CHAPTER I

GENERAL INTRODUCTION
Lymphocytes play an important role in conferring immunity against pathogens. The two types of lymphocytes are, a) B lymphocytes (Bursa/Bone marrow-derived) and b) T lymphocytes (Thymus-derived) which carry out humoral and cell-mediated immune responses respectively. The mature T and B lymphocytes circulate via the blood and the secondary lymphoid organs, which are the sites of lymphocyte-antigen interactions. The secondary lymphoid organs include spleen, lymph nodes and the lymphoid tissues of gastrointestinal, respiratory and urogenital tracts. Apart from lymphocytes, these organs also contain macrophages and dendritic cells that trap and process antigens which are presented to the antigen specific T and B cells.

Lymphoid tissues associated with gastrointestinal, respiratory and urogenital tracts are always exposed to the external environment. They participate in the defense against various viral, bacterial and parasitic antigens that enter the body via the mucosal route. Besredka (1919) proposed the existence of mucosal protective local immune systems which function fairly independent of systemic immunity. A common mucosal immune system has been proposed to be associated with the above mentioned tissues (Bienenstock et al, 1978; Mestecky and McGhee, 1987). When an antigen is administered orally, intestinal lymphoid tissue is stimulated and the sensitized lymphocytes leave the intestine, circulate in the blood stream and colonize the mucosal surfaces throughout the body.

**Gut associated lymphoid tissue (GALT)**

The gut associated lymphoid system has been shown to exhibit humoral or cell-mediated responses or both. The immune response of
this system differs from the responses of regional lymph nodes or systemic lymphoid compartment (Bienenstock and Befus, 1980). GALT is unresponsive to the vast array of complex harmless antigens present in the gastrointestinal lumen, but it somehow recognizes and removes the potentially harmful infectious organisms. Thus, it plays an important role in host defense against harmful antigens gaining entry along with the ingested food on one hand, and in oral tolerance against the harmless food antigens on the other.

GALT is one of the largest lymphoid compartments in the body, and it comprises more than one half of the total lymphocyte population (Walcsman and Ozer, 1976). GALT consists of lymphoid cells organized in discrete structures such as Peyer's patches, and those present in epithelial and lamina proprial layers. The lymphocytes present in between the epithelial cells are called Intraepithelial lymphocytes (IEL), while those disseminated diffusely in the lamina propria are called Lamina proprial lymphocytes (LPL). The subset composition and functions of IEL and LPL differ considerably from each other.

**Peyer's Patches**

Small deposits of lymphoid tissue in the form of nodules located in the submucosal layer of intestine are called Peyer's patches (PP). In PP, lymphoid cells are arranged in a fashion analogous to lymph nodes, with B cell germinal centers surrounded by T cells, interspersed macrophages and dendritic cells (Bloom and Fawcett, 1972). An important characteristic of PP is the presence of a unique epithelium covering the dome region consisting of cuboidal epithelial cells, and specialized antigen sampling cells called microfold cells or M cells.
(Owen and Jones, 1974). The antigens that are transported from gut lumen by the M cells are processed and presented to the lymphocytes by macrophages and dendritic cells of PP (Castro, 1982). PP are the major sites at which the activation and programming of IgA-producing B cells and mucosally-directed T cells are initiated. T cells with both helper and suppressor function are present in PP. Approximately 60% of PP T cells are T helper cells including those which support IgA responses (Hanson and Brandtzaeg, 1989). T cells capable of suppressing IgA production have also been described in PP (Elson et al, 1979). The antigenically primed T and B lymphocytes migrate from PP through the mesenteric lymph nodes and blood circulation and finally home onto the lamina propria and intraepithelial spaces of different mucosal surfaces (Husband and Gowans, 1978; Ottaway, 1990).

**Intraepithelial lymphocytes (IEL)**

Intraepithelial lymphocytes are among the largest populations of T lymphocytes in the body (Mowat, 1984). They are located in very close proximity to the intestinal lumen. IEL in the gut are intriguing cells with poorly understood functions. IEL contain a large proportion of granulated lymphocytes (Guy-Grand et al, 1978; Mayrhofer, 1980; Rudzik and Bienenstock, 1974; Ferguson, 1977; Davles and Parrott, 1981; Cerf-Bensussan et al, 1983). Most of the T cells of IEL have a CD8 phenotype (cytotoxic/suppressor) (Selby et al, 1981; Lyscom and Breuton, 1982; Ernst et al, 1985; Van der Heijden, 1986; Vaage et al, 1990). A small subpopulation of T helper cells (CD4) is also present among IEL. Spontaneous cytotoxic activities like natural killer cytotoxicity and antibody dependent antibacterial activity are associated with large granular T lymphocytes (Herberman, 1981; Timonen et al,
1981; Tagliabue et al, 1982). Almost 50% of T cells of mouse IEL which are cytotoxic are thymus-independent (Klein, 1986). Recently it has been reported that a majority of murine intestinal cytotoxic T lymphocytes express the T cell receptor $\gamma/\delta$ (Bonneville et al, 1988; Viney et al, 1990). In contrast, majority of rat and human IEL bear T cell receptor $\alpha/\beta$ rather than $\gamma/\delta$ (Vaage et al, 1990; Brandtzaeg, 1989).

