الملامح الباثولوجية للايشمانيا الجلدية

وائل تيسير دعبول*

الملخص

خلفية البحث وهدفه: مع الاكتشاف الجديد المتمثل بوجود الأشكال الممشوقة فضلاً عن الممشوقات المولدة لليف في مواقع الأذية عند الإنسان المصاب باللايشمانيا الجلدية (1)، فقد أصبح من المهم بناء الملامح الباثولوجية للمرض في مراحله المختلفة آخذين بالحسبان الاكتشافات الجديدة التي تمت.

البحث وطرائقه: تم تحويل اثنتين وأربعين (42) حالة إصابة باللايشمانيا الجلدية إلى المختبر في دمشق بين كانون الثاني 2004 و تشرين الأول 2007 من أجل التشخيص بالطرائق المجهرية. أخذت شريحتان من كل آفة ولونتا بتلوين رايت. تم تصنيف هذه الإصابات بشكل متدرج و في آنٍ معاً، وفقا للفترة المتوقعة التي ظهرت فيها الإصابة. ومن ثم قمنا بإعادة بناء التصميم للمراحل الباثولوجية المختلفة للمرض بشكل متسلسل.

النتائج: تم تحري الخلايا الليمفاوية ذات الذيل مجهرياً في 41/ 42 حالة وبنسبة 98% من حالات الإصابة المحولة. كما تم تحري الشكل اللايشماني في 42/20 حالة وبنسبة 48% من حالات الإصابة المحولة. أما الأشكال الممشوقة والأشكال الممشوقة المولدة لليف فقد تم تحري وجودها مجهرياً في 42/32 حالة بنسبة 76% من حالات الإصابة المحولة. وقد ظهرت أشكال لهب الشمعة في 42/21 بنسبة 50% من الحالات. وأما الأشكال الكروية والأشكال المضلعة فظهرت في 42/25 من الحالات المحولة وبنسبة 59%.

* ماجستير في علم الأحياء– أخصائي في التكنولوجيا الطبية– أخصائي في التشخيص المخبري.

يتألف النموذج الجديد لباثولوجيا اللايشمانيا الجلدية المعاد بناؤه من خمس مراحل:
المرحلة الأولى: هي مرحلة الكمون، وهي مرحلة الحضانة دون ظهور الأعراض. المرحلة الثانية: وهي مرحلة ظهور الشكل اللايشماني داخل الخلوي. المرحلة الثالثة: وهي المترافقة بظهور الأشكال الممشوقة. المرحلة الرابعة وهي التي تختفي فيها الأشكال اللايشمانية. المرحلة الخامسة وهي مرحلة تشكل الليف.
وبالنتيجة: تؤدي الكريات البيضاء المفصصة دوراً في إزالة الأشكال اللايشمانية المتحررة إلى الأوساط خارج الخلوية. كما أنَّ بعض المجموعات الخاصة من الخلايا اللمفاوية حين تقوم بالاستحالة المتدرجة إلى ما يشبه الأشكال الممشوقة العرطلة فإنها تؤدي دوراً مهماً في العملية المرضية، وذلك من خلال توجيه الأشكال الممشوقة الطفيلية للقيام بعملية توليد الليف مما ينجم عنه حصر الطفيلي المسبب للمرض (وهو أي شكل من مجموع الأشكال المختلفة الموجودة للطفيلي) ومن خلال ذلك الإحاطة بالمرض واحتوائه.

The Pathological Features of Cutaneous Leishmania

Mohammed Wael Daboul*

Abstract

Background and purpose of the study: With the new discovery of the promastigote form and the fiber producing promastigote form in the infected lesion in human with cutaneous leishmania(1), it became important to reconstruct the pathological features of the disease process considering the new findings.

Material and methods:42 cases of cutaneous leishmania referred to the laboratory in Damascus between January 2004 and October 2007 for microscopic diagnosis and from each lesion couple of slides were obtained and stained with Wright stain. Samples were similtinuasly rearranged in series according to the approximate time of the lesion appearance. A reconstruction model of the pathology of the disease sequence was established.

