EFFECT OF DIFFERENT TYPES OF ANTIBIOTICS ON LIBERATION OF MIF

THESIS

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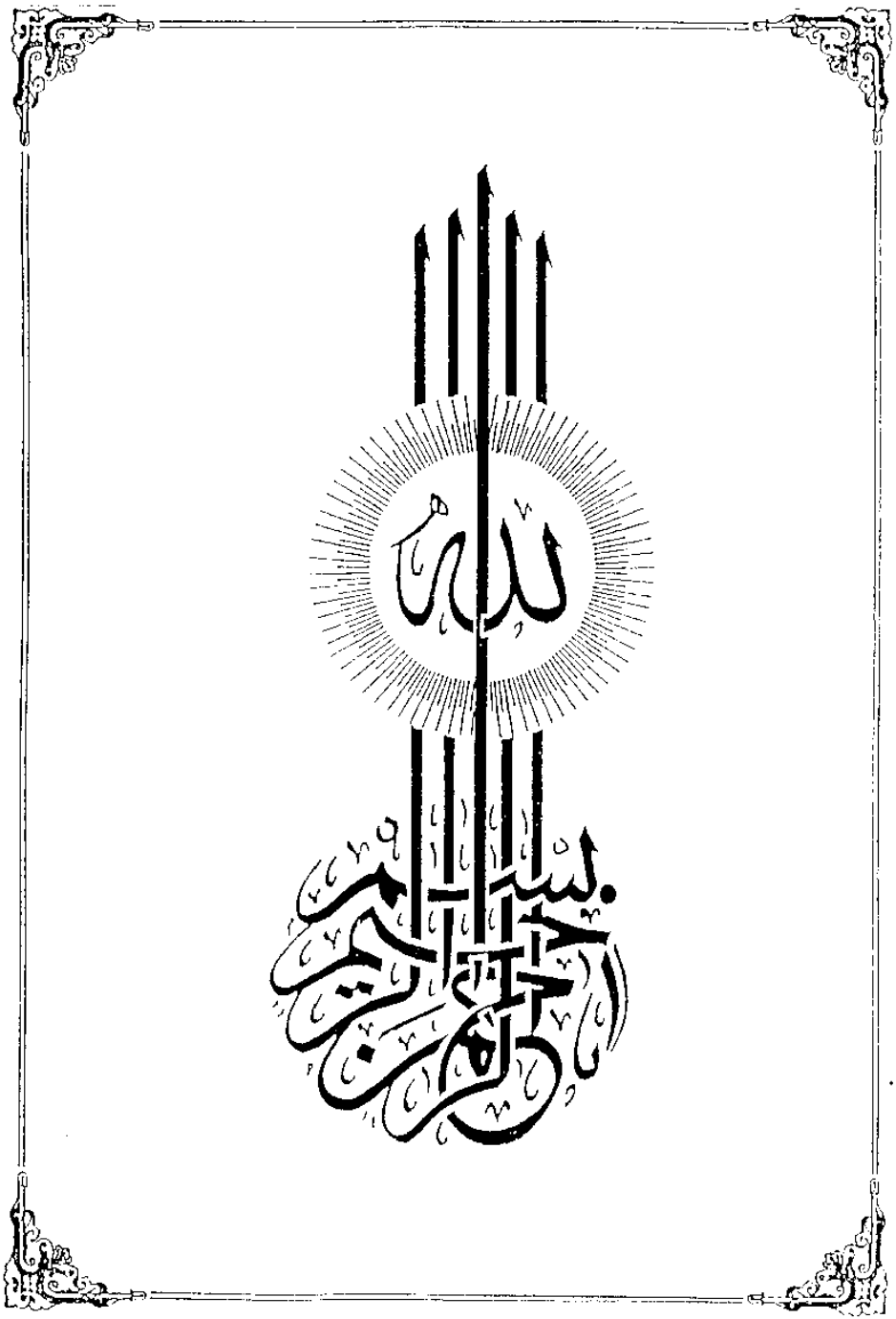

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INTRODUCTION AND AIM OF THE WORK

Introduction :

Cell-mediated immunity which provides the main defense against intracellular organisms, depends upon the interaction of antigen with specific receptors on the surface of T-lymphocytes. One population of T-cells elaborates soluble factors (lympho kines) whose main function is to recruit and activate cells of the mononuclear phagocyte system; another population becomes cytotoxic for target cells bearing the antigen (Roitt, 1980).