As majority of the IEL are cytotoxic T cells, it is implied that they are involved in the protection of mucosa by participating in local cell-mediated immunity; they also participate in epithelial renewal (Cerf-Bensussan et al, 1984; Mowat and Ferguson, 1982).

The intestinal epithelial cells express Class II antigens of the major histocompatibility complex (Wiman et al, 1978; Barlay and Mason, 1982; Mayrhofer et al, 1983; Bland, 1988), which are characteristic surface antigens of antigen-presenting cells.

Lamina Proprial lymphocytes (LPL)

Lamina propria of the intestine contains a large number of lymphocytes and plasma cells, along with a few eosinophils and macrophages. In response to the antigens of gut lumen, IgA committed B cells and T cells which regulate B cell responses, migrate selectively to lamina propria (Fiocchi, 1989).

LPL populations are remarkably different from IEL populations in their composition. LPL consist of heterogeneous populations of cells including B cells, helper T cells (CD4$^+$) and suppressor/cytotoxic T cells (CD8$^+$) (Farrott et al, 1983), while IEL appear to consist
predominantly of T cells with CD8 phenotype. Unlike IEL, very small numbers of large granulated lymphocytes are present in the lamina propria of mice (Tagliabue et al, 1982). The T lymphocytes of LPL have the capacity to provide help for Ig synthesis by B cells as well as suppressor cell activity similar to the lymphocytes of peripheral blood (James et al, 1985; Elson et al, 1985). The predominant class of immunoglobulin secreted by the B cells of lamina propria is IgA (Tseng, 1982; Guy-Grand et al, 1974).

The macrophages of lamina propria play an important role in phagocytosis of bacteria and in antigen processing and presentation (Bull and Bookman, 1977).

**Secretory IgA**

IgA is the major immunoglobulin of intestinal secretions and participates in antibody-mediated defense at mucosal surfaces (Tomasi et al, 1963; Brandtzaeg, 1974; Mostecky, 1987; Nagura and Sumi, 1988). The mucosal secretory IgA consists mainly of dimeric molecules complexed with the J chain and possesses an additional glycoprotein called secretory component (SO (Tomasi et al, 1965). IgA is secreted by the plasma cells of lamina propria and is transported into the lumen through intestinal epithelial cells where the SC secreted by epithelial cells binds to dimeric IgA to form a complex molecule called secretory IgA (slgA) (Brandtzaeg, 1974). The secretory component protects IgA from the proteolysis by gastrointestinal proteases.

The surface of the intestinal epithelial layer is protected by IgA in conjunction with mucus. slgA, effectively blocks both bacterial
adherence and antigen association with the mucosa, thereby reducing or eliminating the mucosal penetration by microbes (McNabb and Tomasi, 1981).\(\text{slgA}\) protects the mucosal surfaces from bacterial and viral infections by neutralizing bacterial exotoxins and viruses. Receptors for Fc portion of IgA on lymphocytes have been reported (Strober et al, 1978) and slgA also participates in antibody-dependent cell-mediated bacteriolytic activity carried out by CD8 subset of T cells (Nencioni et al, 1983). Thus, slgA in the gut has three major roles: antigen exclusion, antibacterial function and participation in cell mediated immunity.

Lymphokines in GALT

Lymphokines are a group of molecules produced by activated T cells and have multiple effects on the growth and function of the cells of immune system and also on other cell types. Immunoregulatory T cell-help is required for IgA responses and also for the function of cytotoxic lymphocytes (Elson and Heck, 1979; Kawanishi et al, 1983; McGhee and Mestecky, 1989). These T cells operate through lymphokines secreted by them. Activation of antigen specific CD4 T cells leads to the secretion of IL-2, IL-4, IL-5 and IL-6 needed for B cell responses and Ig synthesis. The IgM expressing B cells of PP switch over to IgA expressing B cells in presence of IL-4, while IL-5 and IL-6 are needed for the differentiation of IgA expressing B cells in the lamina propria into IgA secreting plasma cells (Kawanishi et al, 1982; 1983). In addition, antigen-activated CD4 T cells modulate the function of cytotoxic lymphocytes with the help of lymphokines such as IL-2, IFN-\(\gamma\) and lymphotoxin (tumor necrosis factor-\(\beta\)). Both CD4 and CD8 T cells require the T cell growth factor, IL-2, for proliferation. IL-2 is

