THE EFFECT OF THE NUMBER OF EFFECTOR ~~CELLS~~ (LYMPHOCYTES) ON THE MIGRATION INHIBITION ~~ASSAY~~

A THESIS

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By

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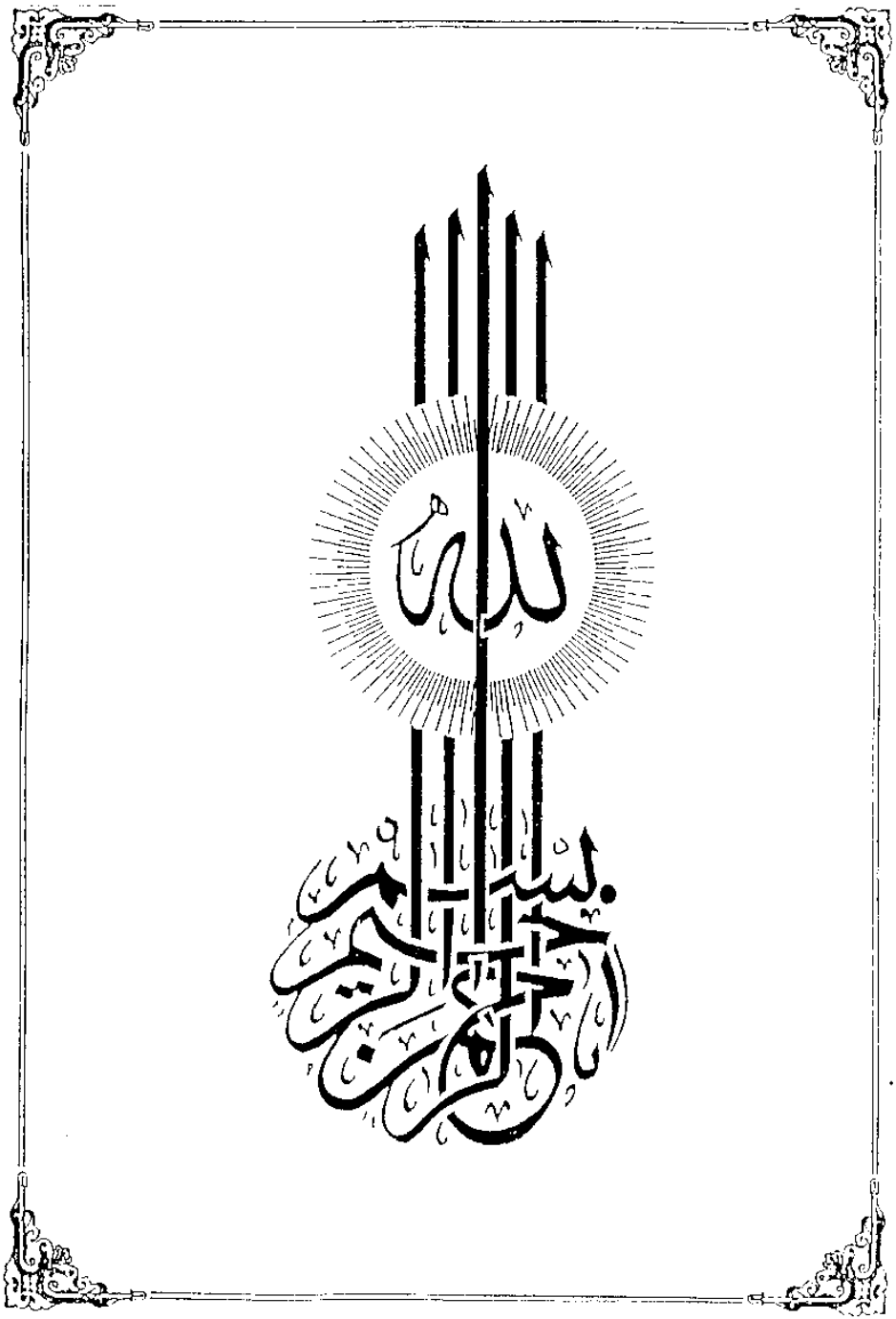
« وقل رب زدنى علمـــــاً »

صدق الله العظيم



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I N D E X

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In vitro, MIF assay was found to correlate well in man with delayed hypersensitivity skin test (Thor and Dray, 1968 and Rocklin et al., 1970). Thus, it has been used extensively as an in vitro parameter of cell mediated immunity.

Various factors may influence MIF assay procedure as the concentration of the antigen or mitogen used, the number of lymphocytes needed for the production of MIF and the medium used for cell culture.

The aim of this study: is to assess the effect of varying the number of lymphocytes on the macrophage migration inhibition using the capillary tube method.

INTRODUCTION AND REVIEW OF LITERATURE

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Sensitized lymphocytes on interaction with specific antigen, or normal lymphocytes after contact with a variety of nonspecific agents (e.g., phytomitogens) are known to produce a number of soluble, pharmacologically highly active substances called lymphokines **(Taylor et al.,1975).**

One of these compunds is the migration inhibition factor (MIF) which retards the migration of macrophages or monocytes from capillary tubes **(Bloom and Bennett, 1966).**

T.lymphocytes represent the main cell population responsible for MIF production in man although there is some evidence that B-lymphocytes are also capable of elaborating MIF **(Rocklin èt al., 1974).**

The MIF system of migration inhibition utilizing macrophages or monocytes as indicator cells has been shown to be distinct from the system of migration inhibtion which uses polymorphnuclear leukocytes as indicator cells **(Rocklin, 1974).**

The inhibition of migration of polymorphnuclear leukocytes is mediated by a separate factor termed leukocyte inhibitory factor, (L.I.F) **(Rocklin, 1980).**

Migration Inhbition Assays:

There are currently two methods available for the assay of human MIF.