Results: microscopically Lymphocytes (with tails) are present in 41 out of the 42 (98%) of the cases referred, Amastigote form is present in 20 cases out of 42 (48%), Promastigote and fiber forming promastigote microscopically are present in 32 out of the 42 cases (76%), Candle flame forms appear in 21 out of 42 (50%) of the cases, and the spherical and polygon forms appear in 25 out of the 42 cases referred at 59% rate.

The reconstruction model of the disease pathology established consisted of five phases:

Phase one is the lag phase: It is the incubation phase with no symptoms appearance. Phase two is the intracellular amastigote appearance phase. Phase three is the promastigote appearance phase. Phase four is the amastigote disappearance phase. Phase five is the fiber formation phase.

Conclusion: Neutrophils have a role in eliminating the amastigotes released to the outer cellular fluid. Some presumed subgroups of lymphocytes by converting into giant promastigote-like forms in shape, have a role in the disease process by directing the parasitic promastigotes into fiber formation process and hence controlling the causing factor (which is the different parasite forms present) and through that eliminating the disease.

* DDS, MSc, MT(ASCP).

Introduction:

Studies of leishmaniasis that were done before lack many of the pathological features of the disease process. Many pathological events are either unclearly elucidated or uncertain or overlooked with out being fully explained. That made the whole pathology of the disease process somewhat ambiguous and unclear.

Cutaneous leishmaniasis presents as a skin ulcer at the site of the sandfly bite and generally heals spontaneously with a scar within three to six months.

Histological examination of the cutaneous lesion sometimes reveals extensive subcutaneous lymphohistiocytic infiltrate with clusters of amastigote within histiocytes (2). Other times it shows granulomatous inflammation with histiocytic infiltrate (3).

Other histopathological studies indicate lymphocytes and plasma cells abundance in the wet ulcerative lesions. The overlying epidermis is hyperkeratotic and subsequently breaks down to form an ulcer covered with dried exudate, dead cells and a mixture of live and dead organisms (4). Through the disease process, over the following months, there is a gradual decrease in the number of amastigotes and macrophages (4), leaving a sporotrichoid granuloma with less lymphocytes, epithelioid cells, multinucleated giant cells and scanty plasma cells in dry nodular lesions (5), (6), (7).

From the immunopathologic point of view: The extent of the disease manifestation is a combination of the parasite pathogenesis and the immune host response. The interactions between the parasite virulence factors and the cell- mediated immunity are not fully understood (8). A study of post-kala-azar dermal leishmaniasis declared that there is an abundance of CD4, which closely interacts with Leishmania antigen present (9).

Another study proposes the presence of three group of antigenic determinants in the parasite**:** First group is the invasive / evasive determinants. They help the parasite to establish infection in the host**.**

Second group is the parasite pathoantigenic determinants. The immune response against such determinants results in immunopathology causing the disease symptoms to appear.

Third group is the vaccine determinants: When the immune system interacts with those determinants they lead to parasites elimination. A

hypothetical model was constructed assuming that the disease virulence is due to interaction between the host's immune system and the leishmania parasite determinants. Recent works revealed the existence of T-cell epitopes in leishmania cytoplasmic molecules elucidating protective immunity (10).

Such studies mentioned above, assume the amastigote as the only form present in vertebrates and hence, it summarizes the whole process of the disease of cutaneous leishmania accordingly. Our findings (1) reveal beside the amastigote, other forms of the parasite are present and missed by those studies. These findings will add more light to the pathology of the disease process.

Additionally, the pathological features under the microscope have to be a reflection to the immunopathologic interaction between the parasite and the host.

Unfortunately going through the literature presented, we do not see such a reflection. In fact, all what is seen is a mix up of deficient pathological elements timely misarranged describing the whole set of the immunopathologic interaction during the disease course.

The purpose of our study was to reconstruct the pathologic features with the aid of the images in the right sequence by adding the new pathological figures discovered and the new data presented (1) and

Locating them into their right position of the sequence on step-by-step basis. This will contribute to a better understanding of the pathology of the disease.

