IN VITRO EFFECT OF DIFFERENT MEDIA ON LIBERATION OF MIF

THESIS

Submitted for Partial Fullfilment of Master Degree
in Bacteriology

BY

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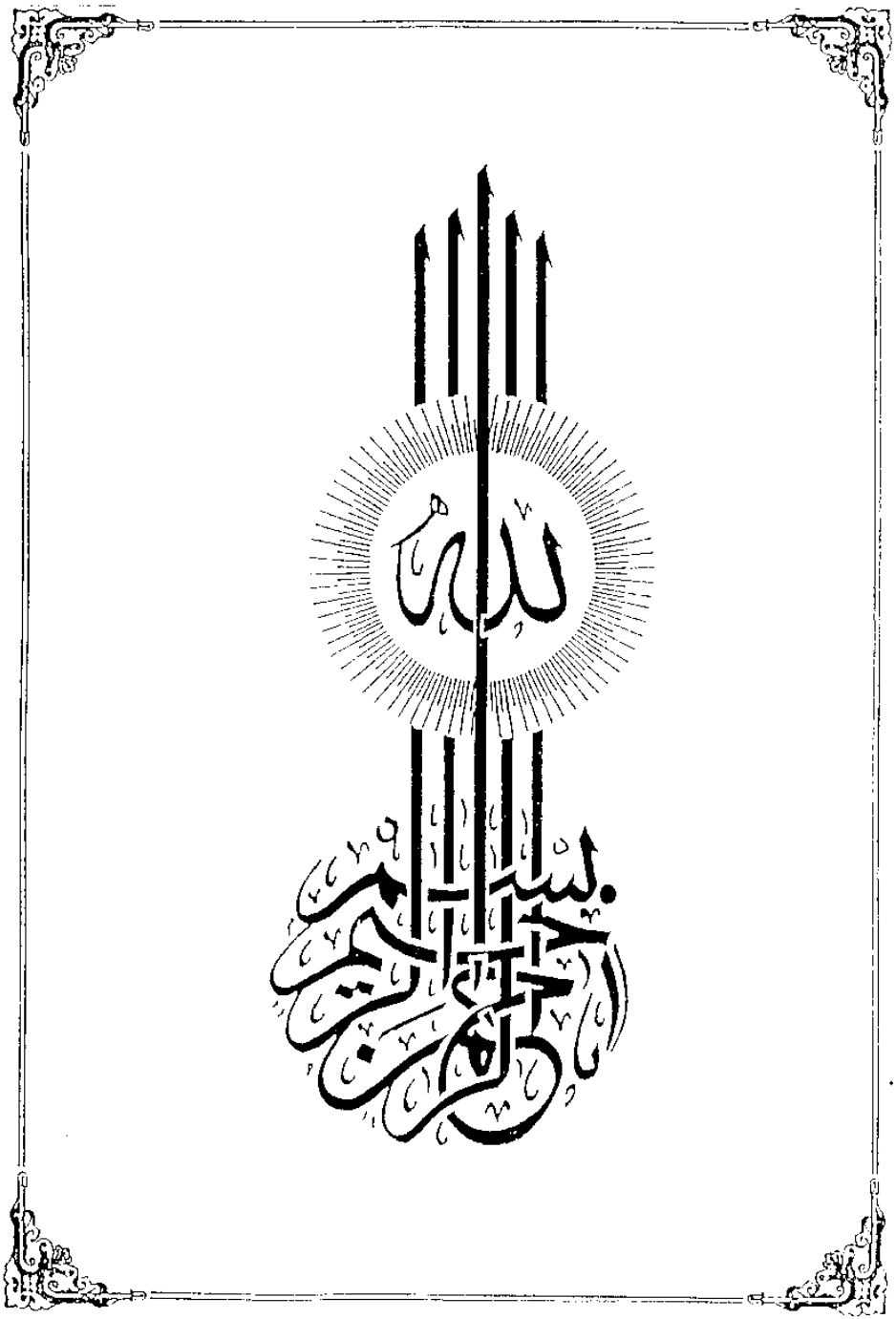
1985

CONTENT



ACKNOWLEDGEMENT

It is my pleasure to express my deep gratitude to Prof. Dr. Zeinab Maged, Head of Microbiology and Immunology Department, Ain Shams University for her continuous encouragement and valuable remarks.

I wish to express my highest appreciation and everlasting gratitude to Prof. Dr. Fatma Mohamed Abdel Fattah, Professor of Bacteriology, Ain Shams University for her tolerant guidance and experienced advise she kindly offered me throughout this work.

