immune responses; they can also bring about change from one type of immune response to another. For example, doses of bovine serum albumin that in the absence of adjuvant induce tolerance, in the presence of adjuvants induce antibody formation (Allison and Davies, 1971). Halpern et al., (1964) have shown that injection of killed suspension of Corynebacterium parvum results in massive proliferation of the cells of the lymphoreticular system. Similar type of cell multiplication and in consequence enlargement of lymphoid tissue was observed after injection of Freund's adjuvant (Moore and Schoenberg, 1964). T cell involvement in adjuvant expression comes from the histological studies of Taub et al (1970) who demonstrated hypercellularity in the thymus-dependent area of draining lymph nodes after footpad injection with adjuvant.

Blastogenesis is one of the indicators of activation of lymphocytes. The initial interaction between lymphocytes and mitogens is the binding of the mitogen to the receptor on the lymphocyte

surface. Normally the lymphocytes after getting stimulated become larger in size or form blast cells and undergo differentiation to become an effector cell. There are many changes in the metabolic activities of lymphocytes between the initial stimulation by mitogens and the start of DNA synthesis. Biochemical studies have shown that there are alterations to a greater or lesser extent, in the activities of almost every metabolic pathway that has been investigated (Key, 1966; Forsdyke, 1967; Cooper, 1968; Ross and Loss, 1970; Cooper, 1972; Andersson and Melchers, 1973). Some of these changes occur more rapidly after stimulation than others, giving the impression of an ordered sequence of events, and many are well established before DNA synthesis starts. Cytomorphological studies show that these metabolic changes result in the enlargement of the cell, and in particular of the cytoplasm, in the conversion of much of the nuclear heterochromatin to euchromatin and in the appearance of prominent nucleoli (Inman and Cooper, 1963; Kay, 1967; Hirschhorn et al., 1969; Andersson et al., 1972; Parkhouse et al., 1972; Cooke and Key, 1973; Fillingame and Morris, 1973; Ferber et al., 1974). The metabolic changes to which most attention has been paid are alterations in protein and RNA synthesis (Epstein, 1966; Turner and Forbes, 1966; Kay, 1967; Cooper, 1968; Cooper, 1969; Berke et al., 1971; Cooper 1972). Both protein and RNA synthesis begin quite rapidly after stimulation and become progressively more marked. Then DNA synthesis is needed for the

cell division. As DNA synthesizing cells are rare in many resting population of lymphocytes, the initiation of DNA synthesis has provided a sensitive low background assay that has become the standard method for determining whether or not lymphocyte transformation has occurred (Bender and Prescott, 1962; Cooper et al., 1963; Marshall and Roberts, 1965; Ribas-Mundo, 1966; Jasinska et al., 1970; Loeb et al., 1970; Zank et al., 1971; Soubil and Panijel, 1970; Hardy and Ling, 1973).

Thus it seemed that measure of blastogenesis and synthesis of macromolecules like DNA, RNA and protein at different hours would help in analysing the process of activation of lymphocytes, especially T cells, in vivo with the stimulation of Con A, PHA and Freund's complete adjuvant. Stimulation has been done with different doses of the substances. Next, the cytotoxic ability of the cells activated with these polyclonal stimulators have been tested in ^{51}Cr release assay to understand the efficacy of in vivo generated effector cells against allogeneic target cells.

Furthermore, in this investigation the morphology and organisation of the lymphoid cells in situ in the secondary lymphoid organs have been studied by analysing the histological sections of spleen and mesenteric lymph node at different hours after in vivo administration of the polyclonal activators. The

histological study hopefully would provide some information whether there would have been any untoward effect to the lymphocytes with such in vivo administration of the polyclonal stimulators.