Material and methods:

42 cases referred to the laboratory in Damascus since January 2004 from consultant dermatologists as being clinically diagnosed as cutaneous leishmaniasis.

From each lesion couple of slides were obtained and stained with wright stain.

A study was done to identify the presence of the lymphocytes with tails among the different samples and cytomorphologically compare them with each other in the cases referred and with typical forms of lymphocytes as controls.

Another study was done to identify and compare the different cytomorphologies of the parasite forms (amastigotes, promastigotes, candle flame shapes, polygonic forms, spherical forms and fiber forming

forms) found and to define their appearance percentage among the different samples.
The approximate time of the lesion appearance at the time of the sample collection was investigated through patient inquiry.
With the time aid of the lesion appearance connected with the pathological features for all the 42 samples similtinuasly rearranged in series according to that approximate time and studied, a reconstruction model of the pathology of the disease sequence was established.
Confirmatory microscopic photos were taken for documentation.

Results:

Table 1_ Appearance of the lymphocytes (with tails)

Total number of samples investigated	42
Total appearance of the lymphocytes (with tails)	41
Percentage of total appearance of the lymphocytes(with tails)	98%

From table-1- we found that the lymphocytes (with tails) are present in 98% of the cases. 41 out of the 42 samples referred are having this type of lymphocytes microscopically showing up. The appearance of such lymphocytes among the different samples is not in the same density. That was related to the disease stage when the sample was collected in the lab.

Table 2_ Appearance of Amastigotes, Promastigotes, Fiber forming Promastigotes, Candle flame forms and polygons and spherical forms.

Appearanc	Amastigote	Promastigote form	Fiber forming Promastigote	Candle flame Polygons	Spherical&
Total appearance	20/42	32/42	32/42	21/42	25/42
Appearance percentage	48	76	76	50	59

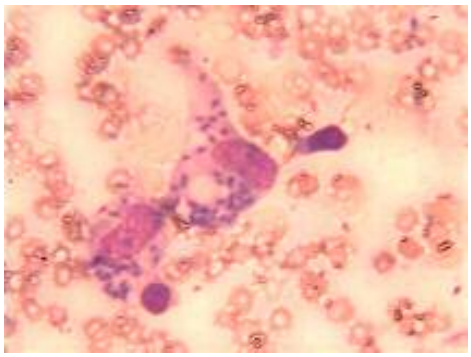
Table –2- reveals that in 20 cases out of 42 (48%) the amastigote microscopically is present. While the appearance of the promastigote and the fiber forming promastigote form is equal. They are present in 32 out of the 42 cases (76%). The candle flame form appears in 21 out of 42 (50%) of the cases and the spherical and polygon forms appear in 25 out of the 42 cases referred at 59% rate.

The reconstruction model of the disease pathology:

Phase one (The lag phase): The disease pathology starts when the infected sand fly during its meal transfers the parasite to the cutaneous area of the human skin. A (lag) incubation period extends from one day

up to several months (11). During which, the promastigote type of the parasite enters the subcutaneous area where it loses its flagella and is engulfed by the macrophage or the phagocyte. The macrophage plays an important role. The parasite assumes its multiplication within that cell while the macrophage starts the recognition process.

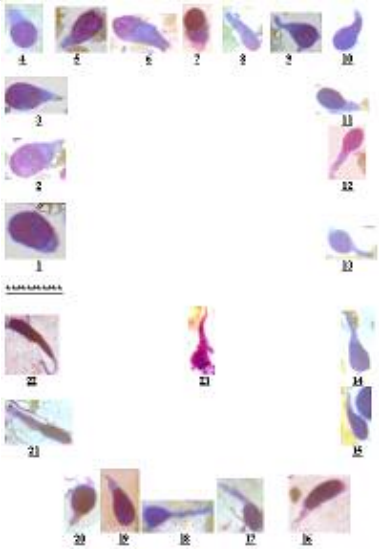
Phase two: The second phase after the lag phase is (the intracellular amastigote appearance phase) where inside the macrophage, the amastigotes begin to show up multiplying and filling the cytoplasm of the phagocytes (Figure 1). Synergistically, the macrophage plays its role as an antigen presenting cell, which presents the amastigote antigen in its surface to other cells of the immune system meaning the B, and T lymphocytes and natural killer cells. Microscopically, the lymphocytes are noticed in large numbers surrounding the infected macrophages at this point (Figure 1).



