ABSTRACTS

Monday, December 10, 2007

Session 1: Leishmania (Molecular biology)

Comparative functional genomics of *Leishmania* species

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Recent completion of the genome sequences of three *Leishmania* species associated with different disease types (*L. major*, *L. infantum* and *L. braziliensis*) is facilitating comparative analysis of their gene content and synteny. While these features show remarkable conservation between the target species, a small sub-set of species-specific genes has been identified for functional study. Microarray analysis has demonstrated that all of these sequences are expressed as low abundance mRNAs. The potential influence of individual genes on parasite tropism in the host is under investigation, using transgenic parasites in *in vitro* and *in vivo* models of infection. Our present focus is on a *Leishmania* orthologue of the bacterial gene encoding cyclopropane fatty acyl phospholipid synthase (CFAS), that is present in both the *L. infantum* and *L. braziliensis* genomes but not in *L. major*.

Leishmania donovani metabolic retooling for life in a new host

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In order to survive extremely different environments, intracellular parasites require highly adaptable physiologic and metabolic systems. *Leishmania donovani* extracellular promastigotes reside in a glucose-rich, slightly alkaline environment in the sand fly vector alimentary tract. Upon entry into human macrophage phagolysosomes,

promastigotes differentiate into intracellular amastigotes. These cope with an acidic milieu, where glucose is scarce, while amino acids are abundant. Here, we use an axenic differentiation model and a novel high-coverage, comparative proteomic methodology to analyze in detail protein expression changes throughout the differentiation process. The analysis identified and quantified 21% of the parasite proteome across 7 time-points during differentiation. The data reveal a delayed increase in gluconeogenesis enzymes, coinciding with a decrease in glycolytic capacity. At the same time, β-oxidation, amino acid catabolism, tricarboxylic acid cycle, mitochondrial respiration chain and oxidative phosphorylation capacities are all up-regulated. The results indicate that the differentiating parasite down-regulates general protein synthesis and shifts from glucose to fatty acids and amino acids as its main energy source. Furthermore, glycerol and amino acids are used as precursors for sugar synthesis, compensating for lack of exogenous sugars. These changes occur while promastigotes undergo morphological transformation. Our findings provide new insight into changes occurring in single-cell organisms during a developmental process.

RNA thermosensors and translation regulation in *Leishmania*

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Translational regulation plays a key role in developmental gene expression in *Leishmania*. Translation control is mediated by 3' UTRs, through mechanisms that are still elusive. We used the HSP83 gene as a model for understanding how stage-specific translation regulation is directed during the parasite life cycle. We mapped the regulatory element in the Hsp83 3' UTR to sequences 201-346. However, this region is required, but not sufficient for conferring preferential translation at elevated temperatures. We present data indicating that the function of this element is structure-dependent. To examine these structural changes, the *in vitro* transcribed RNA region (1-472) was end-labelled and subjected to enzymatic probing of its secondary structure. The mapped positions were used as constraints for structure prediction by UNAFold with Mfold utilities, that provides the probability of each nucleotide to be found in a single- or double- stranded form. In the predicted structure, regions that were shown to be essential for preferential translation cluster on a discrete arm of the RNA. We further identified a single-stranded polypyrimidine-rich region in the Hsp83 3' UTR that is essential for translation at elevated temperatures, and is subject to structural changes during a temperature switch. We propose that this is the basis for a thermosensing mechanism that controls stage-specific translation. We discuss potential modes of translation regulation in *Leishmania*, which are associated also with the basal translation machinery of *Leishmania* and trypanosomes, and report on the evolutionary and developmental changes in the cap binding translation initiation factors (LeishIF4Es).