Lymphokines include mediators affecting the behaviour of macrophages, polymorphs, lymphocytes, and other cell types. These mediators play a role in vivo in the expression of cell-mediated immunity in the skin, in resistance to infection by intracellular organisms and in inflammation. The first of the lymphocyte mediators to be described was macrophage migration inhibitory factor (MIF). It is released from rensitized T-lymphocytes when stimulated by either specific antigen or by non-specific T-cell mitogen like phytohaemagglutinin (PHA) and it acts upon macrophages to inhibit their migration. The production of antigen-induced MIF by human and animal lymphocytes is closely associated with the presence of in vivo cellular hypersensitivity of the host to that antigen and thus related to the development of cellular immune reaction (Rocklin, 1982).

Aim of the Work :

1. To review the different subclasses of T-lymphocytes.
2. To study the effect of some types of antibiotics (Penicillin G, Garamycin) in different doses on the liberation of migration inhibition factor by spleen lymphocytes of the mice.

REVIEW OF LITERATURE

1- The Immune System and its Cellular Components :

The immune system is an extremely complicated one with a variety of roles in maintaining homeostasis and health. Like the endocrine system, it exerts control within the body by virtue of circulating components capable of acting at sites far removed from their point of origin. A normally functioning immune system is an effective defense against foreign particles such as pathogenic microbial agents and against native cells that have undergone neoplastic transformation (Katz, 1982).

The major cellular components of the immune system are the macrophages and lymphocytes.

Macrophages :

Macrophages have a variety of functions in the immune response. They play a central role in the induction of the immune response with respect to the presentation of antigen to lymophocytes (Roitt, 1980). Although macrophages are not currently thought to be specific for any given antigen, their role in concentrating and presenting antigens to lymphocytes is a crucial one (David, 1983). In addition, the macrophages provide several accessory functions which include the production of soluble factor, interleukin 1 (IL-1) which has been shown to be involved in the production of interleukin 2 (IL-2) by helper T-cells (Gillis, 1982).

Lymphocytes :

Lymphocytes are the antigen-specific cellular components of the immune system, acting via receptors on the surface membrane of every immunocompetent cell. Each receptor is highly specific, and different clones of lymphocytes express their own unique specificity. Functional subpopulations of lymphocytes can be classified into two classes having distinct functional capabilities (Katz, 1982).

I. B-Lymphocytes :

B-lymphocytes are bursa-dependent and concerned in the synthesis of circulating antibody. On appropriate stimulation by antigen, the B-lymphocytes develop into the plasma cell series. The mature plasma cell is actively synthesizing and secreting antibody and has a well-developed rough surfaced endoplasmic reticulum characteristic of a cell producing protein for export (Roitt, 1980). B-lymphocytes are found in the bone marrow (bursa of fabricius in birds), blood, peripheral lymophoid organs, and in small quantities in the thoracic duct lymph, they are absent in the thymus (Klein, 1982). Morphologically, B-cells look the same like T-cells. At one time immunologists thought that B-lymphocytes were hairier (had more microvilli on their surfaces) and T-cells were smoother; but it was later found that the number of microvilli depended on the physiological state of the cell, and that when one compared T- and B-lymphocytes in the same state, there was

no morphological difference between them. B- and T-lymphocytes can, however, be distinguished by various other markers; B-lymphocytes lack many of the T-lymphocyte markers and, in addition, possess some of their own (Greaves, et al. 1973) as shown in the following table :

Lymphocytes	Immunofluorescent staining for :	Rosette formation using sheep r.b.c. coated with				Virus receptor	Approx. % of human blood lymphocytes
	Ig	Thy 1 (θ)	Nothing	IgG	IgM	C_3		
T	-	++	++*	+	++	±	Measles	70
B	++	-	-	++	±	++	EB	10-20

Tests for surface markers on B- and T-cells as indicated by Roitt (1980), where asterisk means human T-cells.

According to Katz (1982), B-lymphocytes are classified into :

(A) Precursors of antibody-forming cells B_μ, B_γ, B_α, B_ε.

(B) Memory cells.

(C) Regulatory B-lymphocytes.

II. T-lymphocytes :

T-lymphocytes neither produce circulating antibodies nor give rise to antibody-secreting cells. The most extensive investigations of these cells have been made in the mouse. T-lymphocytes (Thymus-processed lymphocytes) derive their name from