Lymphocytes from all the compartments of gut associated lymphoid tissue contain higher numbers of IL-5 and IFN-γ secreting cells when compared with spleen (Taguchi et al, 1990). LPL which is a major IgA effector site, contains large number of IL-5 producing CD4 T cells and a few IFN-γ secreting cells. In contrast, PP which is a major IgA inductive site contains a few IL-5 producing CD4 T cells (Taguchi et al, 1990). IL-5 and IFN-γ producing cells are also present in IEL (Dillon et al, 1986; Taguchi et al, 1990). IL-2 is secreted only by LPL cells but not by IEL. The major role of IL-2 in the gastrointestinal immune system is probably to expand cell populations through proliferation. IL-2, also enhances the lytic function of natural killer cells and is necessary for the differentiation of cytolytic T cells (Simon et al, 1986; Chen et al, 1986).

**Oral tolerance**

Suppression of immune responses against harmless non-replicating luminal antigens is called "Oral tolerance". Protection against harmful systemic types of immune reactions elicited by IgG, IgE and T cell mediated delayed type hypersensitivity is also afforded by the same suppressive mechanism (Mowat, 1987). This phenomenon of hypo-responsiveness involves multiple immunoregulatory events (Nicklin and
Miller, 1983). Oral tolerance seen in GALT is mediated by suppressor cells. Selective presentation of processed antigens by gut epithelium leads to the generation of specific and non-specific suppressor T cells (Meyer and Shlien, 1987). Though suppression is a general phenomenon induced by soluble non-replicating agents, the responses of IgA against harmful, non-replicating soluble agents are regulated by the action of contra-suppressor T cells which are of CD8 phenotype (Green et al, 1988; Brines and Lehner, 1988).

Oral vaccination

Vaccination offers the best prophylactic approach to build up immunity to a variety of viral and bacterial diseases, especially amongst children. Now-a-days oral vaccination is gaining importance over systemic immunization to protect an individual against enteric infections such as cholera, typhoid fever, Schigellosis, etc. (Holmgren et al, 1982). The stimulation of local gut mucosal immune system is better achieved by oral route rather than through the systemic route (Mestecky, 1987). Based on the concept of a common mucosal immune system associated with gastrointestinal, respiratory and urogenital tracts, oral vaccines against respiratory and urogenital tract infection have also been developed (Holmgren et al, 1992). The functional status of the gut immune system determines the outcome of the oral vaccines.

Mucosal immunity in intestinal diseases

In certain gastrointestinal diseases, striking changes in mucosal lymphocytes and secretory IgA production which results in mucosal damage have been observed (Nicklin and Miller, 1983; Nagura, 1992). In
chronic gastritis, coeliac disease and inflammatory bowel disease. Overproduction of IgG has been reported (Brandtzaeg et al., 1985). In these diseases abnormalities in mucosal T and B cells were also observed (Brandtzaeg et al., 1987). A striking feature of coeliac disease is the increased number of T cells (CD4) in jejunal epithelium (Freedman et al., 1988). The number of \( \gamma/\delta \) T cells is also increased and this may contribute to the villous atrophy and destruction of enterocytes (Halstensen et al., 1989).

Several aspects of immunity are impaired in diseased conditions resulting from nutritional deficiencies, viz., protein-energy malnutrition, vitamin and mineral deficiencies which are common in developing countries. Increased frequency and severity of infections, especially enteric infections have been observed in protein-energy malnutrition and Vitamin A (Vit A) deficiency states (Scrimshaw et al., 1968; Sommer et al., 1984).

Vitamins and immune response

The relationship between Vitamins and immunity is well recognized in recent years. Both humoral and cellular responses are shown to be altered in Vit A (Bendich, 1991), Vit B (Bendich and Cohen, 1988), Vit C (Long, 1950; Zweiman et al., 1966), Vit D (Rook, 1988) and Vit E (Tengerdy and Brown, 1977; Corwin and Shloss, 1980) deficiencies. Among all the Vitamins, Vit A is known as anti-infection Vitamin (Perla and Marmorston, 1941) because of its effect on both epithelial cells and immune system.
Vitamin A

Animals and humans are not capable of synthesizing Vit A. They depend on natural plant sources which contain significant amounts of Vit A precursors such as \(\beta\)-carotene. Vit A plays an important role in metabolism, in addition to its role in photoreceptor mechanism of visual cycle (Moore, 1957). Further, it is essential for the growth, differentiation and replication of the epithelium and mesenchymal tissue (Sklan, 1987). It plays a role in maintaining the structural integrity of cellular and subcellular membranes. It is suggested to be involved in the transport of metabolites across the cell membrane and in bone formation (Mellanby, 1941). Vit A is known to be necessary to maintain the integrity of barriers such as skin, mucous membranes and cilia or tears which comprise the first line of defense against penetration by pathogens. Functional defects of epithelium during Vitamin A deficiency are suggested to result in high frequency of infections.