Mitochondrial origin binding protein UMSBP mediates DNA replication and segregation in trypanosomes

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Kinetoplast DNA (kDNA) is the mitochondrial genome of trypanosomatids. Its major components are several thousands topologically-linked DNA minicircles, whose replication origins are bound by the mitochondrial protein UMSBP. The function of UMSBP has been studied using RNA interference (RNAi). Silencing of the *UMSBP* genes inhibited the initiation of minicircle replication, blocked nuclear DNA division and impaired the segregation of the kDNA network as well as cytokinesis, resulting in growth arrest. These observations revealed the function of UMSBP in kDNA replication initiation and segregation, as well as in nuclear mitosis, and imply its role in linking kDNA replication to nuclear S phase control. UMSBP activity is sensitive to the protein's redox state. The reduced form of the protein is a monomer, which binds the origin sequence, while its oxidation results in the protein oligomerization and inhibition of its DNA binding activity. UMSBP's redox state and its capacity to bind the origin sequence, cycles during the cell cycle, with peaks of activity during S and M-G1 phases, indicating that UMSBP's regulation *in vivo* is mediated through a cell cycle-dependent control of the protein's redox state. The possibility that UMSBP's redox state is controlled *in vivo* by an enzymatic mechanism, which catalyzes its direct reduction and oxidation, was challenged here in a multienzyme reaction, reconstituted with pure enzymes of the trypanosomal major redox-regulating pathway. Coupling of the reconstituted reaction to a UMSBP origin binding reaction, revealed the regulation of UMSBP activity through the opposing effects of tryparedoxin and tryparedoxin peroxidase. In the course of this reaction, tryparedoxin catalyzes the reduction of UMSBP, while tryparedoxin peroxidase catalyzes the direct oxidation of UMSBP, revealing a regulatory mechanism, based on enzymes-mediated reversible modulation of the protein's redox state. Abundance of similar enzymatic systems in various species, suggest that his mode of regulation may represent a more widely used regulatory mechanism, functioning as an enzyme mediated, redox-based biological switch.

Session 2: Leishmania (New targets for vaccines & drugs)

The leishmanization experience revisited

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Long before many of the advanced experimental vaccines were conceived to prevent leishmanial diseases, there was a long history of using the living virulent parasite. Over hundreds of years these were applied in a number of places and in a number of ways in order to produce a lesion which in itself would not be harmful. In the modern era, Russian, Israeli and Iranian investigators acquired considerable experience in applying and developing these techniques. Our own experiments in this field will be described including what we learned about parasite characterization and standardization of the procedure. Useful information was also derived in developing immunological tools to evaluate the process. Although Israel and Russia abandoned the procedure more than 20 years ago, Iran utilized leishmanization on more than a million subjects during the Iraqi – Iranian war. The lessons learned from these studies will be presented.

Modulation of vaccine-induced immunity against cutaneous leishmaniasis by vector sand flies

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The past 10 years has seen a substantial effort to develop a safe and effective vaccine against cutaneous and visceral forms of leishmaniasis. A variety of non-living, recombinant protein, and DNA based vaccines have been shown to confer protection in animal models. Autoclaved *L. major* antigen + CpG is a highly protective vaccine in mice following needle inoculation of parasites, yet a similar vaccine applied in a field setting has consistently failed to protect people against cutaneous leishmaniasis transmitted by sand fly bite. The only proven effective immunization strategy against Leishmaniasis in humans remains a controlled challenge with live, wild-type *L. major* , referred to as leishmanization. Remarkably, no vaccine has ever been evaluated by natural sand fly challenge under experimental conditions. We have recently refined our ability to efficiently transmit cutaneous leishmanisis to mice using infected sand flies, and have produced a clear set of data to indicate that the non-living vaccines that confer powerful protection against needle challenge are ineffective against sand fly challenge, whereas live-vaccination protects against both. Sand fly bites were associated with a massive recruitment of neutrophils to the site of parasite deposition in the skin. Employing a red fluorescent protein (RFP) expressing strain of *L. major* and two-photon

intravital microscopy, we were able to visualize parasite dependent neutrophil migration and subsequent parasite phagocytosis by neutrophils. The infected neutrophils were not apoptotic, contained viable organisms, and were able to initiate infection in mice as efficiently as metacyclic promastigotes. The findings support an infectious process in which neutrophils are the initial cellular target of metacyclic promastigotes, exploited as means of silent parasite entry into macrophages, and may explain the compromised expression of immunity in the vaccinated mice exposed to infected sand flies. The contribution of specific components of vector saliva, as well as the parasite derived, phopshoglycan-containing molecules egested by infected sand flies, to the recruitment and directed migration of neutrophils to parasites in the inoculation site, are being defined.