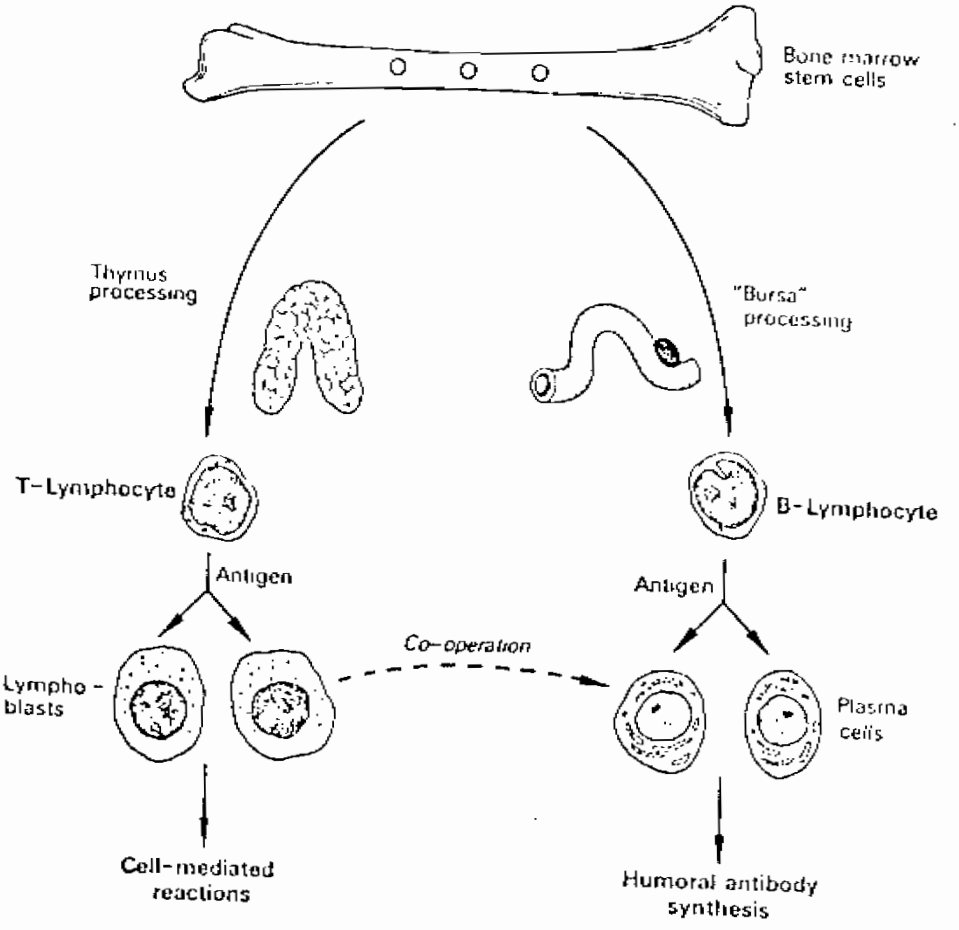

Figure 1. Processing of bone marrow cells by thymus and gut - associated central lymphoid tissue to become immunocomponent T- and B-lymphocytes respectively. Proliferation and transformation to cells of the lymphoblast and plasma cell series occurs on antigenic stimulation.

the fact that they and their precursors, spend a certain amount of time in the thymus. They originate from the same stem cells that give rise to all other blood cells. These stem cells produce progenitor cells, which move into the thymus and differentiate into Thymic lymphocytes (Thymocytes), then they leave the thymus, mature into immunologically competent T-lymphocytes, and begin the life of circulating cells. When a circulating cell encounters a stimulating antigen, it enters an active functional phase. If it is not stimulated, it remains in the body for a certain period and is then eliminated (Klein, 1982).

Function of T-lymphocytes :

T-lymophocytes are responsible for delayed skin reactivity (Mackaness, 1969). They are also responsible for cytotoxic killer activity in cell-mediated lympholysis (CML) (Sondel et al., 1975). These cytotoxic cells can effectively kill neoplastic cells, incompatible transplant tissue cells, and virally infected cells showing viral antigens on their cell membranes. They are also important for protection against slowly growing intracellular pathogens (Bowry, 1977). T-lymphocytes are involved in virtually all regulatory interactions, including helper and suppressor cell function (Reinherz et al., 1979a). T-cells carry out these functions either directly, by cell-to-cell contact, or indirectly, through factors they secrete. The secreted factors can act on different cells

(macrophages, neutrophils, basophils, esinophils, B-cells, and other T-cells) and affect different kinds of functions (recruitment of granulocytes and macrophages, keeping granulocytes at the reaction site, enhancement of granulocyte function, production of tissue damage, and inhibition or enhancement of T-cell and B-cell activities (Klein, 1982).

Classification of T-Lymphocytes :

Although lymphocytes are, by most morphological criteria, a uniform population and for years were regarded as functionally indivisible, it is now clear that they are separable into major functional subgroups (Snell, 1978). Classification has been greatly aided by the discovery of a variety of cell surface markers. The markers currently most used are Thy-1(θ) (Reif and Allen, 1964) which separates T-cells from B-cells, Ly-1, Ly-2,3 (Boyse et.al., 1968) and the Ia family of antigens (Tada et al., 1977). All these antigens show characteristic distribution patterns in different lymphocyte populations and these patterns correlate with known functional groupings.