[A] The direct assay (one step procedure:

It involves the use of sensitized human lymphocytes mixed with guinea pig macrophages or human monocytes in capillary tubes. Specific antigen is added to the system.

MIF is made locally by the lymphocytes, and the MIF then acts upon the macrophages or monocytes within the 24hrs assay period to inhibit their migration(**Rocklin, 1980**).

[B] The indirect assay (Twostep procedure):

In this assay, sensitized lymphocytes are first cultured separately with antigen to produce MIF over a one or two days period. The culture fluid (cell-free supernatant) is then assayed for MIF activity on non immune guinea pig macrophages or human monocytes in capillary tubes.

The main advantage of the direct MIF system is the short time required to perform the test (24hrs and the fact that it is technically less complicated than the indirect method

The main disadvantage with this method is that a negative result, that is no MIF activity detectable in the system, is difficult to inter pret. A negative result may reflect depressed lymphocyte production of MIF or a failure of the indicator cells to respond to MIF.

The main advantage of the indirect method is that it permits a dissection of the lymphocyte - macrophage interaction by testing each component separately. It also has the advantage of easy storage of the mediator for long period so, it can be used for follow up assays. The direct method should be used as a screening procedure. If a positive result is obtained, one may assume that this particular function is normal. If a negative result is obtained, the indirect method should be used so that each component can be tested separately. **(Rocklin, 1980).**

Several in vitro methods are available for assessing inhibition of cell migration by lymphokine containing supernatant including:-

1- Capillary tube method (George and Vaughan, 1962).

2- Tissue explant technique (Rich and Lewis, 1932).
and(Sve Jcar and Johanovsky,1961).

3- Wounded monolayer assay (Houck and Chang, 1973).

4- Macro agarose well technique (Clausen, 1971). and

5- Micro agarose droplet assay (Harrington and Stastny,1973).

CAPILLARY TUBE TECHNIQUE

Inhibition of macrophage migration from capillary tubes is the most widely accepted and best characterized in vitro correlate of delayed hypersensitivity **(Harrington and Stastny, 1973).**

This method was first developed by George and Vaughan in 1962 and subsequently explored in greater details by David and Coworkers in 1964 and by Bloom and Bennett in 1966.

The capillary tube assays have some difficulties. They require a large volume of whole blood and large quantities of antigen. Further, the size of experiments that can be performed on a given test day is restricted by the limited yield of leukocytes from blood specimens and also by the number of time-consuming manual manipulation. Thus, the number of different blood specimens and antigens that can be tested at various concentrations is limited **(Rocklin, 1980).**

It also suffers from considerable variation from one macrophage donor animal to the other and the quantitation of the results obtained is difficult **(Adelman et al., 1980).**

McCoy et al (1976) mentioned several critical comments which must be taken into consideration during performing the direct capillary tube leukocyte migration inhibition assay:-

1- It is critical to avoid any clumping of leukocytes during cell processing. The addition of fetal bovine serum to the media used for resuspending and washing the cells helps to alleviate potential clumping problems.

2- Cutting of capillaries approximately 0.5-1.5 mm below the cell liquid interface appears to remove a large proportion of platelets that are sedimented on top of the leukocytes during centrifugation.

3- The fan of migrating cells usuallay form two distinct zones (inner and outer). The inner zone of cells are both mononuclear cells (45-55%) and polymorphnuclear cells (47-55%) whereas the outer zone mainly contains faster moving polymorphnuclear cells (70-80%) with a smaller number of mononuclear cells (20-30%). It is important to measure the outer zone (polymorphnuclear cells) of migration in the final analysis since the polymorphnuclear cells are largely the indicator cells whose movement is impaired by the mediator,(L.I.F), although measuring and using the inner areas of both control and test groups usually gives comparable migration index values as obtained when using the outer areas.

4- The technique should be as aseptic as possible. Addition of penicillin and streptomycin into the incubation media helps to control bacterial growth. These antibiotics do not appear to interfere with the migration of cells or with inhibitory reactions. (Considerably less contamination has been met with the sterilin plates than with sykes-Moore chambers.)

5- A level of humidified CO_2 incubator should be used to incubate the plates, lack of moisture in the incubator will result in evaporation of media from the wells and in drying of chambers with formation of air bubbles or a change in the tonicity of the media, that may result in erratic migration of cells.

6- The assay is fairly easy to perform and quite reproducible with good stocks of soluble P.P.D.
When employing tumor extract, the technique becomes more difficult to perform. Problems of obtaining false negative values with the tumor antigens can occur.

7- When 3M Kcl extracts of human tumor materials are used, broad dose response studies for each extract should be run in order to establish toxicity and reactive levels.

TISSUE EXPLANT TECHNIQUE

Tissue explant technique was described by **Rich and Lewis (1932).**

This technique was concerned with the migration of macrophages from solid explants in vitro.Spleen tissue or buffy coat cells in a blood from guinea pigs were cultured in vitro. After 24 hours, there was a marked migration of momonuclear cells, mainly macrophages. If the tissue was taken from a tuberculin sensitive animal, the addition of 1:60 dilution of Old Tuberculin was found to inhibit this outgrowth. The outgrowth of cells from normal animals was not inhibited by tuberculin, even in the presence of serum from hypersensitive donors. Lymphocytes appeared less susceptible to the effect of tuberculin than macrophages or polymorphnuclear leukocytes.

Raffel (1948) among many others confirmed these results using guinea pig bone marrow as the source of the explant. Inhibition of macrophage migration from bone marrow of guinea pigs showing delayed hypersensitivity to ova albumin could also be produced when bone marrow cells were challenged in vitro with ovaalbumin.,

Although most of the work on the inhibition of macrophage migration has been with tuberculin, other