Opportunities for drug development in the post-genomic era: mining the *Leishmania* genome for novel drug targets

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In the absence of a vaccine, chemotherapy is the main means of control of leishmaniasis. However, since most current drugs are expensive, difficult to administer, and are facing increasing parasite resistance, there is an urgent need for new drugs. The availability of the genome sequence of several *Trypanosome* and *Leishmania* species provides unequalled opportunities for the identification of novel drug targets against the devastating diseases that they cause. We have identified two such targets. MIX is a protein in the inner membrane of the mitochondria, involved in kinetoplast and cell division and essential for parasite survival *in vivo*. Silencing of the MIX homolog in *T. brucei* by RNAi has demonstrated a similar and important function of MIX in this organism. The second target is a family of magnesium transporters (MgT1, MgT2) present in the parasite endoplasmic reticulum and important for virulence. These proteins show homology to bacterial CorA proteins, the major magnesium transporters and virulence factors in these organisms. Since MIX and the MgTs are uniquely kinetoplastid proteins absent in humans, and play a central role in the biology of these parasites, they make for very attractive drug targets.

Indirubins as potent and selective inhibitors of the protozoan parasite *Leishmania donovani*

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Indirubins known to target mammalian cyclin-dependent kinases (CDKs) and/or glycogen synthase kinase (GSK-3) were tested for their antileishmanial activity using the Alamar blue assay in a 96-well format. In the initial screening, sixteen indirubins were tested at the concentration of 10 μM. Nine of the compounds did not affect parasite growth even when used at 50 μM. 6-Br-indirubin-3'-oxime (6-BIO) was the most potent inhibitor of *Leishmania donovani* promastigotes and amastigotes growth (IC_{50} 0.8 μM). Promastigotes-treatment with 6-BIO induced a sub-G0/G1 phase accumulation and morphological/biochemical changes which lead to induction of cell death exhibiting characteristic features of apoptosis such as nuclear chromatin condensation, externalization of phosphatidylserine and fragmentation of genomic DNA. Since the 6-Br substitution on indirubin backbone greatly enhances the selectivity for mammalian GSK-3 over CDKs, we cloned and expressed in *E. coli* the *Leishmania donovani* GSK-3β homologue (*Ld*GSK-3β) which displays 49% identity and 68% similarity with human GSK-3β. *GSK-3β* genes in different *Leishmania* species were found to be almost identical. Western blot analysis using both a polyclonal anti-ratGSK-3β antibody, directed against the C-terminal sequence CAHSFFDELRDPNVK of rat GSK-3β, and a generated anti-*Ld*GSK-3β showed that the level of expression of the *Ld*GSK-3β in *L. donovani* logarithmic and stationary-phase promastigotes was comparable. Immunostaining of *L. donovani* promastigotes revealed that *Ld*GSK-3β is localized in the cytoplasm and flagellum whereas a more condensed and localized staining was observed in the cytoplasm of axenic amastigotes. The kinase activity of *Ld*GSK-3β was assayed using GS-1, a peptide derived from the GSK-3 phosphorylation site of mammalian glycogen synthase, in the absence or presence of a range of concentrations of indirubins. 6-BIO, 6-Br-indirubin-3'acetoxime, 5-Br-indirubin-3'oxime and 6-Br-5-methyl-indirubin-3'-oxime turned out to inhibit *Ld*GSK-3β at μM concentrations (IC_{50} values 2-3 μM). Since the indirubins tested were more active in cell-based assays than towards *E. coli Ld*GSK-3β, we have generated transgenic *L. donovani* promastigotes overexpressing *Ld*GSK-3β (increased by approximately 2-fold) and compared the susceptibility of control and *Ld*GSK-3β transfectants to 6-BIO. Its activity was almost 2 fold higher in the control transfectants, their respective IC_{50} been of 0.5±0.02 μM and 0.9±0.02 μM. The moderate activity of these compounds towards *Ld*GSK-3β suggested that they may target CRK3, the CDK1 homologue in *Leishmania*. Indeed 6-BIO was at least 10 times more active against CRK3, purified from transgenic *L. mexicana* parasites (kindly provided by Dr. Grant), suggesting that the observed selectivity of 6-Br indirubins for mammalian GSK-3 over CDKs is not valid in *Leishmania*. Structural studies confirm the level of homology of *Ld*GSK-3β with human GSK-3β but most importantly predict the existence of functional/structural differences in their active site that are sufficient to explain the lower inhibitory activity of these compounds towards *Ld*GSK-3β. Thus, the theoretical models of *Ld*GSK-3β as well as that of CRK3 open up the possibility of designing *Leishmania*-selective indirubins targeting both *Ld*GSK-3β and CRK3.

