بسم الله الرحمن الرحيم

شبكة المعلومـــــات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

﴿ قـــســـم ﴾

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
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15 – 25c and relative humidity 20-40 %

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بالرسالة صفحات

لم ترد بالأصـــل

THE EFFECT OF INTERLEUKIN-12 AS AN ADJUVANT ON AUTOCLAVED LEISHMANIA VACCINE IN EXPERIMENTAL CUTANEOUS LEISHMANIASIS

Thesis

Submitted to the Faculty of Medicine,
University of Alexandria .
In partial fulfilment of the requirements of the degree of

Doctor of Parasitology

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2001

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TO MY FAMILY

ACKNOWLEDGEMENT

It is a great pleasure to express my deepest gratitude and indebtedness to ***Professor Dr. Magda Youssef Michel,*** Professor of Parasitology, Faculty of Medicine, Alexandria University, who patiently devoted much of her precious time and effort to give me helpful advice, generous co-operation and excellent supervision.

I would like to extend my thanks to ***Professor Dr. Eman Helmy Hegazy***, Professor of Parasitology, Faculty of Medicine, Alexandria University, for her unlimited efforts. She gave me a lot of knowledge, practical experience and time.

I feel also grateful and thankful to ***Dr. Eman Dorry Hussein El-Kerdany***, Assistant Professor of Parasitology, for her great help, as her interest in the subject were very obvious in her alert guidance, unlimited co operation. She devoted too much of her time to this work.

My sincere thanks to ***Dr. Maha Mohamed El-Sayed Eissa***, Assistant Professor of Parasitology, for her collaboration and guidance in the culture of *Leishmania* parasite in the practical part of this work.

I would like to acknowledge with great thankfulness ***Dr. Fouad Mahmoud Fathy El-Sayed***, Lecturer of Parasitology, for his great help in ELISA test.

My special thanks to ***Dr. Eman Abou El-Naga***, Assistant Professor of Parasitology, Faculty of Medicine, Alexandria University, for providing the *Leishmania* strain.

My deepest appreciation and gratitude to my colleagues and all members of Parasitology Department who gave me a helpful hand to accomplish this work.

CONTENT

INTRODUCTION

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Leishmaniasis is a disease caused by intracellular parasite of order Kinetoplastida, family Trypanosmatidae, genus *Leishmania*.[1]

Most forms of leishmaniasis are zoonotic, and humans are infected only secondarily. The great majority of the *Leishmania* species are maintained in nature by mammalian reservoir hosts such as rodents, dogs, wild cats, Jackals, foxes, sloths, hyraxes and other carnivorous animals [2]. The insect vectors are over 50 species of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World.[2]

In humans, infection with *Leishmania* results in a spectrum of disease depending upon the species involved and the efficiency of the host's immune response towards the parasite [3]. A variety of clinical presentations including cutaneous (CL), mucocutaneous (MCL) and visceral leishmaniasis (VL) are well known forms of leishmaniasis. Except for the visceral variety, leishmaniasis is not associated with mortality, but sometimes produces disabling lesions and disfigurement leading to social repulsion with its economic implications.[4]

Morphology of *Leishmania*

Leishmania has two known cycles of reproductive development, one in the gut of the sandfly where the flagellar promastigote stage exists and another in vertebrate tissue where only the amastigote form is found.[5]

Under light microscope, amastigote form appears as an ovoid or rounded bodies of three to five micrometer, living intracellularly in monocytes, polymorphonuclear leucocytes or endothelial cells. In stained preparation, with Giemsa, the cytoplasm is pale blue in color, the nucleus is relatively large and stains red and at right angle to it, is a deep reddish violet rod-like body called "the para-basal body". A dot like blepharoplast may be seen near the para-basal body, a delicate thread connects the two organelles and an axoneme arises from the blepharoplast and extends to the anterior tip.[6]

The promastigote form, which is spindle in shape, is present in the gut of the sandfly or in culture. The kinetoplast assumes a position at the anterior end and a long free anterior flagellum arises from it. The early promastigotes are short ovoid or pear-shaped, 5-10 μm long by 2-3 μm wide. The fully developed ones are long and cylindical, measuring 10-15 μm by 1-2 μm wide. The nucleus is central and the kinetoplast lies transversely near the anterior end.[6]

Electron microscopy revealed that both forms (amastigote and promastigote) are surrounded by typical trilaminar unit membrane and have a subpellicular microtubular system which acts as a cytoskeleton. It helps in preserving the shape and flexibility of the parasite and serves to anchor intrinsic plasma membrane proteins. The flagellum arises from short peripheral fibrils and ends in a plate from which the axoneme begins, and by which it leaves the body in an invagination of the cytoplasm "the flagellar pocket". This pocket is not a true cytostome, it has coated vesicles which appear to be involved in pinocytosis. The kinetoplast appears as an electron-dense granular

band. It is a specialized portion of the mitochondrion which contains supercoiled DNA (kDNA), which exists as a network of minicircles and maxicircles. The kinetoplast lies on the margin of a well developed flagellar vacuole which may be the source of soluble antigens. The nucleus has a central karyosome and clumps of peripheral chromatin. The nucleus is bounded by a bilaminar envelope perforated by pores 80-100 nm which connect nucleoplasm and cytoplasm.[7,8]

Life cycle of *Leishmania*

In the sandfly

Ingested amastigotes transform into promastigotes in the midgut and hindgut in New World sandflies (peripylarian development) or in anterior portion of alimentary tract in Old World sandflies (suprapylarian development). Over the next 4 – 7 days they migrate to the hypostome, developing into infective metacyclic form. During this process, three molecules in particular are heavily expressed on the surface membrane and are thought to be important for infectivity. The first molecule is lipophosphoglycan which forms a matrix or glycocalyx that protects the promastigote from lysis by host complement and helps to establish the parasite in the macrophage.[9] The other two molecules are the major glycoprotein gp 63[10] and an acid phosphatase [11].Promastigotes are inoculated with sandfly saliva which increases infectivity[12] (Fig 1)

In the host

Mouth parts of sandflies tear the host tissue, creating a tiny pool of blood from which they feed and into which promastigotes are deposited. In vitro experiments suggest that lipophosphoglycan and gp63 bind to specific lectin receptors on the macrophage surface membrane through their carbohydrate moieties. Promastigotes activate complement through its classical pathway. Complement three b (C3b) is deposited on its surface and recognized by macrophage receptors. The macrophage extrudes its membrane to engulf the promastigote forming a parasitophorous vacuole containing lysosomal hydrolases, cathepsin and B-glucuronidase which are secreted by macrophage. Promastigotes are transformed into amastigote form and multiply in the cells of reticuloendothelial system and lymphoid cells of the skin.[13] (Fig I)

Parasitization of the macrophage down regulates the host' s cell-mediated response, by reducing the expression of class II major histocompatibility complex (MHC) molecules on the macrophage surface and increasing the requirement of interferon-γ for microbial killing. The amastigote form resists killing by oxygen radicals, which are the classical end- mechanism of cell- mediated immunity. [14]

Several leishmanial antigens are expressed on the macrophage surface. Gp63 seems to be important for the induction of successful immune response, while carbohydrate moiety of lipophosphoglycan may be immuno-suppressive[15] Multiplication of both forms is by binary fission.