Vitamin A deficiency

Vit A deficiency as a cause of Xerophthalmia and blindness is very well established. Vit A deficiency results in keratinizing metaplasia (xerosis) of mucus-secreting epithelial tissues (Wolbach, 1937). The epithelium of intestinal mucosa does not normally keratinize, but shows a decline in goblet cells (De Luca et al, 1969; Rojanapo et al, 1980). Reduction in epithelial cell turnover in the small intestine of Vit A-deficient rats was reported (Zile et al, 1977). Loss of protective mucoid secretions compromises the surface integrity of epithelium resulting in enhanced susceptibility to microbial invasion. Various specific and non-specific components of
immune response were shown to be impaired in Vit A deficiency (Nauss, 1986; Vyas and Chandra, 1984).

**Vitamin A deficiency and susceptibility to infection**

Studies in Humans:

Vit A deficiency has been recognized as a major cause of childhood morbidity and mortality in the developing countries. It is well established that Vit A deficiency enhances susceptibility of humans to various types of infections (West et al, 1989). Respiratory tract infections and diarrhea are very frequent in children associated with low levels of serum Vit A (Arroyave and Calcano, 1979). A depressed serum Vit A level has been reported to be associated with pneumonia, rheumatic fever, measles and chicken pox (Shank et al, 1944; Jacobs et al, 1954; Sommer, 1982; Arroyave and Calcano, 1979). Studies on Indonesian and Indian children showed that mild Vit A deficiency is associated with increased mortality due to increased respiratory and diarrheal infections (Sommer et al, 1984; Milton et al, 1987). From these reports, it is apparent that Vit A deficiency increases the susceptibility to infection.

Few attempts were made to study the basis of immunological defects associated with the increased incidence of infections in Vit A deficiency (Bhaskaram and Reddy, 1975; Srisinha et al, 1975; Semba et al, 1991; Semba et al, 1993). As Vit A deficiency in humans is usually associated with protein calorie malnutrition, the interaction of Vitamin A with immune system is better understood using experimental animals.
Studies in experimental animals:

Increased susceptibility of Vit A-deficient animals to various infections like *Mycobacterium tuberculosis*, *Salmonella typhimurium*, *Pseudomonas* aureginosa, etc., has been reported (Scrimshaw et al., 1968). Atrophy of thymus and decreased cellularity of spleen was observed in Vit A-depleted rats (Wolbach and Howe, 1925). Depressed cell-mediated and humoral immune responses were observed in experimental Vit A-deficient animals (Vyas and Chandra, 1984). Phagocytosis, mitogen induced lymphoproliferation, delayed type hypersensitivity and specific antibody responses were suppressed in Vit A-deficient animals.

The majority of studies in Vit A-deficient humans and animals mentioned above deal with the immune function of splenic and peripheral blood lymphocytes. Information on the immune system associated with mucosal surfaces is scanty.
AIM AND SCOPE OF THE PRESENT WORK

It is very well established that Vitamin A modulates immune responses in experimental animals as well as in humans. The dietary deficiency of Vitamin A exists to a significant extent in developing countries like India. Vit A deficiency per se, may influence the host-pathogen interaction and impair the outcome of prophylactic vaccination programmes. Oral vaccination has been gaining importance in recent years to combat a variety of bacterial and viral infections. In order to achieve effective oral immunization, the humoral and cellular responses of orally administered vaccines at the gastrointestinal level has to be understood. This will require the phenotypic and functional characterization of intestinal B and T cells as well as their response to antigens. As pointed out above, Vitamin A deficiency could seriously impair the outcome of oral a vaccine in a deficient individual by affecting the function of intestinal lymphocytes.

The main objective of the present study has been to assess the immune response of the intestinal lymphocytes in experimental Vitamin A deficiency using a rat model in terms of

a) the function of intestinal intraepithelial lymphocytes as determined by

i. total number of T cells and subsets,

ii. polyclonal mitogenic and antigen specific activation of T cells,

iii. natural killer cell activity and

iv. \( \text{slgA-mediated} \) anti-bacterial activity,
b) the humoral response of intestinal lamina proprial lymphocytes as determined by

i. total number of \textit{IgA} positive B cells,

ii. \textit{mitogenic} and antigen specific activation of B cells

and

iii. induction of antigen specific \textit{IgA} secreting plasma cells by bacterial antigens.