I wish to express my deep and sincere gratitude to Dr. Laila El Sayd Soliman Assistant Professor of Bacteriology Ain Shams University for her continuous valuable advice, her constant efforts, her encouragement and her close supervision throughout study.

I am sincerely grateful to Dr. Rasha Y. Khalil, Assistant Professor, Ain Shams University, for her valuable support and help throughout this work.

Finally I wish to thank all the staff of Bacteriology Department for their kind co-operation.

INTRODUCTION AND AIM OF THE WORK

The cell mediated immune response depends on two effector mechanisms, the generation of cytotoxic cells and the release of biologically active soluble lymphocyte activation product termed lymphokines (Roitt, 1977).

Lymphokines act on macrophages, polymorphs, lymphocytes and also on other non-lymphoid cells.

The production of soluble mediators by lymphocytes from normal subjects generally correlates positively with in vivo state of cellular immunity. Patients with depressed cellular immunity do not produce these mediators in response to antigenic stimulation.

One of the most studied and best characterized lymphokines is the migration inhibition factor (MIF), which has been shown to limit the normal wandering of macrophage on glass or plastic surfaces (Rich and Lewis 1932). The MIF assay has been used for the detection of sensitized lymphocytes in patients with certain disease whose pathogenesis may involve an immune mechanism (Rocklin, 1983).

MIF assay is affected by several factors as the type of media used, the concentration and type of mitogens and also by the concentration of lymphocytes needed for its production.

Aim of Work

Is to test the effect of different fluid media (Hank's and RPMI 1640), on the result of migration inhibition test.

The Immune Response

Roitt (1977) stated that, when an antigen enters the body, two different types of immunological reactions occur: humoral and cell mediated. In humoral immunity, a specific circulating antibody is induced, which rids the body of the antigen. Humoral immunity is responsible for the immune response to simple chemicals and microorganisms. Hypersensitivity resulting in tissue injury also may be mediated by circulating antibodies and has been subdivided by Gell et al. (1975) into immediate, anaphylactic, cytotoxic and complex mediated hypersensitivity. In cell mediated immunity (CMI) the immunocompetent cells are activated leading to destruction of foreign targets. Cell mediated immunity is responsible for the rejection of foreign organ transplants, delayed hypersensitivity, graft-versus-host reaction and tumour immunity (Gell et al., 1975). Both cell mediated and humoral immune responses are induced by antigen. In addition to these induced resposes, the bodh has natural resistance which is the first line of defence.

This natural resistance appears to be mediated by a special population of cells, called natural killer (NK) cells (Perlamann 1976).

Cells involved in immune response:

The major cellular components of the immune system are the macrophages and the lymphocytes. The initiation of immune responses requires the participation of cells which present antigen, elaborate immunoregulatory molecules, and maintain lymphocyte viability. Although endothelial cells and fibroblasts may sometimes

serve this accessory function, it has been recognized to be a role primari played by cells of the mononuclear phagocyte lineage (Lipsky & Kettman, 1982). Monocytes/macrophages (MO's) have been shown to be required for antigen and lectin induced lymphocyte proliferation as well as the production of antibody and certain lymphokines (Ellner et al., 1976 and Wahl et al., 1975). Macrophages themselves have a veriety of functions in the immune response. Although macrophages are not currently thought to be specific for any given antigen, their role in concentrating and presenting antigen to lymphocyte is a crucial one (David & Katz, 1983).

Macrophates

Activated macrophages obtined from immune animal have altered morphology and metabolism and tthey show enhanced capacities to deal with a number of microorganisms (Lurie, 1964 and Mackaness, 1964). A number of studies have shown that the lymphocytes are involved in these activation processes (Lane & Unanne, 1972). Their action can be partially explained by the production of soluble mediator such as macrophage activating factor (MAF) which increases the capacity of macrophage to kill the bacteria and tumour cell (Fowles et al., 1973 and Piessens et al., 1975). This factor can not be distinguished from migration inhibitory factor MIF (Bloom and Bennett, 1966, DAvid, 1966 and Nathan et al., 1973). The macrophages secrete

secrete several biologically active mediators capable of regulating the type and magnitude of both T and B lymphocyte response. The macrophages are believed to provide several accessory functions in specific immunological amplification which include at least antigen presentation and the production of soluble factor, interleukin 1 (IL_1) which in turn has been shown to be involved in the production of interleukin 2 (IL_2) by helper T cells (Gillis et al., 1982 , Farrar et al., 1982 and Mizel, 1982).