(Figure 1)

Once the parasite is recognized by such cells, the lymphocytes morphologically and microscopically seem to behave somewhat different than the usual lymphocytic immune reaction. The usual immune reaction in cell-mediated immune response is characterized by T cells activation in to helper, suppressor or cytotoxic T lymphocytes which morphologically show if at all, a mild to a moderate enlargement changes in those T lymphocytes. The B lymphocytes usually are increased in size converting last to the plasma cells with the nucleus positioned at one pole of the cell. In both cases, morphologically, there are no cytoplasmic protrusion or extension changes out of those lymphocytes. Here, may be due to its unique distinction as being the only parasite that infects the macrophage and utilizes it for its own multiplication, major morphologic

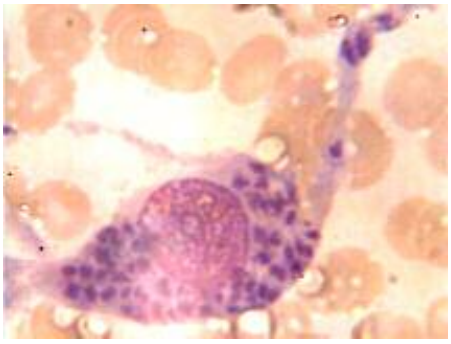
changes take place on a time basis within the lymphocytes. A polarization of the nucleus and an elongation of the cytoplasm within the lymphocytes are recognized. Later, the nucleus becomes more condensed and the cytoplasm becomes like a pale blue tail protruding out from the cell. Afterwards, the nucleus becomes further more condensed like a plain dark piece and it is impossible at that stage to microscopically differentiate any of its nucleus components. Here the cytoplasmic tail becomes even thinner and more elongating while the whole size of what is believed to be a lymphocyte becomes overall smaller. Then, the cytoplasmic elements inside the lymphocyte tail disappear leaving it like a flagella looking sheath while the condensed nucleus appears in the other pole. The new-formed structure looks in shape very much like a giant type of the promastigote parasite. From the photos taken for those changes, a schematic diagram of the lymphocytes conversion in a sequence was built up (Figure1A).



(Figure1A)

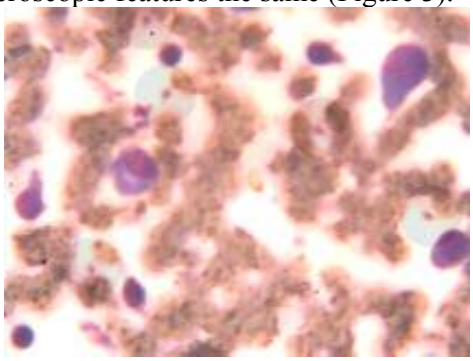
In this stage also, macrophages with the amastigotes multiplying inside are markedly noticed. Many of the macrophages become giant in size and some multinucleated cells appear with the amastigotes showing inside their cytoplasm. These phagocytes together with the different lymphocytes constitute the core microscopic features of our second stage

(Figure 1). Interestingly enough, many of the macrophages seen tend to form elongating cytoplasmic tails protruding out. This phenomena Is called (The tail phenomena) (Figures 2,10). In this stage neutrophils are occasionally seen.