T-lymphocytes can be divided into subclasses according to their function and to the cell-surface molecules they express;

I. T-lymphocytes can be divided into two major functional categories :

(A) Regulatory T-lymphocytes may amplify (helper cells) or supress (suppressor cells) the responses of other T-lymphocytes

or of B-lymphocytes. Helper T-cells are generally of the Lyt-1$^+$ phenotype, suppressor T-cells generally express the Lyt-2,3$^+$ phenotype (Cantor and Boyse, 1975).

(B) Effector T-lymphocytes include lymphocytes,responsible for : (1) delayed hypersensitivity (DTH), (2) mixed lymphocyte reactivity, and (3) cytotoxic T-lymphocytes (CTL or Killer cells). These cells are responsible for cell-mediated reactions as delayed cutaneous hypersensitivity responses, rejection of foreign tissue grafts and tumors, and elimination of virus-infected cells. Cytotoxic T-lymphocytes participate in the latter responses. Rejection of foreign tissues also involves T-cells that undergo rapid proliferation in mixed lymphocyte reaction (MLR). These cells can be distinguished by their Lyt phenotype. The MLR cells is the Lyt-1$^+$ phenotype, the CTL of Lyt-2,3$^+$ phenotype, delayed cutaneous cells are also of the Lyt-1$^+$ phenotype. Thus, T-cells performing helper, delayed cutaneous hypersensitivity, and MLR functions are all of the Lyt-1$^+$ phenotype (Katz, 1977).

II. Classification according to the cell-surface molecules they express :

Of the many antigens present in the lymphocyte cell membrane, the most useful for T-cell classification have proved to be antigens of the Lyt series, especially those controlled by Lyt-1 and Lyt-2,3 loci, described by Edward A. Boyse and his co-workers (1968). Depending on the expression

of these two loci, Cantor and Boyse (1975) classified T-lymphocytes into three subclasses Lyt-1$^+$ Lyt-2,3$^-$; Lyt-1$^-$ Lyt-2,3$^+$; and Lyt-1$^+$ Lyt-2,3$^+$. Most helper T-cells are Lyt-1$^+$ Lyt-2,3$^-$, most cytotoxic and suppressor cells are Lyt-1$^-$, Lyt-2,3$^+$, and not fully differentiated T-lymphocytes are Lyt-1$^+$ Lyt-2,3$^+$.

Cells of Lyt-1 subclass mediate DTH reactions and helper activity, while cells of the Lyt-2,3 subclass express cytotoxic activity and suppressive activity but not DTH and in some cases inhibit this response (Huber et al., 1976).

According to the presence or absence of Fc receptors for IgG or IgM, human T-lymphocytes have been subdivided into three subpopulations : Tμ cells, which possess receptors for Ig M; T$_\gamma$ cells which have receptors for IgG; and T$_\varphi$ cells which lack receptors for either immunoglobulin (Ferrarini et al., 1975). The T$_\mu$ cell population contains cells that act as helpers in the pokeweed mitogen-induced differentiation of B-cells to immunoglobulin-synthesizing and- secreting plasma cells. In contrast, the T$_\gamma$ cell population, when activated by immune complexes, contain cells exhibiting suppressor activity in the similar system (Moretta et al., 1977).

The method used for isolation of the T$_\mu$ and T$_\gamma$ cells employs their rosette formation with ox erythrocytes coated with rabbit IgM or IgG anti-ox RBC antibodies followed by centrifugation on a ficoll/hypaque cushion (Moretta et al., 1977). The separation of T$_\mu$ and T$_\gamma$ lymphocyte subpopulations

could also be done by density gradient electrophoresis (Platsoucas et al., 1979). Reinherz and others (1979b) have reported that it has become possible, with the use of auto-antibodies, heteroantisera, and monoclonal antibodies, to divide the human T-cell population into at least three subsets : (a) the TH_2^+ population accounts for approximately 20% of T-cells in peripheral blood and contains the cytotoxic and suppressor populations;(b) the OKT_4^+ population, which is distinct from TH_2^+ population, accounts for greater than 50% of the peripheral blood T-cells and contains the inducer for T-T interactions as well as the inducer for T-B interactions (Evans et al., 1978); and (c) the third subset of cells that has been identified with JRA antisera.preliminary evidence suggests that this third subpopulation is unreactive with TH_2 antisera and represents a feedback regulator (Strelkavskas et al., 1978).

Two major subpopulation of peripheral blood T-cells has been defined by the OKT series of monoclonal antibodies : a) the OKT_4^+ cells are approximately 65% of the peripheral blood sheep erythrocyte-rosette forming cells and are helper or inducer cells; and b) the OKT_5^+ and OKT_8^+ present cells which exhibit suppressor or cytotoxic function and comprise approximately 30% of the peripheral blood sheep erthrocyte rosette-forming cells (Reinherz et al., 1980). These two populations correspond to that previously defined by heteroantisera TH_2^- helper and TH_2^+ cytotoxic population (Evans et al., 1978).