Recent studies have focused on the importance of hormone like, antigen non-specific factors produced by macrophages. These soluble factors have a wide range of biologic activities. One of the best characterized of these factors is interleukin 1, previously called lymphocyte - activating factor (Blyden and Handschumaker 1977, Gery et al., 1981,, Mizel et al., 1978 and Mizel & Mizel, 1981). The human IL_1 is a macrophage-derived, low m.w. (15,000) protein that can induce thymocyte proliferation (Blyden & Handschumaker, 1977). Many studied showed that IL_1 is biochemically and functionally similar to the monocyte-derived factor, leucocyte pyrogen (LP)[3] (Rosenwasser et al., 1979, Murphy et al., 1980 and Sztein et al., 1981).

Lymphocytes:

Lymphocytes are antigen-specific cellular components of the immune system, acting via receptors on the surface membrane of every immunocompetent cell.

Functional subpopulation of lymphocytes are of two classes having distinct functional capabilities:

I- T lymphocytes

(A) Regulatory T lymphocytes:

1- Helper cells (TH).

2- Suppressor cells (TS)

(B) Effector T lymphocytes:

1- Delayed hypersensitivity (DTH) (T_{DTH})

2- Mixed lymphocyte reactivity.

3- Cytotoxic T lymphocyte (CTL or Killer cells) (TK).

II- B lymphocytes

(A) Precursor of antibody-forming cells.

(B) Memory cells.

(C) Regulatory B lymphocyte

(David & Katz, 1983).

T lymphocytes neither produce circulating antibodies nor give rise to antibody-secreting cells.

Recently, rapid advances have been made in separating human T cells into functional subsets, primarily by isolating T cells subsets on the basis of their possession of FC receptors for different Ig classes (Moretta et al., 1976 and Pichler & Border 1981) or their reactivity mono clonal antibodies (Reinherz et al., 1979 , Reinhers, 1980 and Thomas et al., 1980).

Initially it was thought that only the T cell subset lacking FC receptors for IgG and IgM (T_{G-M}) and the OKT_8 subset

were cytotoxic effectors in allogenic cell mediated cytotoxicity (CMC) (Leopardi & Rosenau, 1982 ; Pichler & Broder, 1981 and Reinherz et al., 1980). It was later observed that OKT_4 + cells can also be cytotoxic and that the stimulating antigen determines which subset, as defined by monoclonal antibody, contains the aggressor cells (Meuer, 1982).

The T cell subpopulations differ in ttheir content of specific T cell surface antigen (Ledbetter, 1979). The mouse, T_H and T_{DTH} express the LyT-1 antigen, whereas T_K and T_S express LyT_2 and LyT_3 antigen. However, it has recently been found that LyT_1 antigven is expressed in different quantitative amounts on different T cell subpupulations (Ledbetter & Herzenberg, 1979).

The direct T cell view has become modified by the proposition that various activities of sensitized lymphocyte may be partly expressed or amplified indirectly by means of soluble non-antibody mediators appearing as a result of antigen lymphocyte interaction (Lawrence and Landy, 1969). The proposition was based initially on confirmation of cell free transfer of delayed hypejrsensitivity in man (Lawrence, 1960) and on the confirmation that products of antigen-lymphocyte in the guinea pig were able to inhibit the migration of guinea pig macrophages in vitro (Bloom & Bennet, 1966; David, 1966 and Dumonde, 1967).

Since 1967, it has become evident that soluble non-abtibody products of lymphocyte activation in vitro possess a number of biological activities which appear relevant to the expression of cellular immune response in vitro and in vivo (Dumond et al.,1968

and Lowrence & Landy, 1969). These lymphocyte activation products are mediators of cellular immune response, that are involved in both expression and regulation of lymphoid cell activity and represent an indirect pathway by which T cell function is expressed (Dumond, 1972).

Cell mediated immunity

Cell mediated immune response has been known since the time of Jenner and Kock in the 19th century, but appreciation of its importance in disease, and perhaps more significantly in health, has only recently become possible.

Bowry (1977) stated that the terms cell mediated immunity and delayed hypersensitivity are used rather confusingly sometimes used synonymously but it is better to restrict the latter to the actual tissue damage resulting from the cellular immune response, leaving the cell mediated immunity to cover the entire spectrum of reactions involved in the response, which range from the definsive to the tissue destruction.