Session 3: Leishmania (New drug development)

The US Military Infectious Disease Research Program (MIDRP) Leishmaniasis Research Program Update: The Therapeutics Portfolio

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Following the US lead military invasion of Iraq in March 2003, the US military began seeing cases of cutaneous leishmaniasis (CL) in the fall of 2003. The vast majority of these cases were typical CL caused by zoonotic *L. major*. The sudden increase in cases led to the re-establishment of a an active research and development program in the US military focused on improved vector control measures (Program Area U) and improved diagnostics and therapeutic agents for case management (Program Area P). The current status of the case management Program Area P will be presented to include: 1) a point of care, non-microscopic dipstick diagnostic device, 2) improved field friendly PCR platforms, 3) An interferon gamma release assay to detect asymptomatic infections, 4) status of a Leishmania skin test antigen, 5) drug discovery and development, 6) topical paromomycin 7) new oral drugs for the treatment of severe or complicated leishmaniasis, and 8) status of our efforts to get sodium stibogluconate approved by the US FDA. An overall view of the worldwide status of diagnostic test and drug discovery directed at CL and where the US military program can contribute to the known capability gap will be reviewed.

Clinical Development of Topical Paromomycin Cream for the Treatment of Cutaneous Leishmaniasis

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Cutaneous leishmaniasis (CL) is a disfiguring parasitic disease that occurs throughout the Middle East and the Mediterranean and in the Americas from Texas to Argentina. More than 90% of the world's cases of CL are in Iraq, Iran, Afghanistan, Syria, Algeria, Saudi Arabia, Brazil and Peru, and it is a common infection among US personnel working in these regions. Current standards of care are often costly, require repeated intravenous injections, and result in thousands of lost work hours. In the United States, the standard treatment often involves administration of the non-FDA-approved drug Pentostam™, under investigational status. Unfortunately, Pentostam™, an organo-antimony based drug, has a significant toxicity profile, and is usually not an appropriate therapeutic choice for treating non-complicated CL. The United States Army Medical Research and

Materiel Command, in partnership the Institut Pasteur, Paris and the Institut Pasteur of Tunis, along with the commercial pharmaceutical manufacturer Teva, U.S.A., is currently developing WR279396, a topical preparation containing paromomycin and gentamicin, as a new first line chemotherapeutic drug for non-complicated CL that may be easily used and self-administered. Clinical development of this combination topical cream necessitates the coordination of multinational efforts in order to meet regulatory requirements for drug approval in the US, Europe, and Tunisia, the sites of all the clinical trials. Phase 1 and 2 trials are completed and Phase 3 trials are due to begin by the end of 2007. WR279396 offers great potential as a new simple, easily applicable, and inexpensive topical therapy for this neglected disease.