(Figure 2)

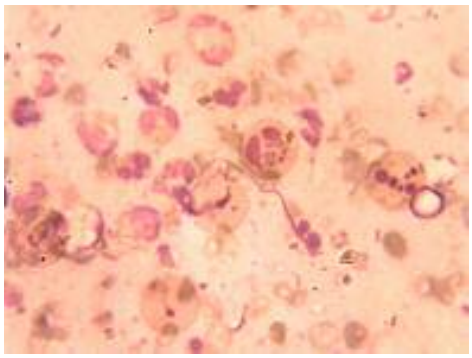
Reaching the end of this stage, the macrophages membrane tears out and the amastigotes are released in a large number into the extra cellular fluid leaving other microscopic features the same (Figure 3).



(Figure 3)

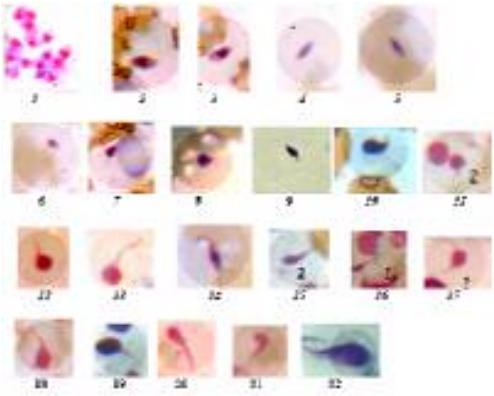
Phase three: (The promastigote appearance phase): In this phase, the already released amastigotes are present in the extra cellular fluid, which stimulate an acute phase inflammatory reaction characterized by neutrophils accumulation for phagocytosing and amastigote killing. Other figures present are: decrease number of the lymphocytes, plasma cells, and what are known as lymphocytes (with tails) and the presence of what looks like giant promastigotes. We may also see a reducing number of macrophages with the amastigotes inside. At this stage, in the extra

cellular fluid, the amastigotes appear at different shapes from round to oval and to spindle while the chromatin inside is either spreading or condensed and taking a polar position. Interestingly, candle flame figures erupt out of those amastigotes, taking the spindle shape with a tail protruding out. At a later time on their development, some of those candle flame figures become polygonic in shape reaching in maximum the RBCs size. They become difficult to differentiate from regular lymphocytes. Other growing parasites from the candle flame appear round in shape reaching also the RBC size. Some of those candle flame figures and the amastigotes too are phagocytosed by the neutrophils. Others continue in their development. We may later see many destroyed neutrophils as a result of such parasitic neutrophilic interaction (Figure 4)..



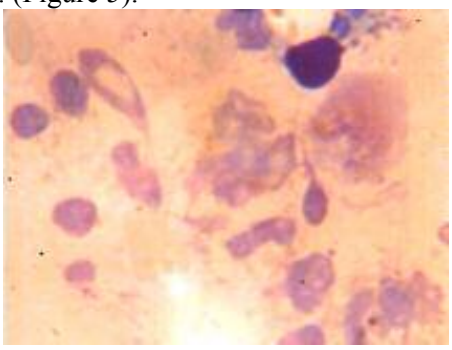
(Figure 4)

From that time on, some of those candle flame figures that have the spindle shape and the tail become more enlarged taking the shape of small promastigotes. Later on, the developing promastigotes are manifested at many different cytomorphologies (see figure 2A indicating the amastigote development into promastigote) making this phase by large, the promastigote appearance phase



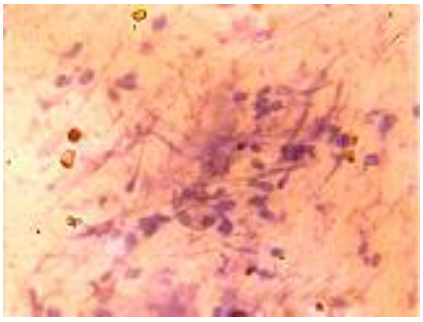
(Figure 2A)

Phase four. (The amastigote disappearance phase): At this phase the neutrophils have already consumed the extra cellular amastigotes so the amastigotes are rarely seen in the extra cellular fluid while the macrophages and the multinucleated giant cells with the intracellular amastigotes tend to disappear, leaving the screen at the end of this phase with few neutrophils and plasma cells and few of the different lymphocytes including the taily ones with lymphocytes originated giant promastigotes and increased number of the parasitic promastigotes and polygonic figures. (Figure 5).