Mechanism of cell mediated immunity(CMI)

The T cell response to antigen involves a series of cellular and lymphokine-mediated events which culminate in T cell proliferation and the emergence of specific effector T lymphocyte. Antigen binding to its specific T cell receptor, in the presence

of the macrophage-derived interleukin, 1(IL_1), triggers the production of interleukin 2(IL_2) and the generation of cell surface IL_2 receptors (Andersson et al., 1979); Larsson, 1980; Smith et al., 1980 and Ruscetti & Gallo, 1981). Subsequent T cell proliferation is dependent on the interaction of IL_2 with IL_2 receptors (Larsson, 1981; Morgan et al., 1976 and Smith et al., 1979).

Interleukin 2 present in culture supernatent can be accurately measured by using a simple and reproducible bioassay (Gillis et al., 1978). IL_2 has been purified (Mier & Gallo, 1982; Watson et al., 1982 and Welt, 1982), sequenced (Robb et al., 1983) and molecularly cloned (Taniguchi, 1983).

This lymphokine has permitted the long-term growth and cloning in vitro of tumour cells (Poiesz et al., 1980), and of normal T cells with helper, suppressor, and cytotoxic functions (Kurnick et al., 1979; Ruscetti et al., 1977 and Schreierr et al., 1980). The presence of specific receptors for IL_2 was initially suggested by the ability of activated cells to adsorb IL_2 activity from crude supernatent preparations (Bonnard et al., 1979; Coutinho et al., 1979; Smith, 1980 and Kern, 1981), and was then directly demonstrated and measured using purified, radiolabelled IL_2 (Robb, 1981).

Biological and clinicl importance of cell mediated immune response:

The biological and clinical importance of cellular immune response lie in its role in maintaining resistance to facultative and obligate intracellular infection, in mechanisms of delayed hypersensitivity and allograft rejection, in restricting tumour growth, in its association with autoimmune disease, and in its role in facilitating antibody production. It is now customary to apply the term specific cell mediated immunity to denote those biological and clinical phenomena which seem to result from interaction between sensitized lymphocyte and specific antigen, and in which no obligatory role can be found for classical humoral antibodies (Turk, 1967).

Functions of cell mediated immunity:

These can be summarized in:

(1) Leads to protection and defence against facultative and obligate intracellular organisms including viruses, fungi and parasites (Wheelock and Toy, 1973).

(2) Plays an important role in auto-allergic disease (Svell, 1975).

(3) Responsible for delayed skin reactivity (Mackaness, 1969).

(4) T cells recognize the cells of cancer as non-self antigen and destroy them (Berrstein et al., 1971) "immune servellance".

(5) Cell mediated immunity is largely, but not solely, responsible for transplant rejection (Bowry, 1977).

(6) Regulation of immune system e.g., the helper T cells promote B cell response to the majority of antigens (Bowry, 1977).

Lymphocyte activators:

Nowell (1960), demonstrated that an acquous extract of kidney been, Phaseolus Vulgaris (Phyto haem agglutinin, or PHA) was able to produce large, dividing, blast like cells in culture of human peripheral blood. Following confirmation by several workers (Carstairs, 1961 & Hasting et al., 1961), it became evident that the precursor cell of these blasts was the small lymphocyte. It was soon demonstrated that in addition to PHA, other activators are capable of inducing the formation of blast cells from small lymphocytes (Douglas, 1971; Hirschhorn, 1965; Horrnung & Fritchi, 1971; Keiser et al., 1971 and Plate & Amas, 1971).

Many of the known lymphocyte activators can be classified into two groups, specific and non-specific.

Non-specific activators (e.g., PHA) stimulate a sizeable proportion of the lymphocytes of all normal individuals and induce detectable biochemical and physiological changes within 1 hour after addition to lymphocyte suspensions. Specific activators (Antigens), on the other hand, stimulate only lymphocytes from sensitized individuals (Hirschhorn, 1965).

Some activators do not readily fall into the above classification. Stimulation by allogenic lymphocyte (the mixed lymphocyte culture response) which possessing many of the characteristics of specific activation, does not require presensitization of the donor. The proportion of lymphocytes which show morphological evidence of stimulation by specific activator is less, and detectable changes in these cultures occur about 3 days later, than those of non-specific stimulated ones. Thus for, there is no evidence that there is any qualitative differences in the biochemical and physiological changes induced by specific and non-specific activators. Present evidence indicates that the differences in time and degree of response are quantitative in nature and are related to the relative proportions of cells in the culture which are initially responsive to the activator.

The activation of lymphocyte for lymphokine production by specific antigen is a property of T cells. However, B cells as well as T cells may be activated by agents that act non-speficically, agents as PPD and endotoxin lipo polysaccharides are known as B cell mitogens (Yoshida et al., 1973), also agents previously considered to be only T cell mitogens, such as pokeweed mitogen, phytohaemagglutinin and concanavalin A.