Immune intervention in leishmaniasis

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A variety of balanced immune responses are needed to overcome leishmanial infections. These include: an appropriate cytokine response; development of CD41 Th1 cells that promote effective cell-mediated immunity, activation of macrophages by interferon-□; and a consequent production of reactive oxygen intermediates, nitric oxide (NO) and tumor necrosis factor-□. Both therapeutic and immunological interventions can affect the outcome of leishmanial infections. Chemotherapy may alleviate leishmaniasis by reducing the parasite load, by changing the leishmanial antigens exposed to the immune system, thus altering cytokine cascade, and by affecting the immune response directly. For example, parasite components reduce the oxidative burst and apoptosis of host cells, prolonging parasite survival. Similarly, a widely used curcumin acts as an immunosuppressor, which reduces host NO synthase and NO, which in turn supports parasite development. In contrast, injection of immune stimulators like IL-12 and CpG enhances Th1 responses and consequently reduces leishmanial infection. Likewise, another food additive, *Sambucol* extract, elevates inflammatory cytokines and slows in vivo development of *Leishmania major* in *Balb/C* mice. Amphotericin B, a first line anti-leishmanial drug, kills the parasites directly by interfering with ergosterol metabolism. However, its role as an immunomodulator is also expressed by affecting the parasites within the host cell and by various degrees of in vivo toxicity, especially in the kidneys. This toxicity may be moderated without affecting the anti-leishmanial activity by coupling AmB to various carriers. AmB is slow- released from the prodrug so that enough active compound is available to prevent parasite development without inflicting toxic effects. In conclusion, an anti-leishmanial drug may be more effective if it a. acts directly against the parasite, b. it is not toxic and c. it induces an optimal Th1 protective response. However, the effect of such a drug on other immunopathological diseases should be considered in view of possible concomitant infections.

Leishmania major: Characterization and anti-leishmanial activity of Israeli plant extracts against promastigotes and amastigotes

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Introduction: In this study we examined the anti-leishmanial activity of extract made from plants collected in Israel on the development of *Leishmania major* promastigotes and amastigotes *in vitro* in culture and *in vivo* in experimentally infected mice.

Objectives: To isolate and to characterize specific plant compounds with anti-leishmanial activity.

Study Design: 50% methanolic extracts were prepared from the leaves and stems of plants, collected at natural habitats, in Israel. In certain cases further 2 extraction procedures have been performed, including: a) methanolic treatment, followed by dichloromethane extraction at acidic and basic conditions. b) Water extraction, boiling at acidic condition, followed by xylene treatment at acidic and basic conditions. The anti-leishmanial activity of the crude and the partially purified compounds against promastigotes was determined using colorimetric method (MTT). The effect on intracellular amastigotes in macrophages was determined microscopically, before and after Gimsa's staining. The anti-leishmanial activity was also determined *in vivo*, in Balb/c mice. The efficacy was determined by measuring the lesion size and by the demonstration of the parasites in smear and culture made from the edge of the lesion.

Results: Of the 57 plant extracts examined, belonging to 28 different families, 10 were highly effective against *Leishmania* promastigotes and amastigotes. None of these plants totally eliminated the intracellular amastigotes, except one (AG) that was almost as effective as paromomycin - the gold standard drug. AG was found to be an alkaloid(s) of <14,000 kDa, thermo-stable and resistant to methanol, dichloromethane and xylene treatment. The IC_{50} for promastigotes and amastigotes of the partially purified compound was 2μg/ml and 0.21μg/ml, respectively. A total elimination of the intracellular amastigotes (with no toxicity to the macrophages) was achieved with AG at 8μg/ml, within 3 days of treatment. AG administrated into Balb/c mice caused a delay in parasite development, and consequently affected the mice survival. A synergistic effect of AG combined with paromomycin on intracellular amastigotes was further demonstrated.

Conclusions: High anti-leishmanial activity was demonstrated with 10 of the plants examined. One of them (AG), that caused a total elimination of the intracellular amastigotes (IC_{50}=0.21μg/ml) *in vitro*, was also partially effective *in vivo* in experimentally infected mice. Additional studies will be required for further analysis and evaluation of the potential therapeutic use of the results.