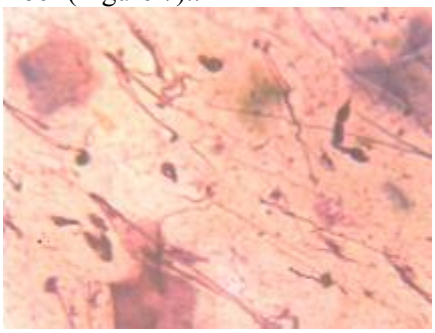
(Figure 5)

Phase five (The fiber formation phase): In this phase the parasitic polygons together with the lymphocytic giant promastigotes and the parasitic promastigotes start gathering and establishing the core elements for fiber production (Figure 6).



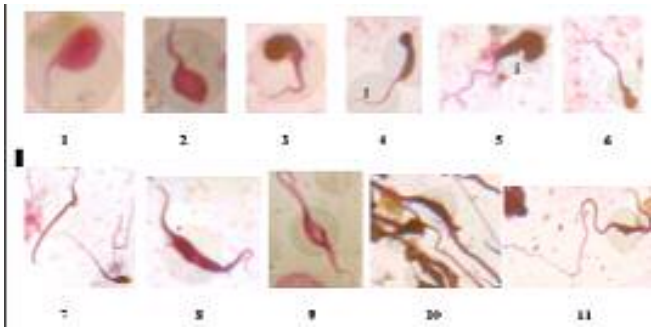
(Figure 6)

Those parasitic promastigotes begin producing thick fiber structure from their flagella area until they are terminated with a thinning nucleus in a middle of a whole fiber (Figure 7)..



(Figure 7)

By this way, those formed parasitic promastigotes are involved in fiber formation and got embedded in the center of hairy like fiber elements which cause the disease to be controlled by controlling the causing factor which is the parasite and hence terminate the illness (Figure 3A illustrates the fiber formation on a step bases) .



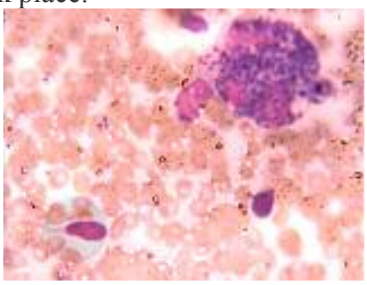
(Figure 3A)

Discussion:

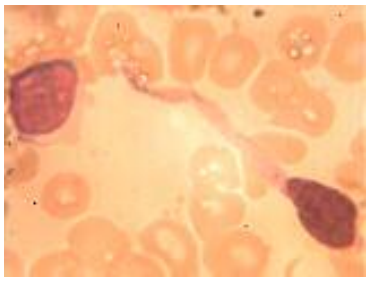
One may question the credibility of such lymphocytes (with tails) whether being originated from lymphocytes or from the parasite during its development sequence or may be originated from the fibroblasts. We had this dilemma in mind. Regarding their being from a parasite origin, when going back through the disease process, it was found that the lymphocytes (with tails) appeared early on phase two and that is much earlier before the amastigote had converted in to any promastigote form. The appearance of the polygonic forms was declared in the middle of phase three. Those polygonic forms are the only forms, which may look like and may be morphologically confused with lymphocytes but they were grown later within the disease process. In addition to that, those lymphocytes in tails show up in their highest count in phase two when the amastigotes are still inside the macrophage and no other figures are seen in the start of the disease process except those macrophages and the different lymphocytes. And, as with regards to the fibroblasts origin, none of the literature reviewed had motioned any fibroblasts or fibrocytes appearance especially in the early stages of the infection. Besides, the cytomorphologic manifestation of fibroblasts is far different than those presumed lymphocytes with tails appearance. Microscopically, the fibroblast nucleus is usually centralized and the cytoplasm is surrounding, giving the cell a spindle shape with a fiber protruding out from both polls. So one can assume that such lymphocytes (with tails) were developed from those lymphocytes surrounding the macrophages. Immunohistochemical studies should be followed for further

identification of those cells, which are beyond this cytomorphologic study.

