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Review Article

"Vector-Host-Parasite" Inter-relationships in Leishmaniasis: V. Focus on the feeding medium of *Leishmania* parasites *in vivo* and *in vitro*

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ABSTRACT

Leishmaniasis infections in humans comprise a spectrum of diseases with a wide diversity of manifestations, caused by different species of the genus *Leishmania*. This manuscript introduces a hypothesis for understanding the importance of the kind of blood in the feeding media of *Leishmania* species *in vivo* and *in vitro,* since, there are major differences in blood proteins among different vertebrate species. This hypothesis is based on the following evidences: The blood taken with the amastigotes by the sand flies, *in vivo*, differs from that used for parasite cultivation, *in vitro, in* physical and chemical properties, concentration and the degree of digestion. *Leishmania* sp. obtain their nutritional requirements from the culture media. Clearly, the specific requirements for the differentiation of a particular *Leishmkania* species differ from one species to another. The differences in blood proteins may affect the molecular processes, which lead to stage differentiation in *Leishmania,* since proteins are of the utmost importance in biological processes. Additionally, the phenotype and / or genotype of pathogen can change dramatically following culture in an artificial system. Furthermore, it has been proven that amino acids and small peptides are the principal growth substrates for *Leishmania* spp. and some amino acids play an important role in cellular physiology. These evidences indicate that the type of blood proteins is important for producing infective or non- infective stages within a single life - cycle. Ideally, therefore, the *in vitro* culture media should duplicate the natural reservoir host blood constituents in the sand fly gut. In our opinion the use of unsuitable blood *in vitro* culture may have an effect on parasite differentiation that may appear gradually by prolonged pass aging. This may explain why promastigotes, which are extensively subpassaged in vitro, lose their virulence. This suggests that the loss of infectivity with prolonged culturing may be due to overgrowth by the non- infective population as a result of utilization of unsuitable blood. Therefore it is suggested that there is a subtle factor(s) in each kind of blood

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that is highly significant for parasite differentiation of the *Leishmania* stages to the infective stage inside the sand fly gut. This concept may open a new approach for a better understanding of *Leishmania* cultivation and transmission problems by laboratory infected sand flies.

KEYWORDS: *Leishmania*, culture media, amino acids, differentiation, feeding media, promastigotes, infective stage, transmission.

INTRODUCTION

Leishmania are parasitic protozoa with a digenetic life cycle, proliferating as extracellular flagellated promastigotes in the digestive tract of the sand-fly and as intracellular amastigotes in the phagolysosomal vacuoles of mammalian macrophages. They comprise a complex of species and subspecies affecting man in both the Old and New Worlds. Although morphologically similar, they are capable of generating a wide spectrum of different clinical manifestations, from self-limited cutaneous leishmaniasis to the potentially fatal Kala-azar (visceral leishmaniasis).

Physiological and biochemical differences exist not only among different species in the genus *Leishmania* (Poorman & Janovy 1969), but also among the life-cycle stages of single species (von Brand 1966 a & b). Furthermore, previous reports suggest that *Leishmania* promastigotes differentiate from a non-infective to an infective stage during growth within the culture medium and within the sand-fly vector (Sacks & Perkins 1984).

The increased appearance of stress-protein-related molecules is accompanied by adverse growth conditions to which the parasites are subject during differentiation; however, the functional role of the proteins has not yet been elucidated (Sacks & Perkins 1984). The differentiation of *Leishmania* involves both qualitative and quantitative changes in various biochemical parameters (Chang *et al.* 1990). The correlation between parasite differentiation and the expression of a variety of genes has been described in several species of *Leishmania* (Cairns *et al.* 1989; Lohman *et al.* 1990). However, mechanisms which control such changes and their effect on leishmanial differentiation remain to be defined (Joshi *et al.* 1993).

Blood requirements for *Leishmania* parasites *in vivo*

In the vertebrate host, *Leishmania* amastigotes feed on the contents of the macrophages in infected tissues, an optimal habitat for replication (Antoine 1995). In the invertebrate vector, they are taken up with whole blood of the infected vertebrate host into the sand-fly gut. As digestion of blood takes place, the protein macromolecules are converted into smaller peptide fragments and amino acids. Feeding of parasites on these digested materials may play an important role in parasite differentiation. Clearly, the nature of food for amastigotes in vertebrate host differs from the nature of food in the invertebrate vector. In the former case, amastigotes are present in one form, but in the latter case, the amastigotes can differentiate into successive forms of promastigotes and paramastigotes according to the kind of digested blood proteins (Daba *et al.* 1997b) taken by the parasites. The variety of forms of promastigotes and paramastigotes observed in the life-cycle of *Leishmania* supports the notion that changes in antigenicity of the parasites may occur in the sand-fly (Molyneux & Killick-Kendrick 1987). This