Second, the question of the giant promastigotes origin from those lymphocytes (with tails): From the diagram attached (Figure 1A) and hundreds of photos taken (Figures 9,10), it is quite sure that such conversion from the lymphocyte (with tails) to the giant promastigote took place.



(Figure 10)



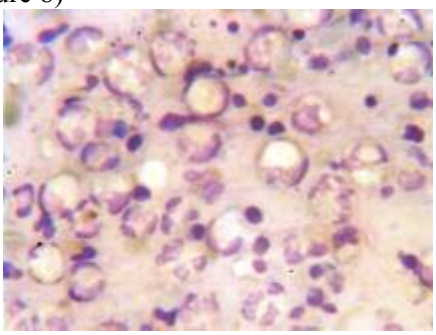
(Figure 9)

But the question is what role does this conversion play in the disease sequence?

Our hypothesis is that from the immunologic point of view, first, the morphology of those lymphocytes (with tails) and macrophages is unique, that we are uncertain of any such changes in the morphology of both lymphocytes and macrophages like this has occurred in any other condition. Second, the parasitic promastigote produced by the amastigote seems to be so large for the neutrophile to phagocytose. In such case, the alternative process of the disease control seems to be in the direction of the parasite surrounding and embalming by hairy fiber formation. Here, an immunologic interaction takes place among the three figures the parasite in one hand and the macrophages and some subgroup of lymphocytes in the other hand. This subgroup of the lymphocytes becomes activated and is converted to lymphocytes (with tails) . In their conversion process to huge size promastigotes, lymphocytes (with tails) had an obvious role to play. That role could be an educational role for the parasitic promastigote towards producing the fiber like materials so that the disease will control itself.

Other studies totally overlooked the neutrophils role in the disease process. In fact neutrophils, are best specialized in phagocytosing process

while macrophages are considered less efficient than PMNs (polymorphnuclear neutrophils) at killing bacteria, and the mechanisms of killing are not as well understood (12). Accordingly, one can assume that those PMNs must have a strong role in phagocytosing and destroying many of the amastigotes released after membrane rapture of the infected macrophages (Figure 8)



(Figure8)

Otherwise, it is not possible to explain the amastigotes disappearance from the infected tissues after their release in that huge number into the extra cellular fluid.

Researchers always relied on one fact that the only type of parasite present in the vertebrate including human being infected with leishmania is the amastigote form and they formulated all their pathological and immunologic understanding of the disease process on such an incomplete fact (2,3,4,5)

The presence of such an amastigote and its development into a promastigote form in the vertebrate may modify our understanding to the pathology, life cycle and hence our approach for controlling and treating the disease. Talking about the disease, it not only is the cutaneous type of leishmaniasis but the visceral leishmaniasis (VL) as well as the mucocutaneous leishmaniasis. All the three parasite types share similar morphology. And though, we did not check the presence of the promastigotes in the other tow types meaning, the mucocutaneous and the visceral leishmaniasis due to limitation of resources, the similarities in morphology among those different parasites make them not even possible to differentiate their types by mean of microscopic exam (4). That allows us to predict similarities to our findings in the cutaneous leishmania in

both types of the parasites. Those similar findings are the appearance of such promastigotes at their different stages of development starting from their amastigote conversion into promastigote form and ending with fully mature promastigote appearance inside the infected location of the human host.

Our new discovery with respect to Leishmania parasites in general through the cutaneous leishmania species in particular allowed us the following advantages:

1- A better understanding for the disease process in its all stages.

2- A better understanding to the pathology of the disease and the macrophages, neutrophils and lymphocytes role in it.

3- A 100% sensitivity and high specificity for leishmania detection by the microscopic method.

4- A better understanding of the parasite life cycle and the role of both the victor and the host.

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