suggests that the type of blood in the latter case is important for parasite differentiation in the sand-fly gut. If the digested blood proteins are suitable for the development of one species of *Leishmania* to the infective stage, the host blood is considered a reservoir host, and the sand-fly can transmit the disease. If the blood is unsuitable for the development of such species of *Leishmania* to the infective stage, the host blood is considered an accidental host, and in this case the sand-fly cannot transmit the disease (Daba *et al.* 1997b). This may explain why transmission attempts using *Phlebotomus papatasi* infected with *L. major* from infected hamsters were unsuccessful, despite the presence of parasites within the mouthparts (Shehata *et al.* 1988). Furthermore, studies by Schlein *et al.* (1983) and Daba *et al.* (1997a) revealed that turkey blood arrests leishmanial parasite development inside the sand-fly gut. In this case, the host blood is not considered a host for *Leishmania* in nature.

On the other hand, results of protein electrophoresis of blood samples from various species (Daba *et al.* 1997c) showed that there are major differences in blood proteins among different vertebrate species. Given this difference, and the prior evidence of the effect of different blood meals on parasite differentiation, a logical inference is that the source of blood meals *in vivo* affects the biology and physiology of *Leishmania* species inside the sand-fly gut. Each kind of natural host blood has a particular effect on the differentiation of each leishmanial species, with natural reservoir host blood being the key factor for development of the parasites to the infective stage (Daba *et al.* 1997b). Clearly, the specific requirements for the differentiation of a particular *Leishmania* species differ from one species to another. These differences in blood proteins may affect the molecular processes which lead to stage differentiation in *Leishmania,* since proteins are of the utmost importance in biological processes (Bell *et al.* 1970).

Based on the above information it is very plausible that each kind of blood meal taken by the sand-fly has direct effects on differentiation of each leishmanial species.

Blood used in cultivation of *Leishmania* parasites *in vitro*

Different culture media have been used for *in vitro* cultivation of the *Leishmania* promastigote stage in the sand-fly vector. These media can be classified as biphasic, semi-solid or liquid media. All principal biphasic, semi-solid and some liquid media require defibrinated rabbit blood as an important factor for maintenance and replication of the parasites (Marin *et al.* 1982). Most other liquid media require fetal bovine or calf serum as an essential factor for leishmanial growth. Some of these culture media promote good growth without affecting survival of certain stocks of *Leishmania*, while other media can not support parasite growth for more than few passages (Berens *et al.* 1976). Comparison of promastigote growth on modified media indicate that small changes in the composition of media can have drastic effects on parasite growth (Marin *et al.* 1982; Shaw & Lainson 1981). Additionally, the phenotype and/or genotype of a pathogen can change dramatically following culture in an artificial system (Randolph & Nuttall 1994).

The blood taken with the amastigotes by the sand-flies, *in vivo,* differs from that used for parasite cultivation, *in vitro*, in physical and chemical properties, amount, concentration and the degree of digestion. It seems to us that *in vivo,* the natural host blood is mainly digested by two means: the sand-fly gut digestive enzymes; and digestive enzymes excreted by the parasite. However, *in vitro*, only trace amounts of the diluted blood is digested by leishmanial digestive enzymes. *Leishmania* obtain their

nutritional requirements from the culture media. It has been proven that amino acids and small peptides are the principal growth substrates for *Leishmania* species (Krassner & Flory 1971; Mukkada & Simon 1977; Law & Mukkada 1979). Moreover, some amino acids play an important part in cellular physiology (Lehninger 1975). This indicates that the kinds of amino acids and small peptides are responsible for parasite differentiation to produce relatively infective or relatively non-infective organisms within a single life-cycle. Ideally, therefore, the *in vitro* culture media should duplicate the natural-reservoir host-blood constituents in the sand-fly gut. In our opinion the use of unsuitable blood in *in vitro* culture may have an effect on parasite differentiation that may appear gradually by prolonged passaging. This may explain why promastigotes which are extensively subpassaged *in vitro* can lose their virulence (Randolph & Nuttall 1994; Jaffe *et al.* 1984). This suggests that the loss of infectivity with prolonged culturing may be due to overgrowth by the non-infective population as a result of utilization of unsuitable blood. Promastigote research is best conducted using *Leishmania* recently isolated from stocks carried in animals or with promastigotes cryopreserved in liquid nitrogen within 2 passages after primary isolation. This original parent wild-type population contains fully virulent infective clones (Jaffe *et al.* 1984).

Moreover, the difference in culture media may affect the molecular processes which lead to changes in isoenzymes of leishmanial promastigotes that appear due to repeated subpassaging (Jaffe *et al.* 1984). This may explain the occurrence of some unknown *Leishmania* species which do not match with any of the known WHO stock species (Kreutzer *et al.* 1987).

Unfortunately, the role of the blood of natural-reservoir hosts inside the sand-fly gut has been neglected in most previous studies concerned with mammalian *Leishmania* parasites in sand-flies. The "vector-parasite" interaction was studied using the blood of laboratory animals (Killick-Kendrick *et al.* 1988) or other nutritive media (Tesh & Modi 1984; Walters *et al.* 1989 & 1992). These artificial nutritional substances are quite different from the whole blood of the natural-reservoir host(s), which the sand-flies consume with the amastigotes without discrimination. Data based on the use of laboratory hosts give inaccurate results for the natural epidemiology of the disease (Randolph & Nuttall 1994). For example, a culture medium may support excellent growth, but these forms may be non-infective. Use of such a system may lead to such misinterpretation. Recent research (Daba *et al.* 1997b) has revealed that blood from certain species promoted good growth of parasites inside the sand-fly gut, seeming not to affect the survival or viability of parasites, but instead having an effect on the differentiation of the parasites. This may explain why many sand-flies from India and Israel fed on both animals and man failed to transmit leishmaniasis, although on dissection they were shown to have massive foregut infections (Molyneux 1977).

In conclusion, it is clear that each *Leishmania* species has its specific nutritional requirements to differentiate to the infective stage inside the sand-fly gut, and that the types of blood proteins play a role in the stage differentiation. The kind of blood meal taken with the amastigotes by the sand-flies is a critical factor for parasite differentiation. Successful transmission depends on the presence of the infective stage, not on the rate of growth of non-infective promastigotes. Therefore, the failure of many laboratory trials to obtain the infective stage of many *Leishmania* species in the sand-fly gut may be attributed to the use of blood of laboratory animals or other feeding medium

which may possess inhibitory factor(s) or may lack some essential components needed for the development of parasites to the infective stage.

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الملخص العربى

علاقة العائل بالطفيلى: إلقاء الضوء على الوسط الغذائى لطفيل *الليشمانيا* فى الطبيعة والمعمل

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هذا البحث يلقى الضوء على الوسط الغذائى الذى يجب أن يتغذى عليه طفيل *الليشمانيا* فى المعمل كما يحدث فى الطبيعة حيث أن الحشرة تأخذ الطفيل مع دم العائل الخازن والذى يختلف فى تركيبه البروتينى تبعاً لنوع العائل. وقد ثبت من الدراسات السابقة أن نوع الدم يؤثر على شكل الأطوار فى دورة الحياة لطفيل *الليشمانيا* داخل الحشرة أى أن لكل نوع من أنواع *الليشمانيا* الدم الملائم لنموه للطور النهائى المسبب للمرض ومن هنا كانت أهمية دراسة هذا العامل لتربية الطفيل معملياً حتى لا يتحول بتكرار التربية إلى سلالة غير قادرة على العدوى وهى الظاهرة المعروفة للعاملين فى هذا المجال ولم يكن معروفاً لها سبب قبل هذه الدراسة. كما يفسر هذا البحث فشل حدوث العدوى معمليا وذلك لاستخدام حيوانات تجارب ذات تركيب بروتينى للدم غير مناسب لنمو الطفيل للطور النهائى المسبب للمرض وبالتالى لا يحدث نقل للمرض بالرغم من وجود الطفيل داخل الحشرة. ومن هنا يتضح لنا أن وجود الطفيل داخل الحشرة لا يعنى قدرتها على نقل المرض ما لم يوجد الطور النهائى (الطور المعدى) المسئول على نقل المرض. ولذلك يتضح أن الوسط الغذائى يجب أن يتوفر فيه العناصر الهامة لنمو الطور المعدى وليس العناصر التى تعطى الفرصة لنمو الأطوار غير القادرة على نقل المرض. هذا المفهوم سوف يفتح المجال أمام الباحثين للتوصل للطريقة المثلى لعمل مزارع *الليشمانيا* والتغلب على الصعوبات التى كانت تواجههم فى تجارب العدوى معمليا.