Liposomal amphotericin B (AmBisome) for the treatment of cutaneous leishmaniasis due to *Leishmania braziliensis*

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Background: Travel to Latin America has become increasingly common, including to the rural and jungles areas that are endemic to New World leishmaniasis, including the *Leishmania viannia* complexes. Almost all leishmaniasis cases among Israeli travelers were acquired in the Amazon Basin in Bolivia where the endemic species is *L. (V.) braziliensis*. Treatment of cutaneous *L. (V.) braziliensis* infection is with systemic pentavalent antimonial compounds (SSG), at a dosage of 20 mg/kg for three weeks. In our experience, this course is effective in achieving clinical cure in 75% of cases. For the failure cases, we assessed treatment with a liposomal amphotericin compound (AmBisome).

Methods: A prospective evaluation was performed, for cutaneous leishmaniasis due to *L. braziliensis*, proven by PCR. A dose of 3 mg/kg AmBisome was given for 5 consecutive days, with a 6^{th} dose on day 10. All doses were given in an outpatient setting.

Results: 13 consecutive patients received this treatment. All were returned travelers, most of them (12/13) infected in the Amazon region of Bolivia. 9 were males and 4 female, their mean age was 24.8 years. Five cases were failures of a full course of SSG; and 8 patients had the AmBisome treatment for primary lesions. None had mucosal lesions. All received the same schedule and achieved a complete cure within less than 1 month. Mean follow up of 12 months (range 6- 16 months) revealed no relapse. Side effects were mild, and no one had to terminate treatment prematurely. A comparison of the cost of treatment for AmBisome vs. SSG shows that despite the high cost of AmBisome, the expense for the total treatment with AmBisome is less than with SSG: 45% less if SSG was given in an inpatient setting; 15% less when SSG was given in an outpatient setting.

Conclusion: The efficacy of AmBisome in *L. braziliensis* infection has never been tested systematically. Anecdotal reports reported mixed results in different regimens. Our experience, although is in limited number of patients, was very successful, well tolerated and demonstrates the potential of this drug in curing *L. braziliensis*. The major drawback of using it, is its high cost. However, the significant reduction in hospitalization days (inpatients or outpatients) and accessory blood tests required with antimonial treatment makes AmBisome treatment more cost effective. These results should encourage the continued evaluation of this agent for relapse and for primary infection of *L. (V.) braziliensis*.

Session 4: Leishmania (Vector biology and transmission)

Stage-specific attachment of *Leishmania* promastigotes to the gut epithelium in permissive vectors

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Attachment of *Leishmania* promastigotes to the midgut epithelium of their sand fly vectors is an essential part of the life cycle. Whilst many parasite species can undergo a limited period of development in a blood meal for a few days, only true vectors can support attachment and proper establishment of the parasite. Such attachment enables the promastigotes to avoid expulsion from the gut when the remains of blood meal digestion are defecated by the female fly. This establishment phase is also an essential pre-requisite to subsequent development of mature transmissible infections in the anterior midgut and, therefore, continuation of the life cycle. Attachment occurs when promastigotes insert their flagella into the microvillar border of the midgut epithelium and anchor themselves to the gut wall. Given the essential role that establishment plays in the life cycle considerable effort has been invested in determining the underlying mechanisms, both from the standpoint of basic science and as a potential transmission blocking strategy. The paradigm for these studies has been the work performed with *Leishmania major* in *Phlebotomus papatasi*. A series of elegant studies performed mainly by Sacks, Turco, Beverley, Kamhawi and colleagues began with the identification of lipophosphoglycan (LPG) as the parasite surface molecule involved in midgut binding and culminated in the recent identification of a corresponding galectin receptor (PpGalec) in *P. papatasi*. PpGalec possesses carbohydrate binding domains and serves as the midgut receptor for LPG galactose ligands on the promastigote surface. In other species the role of LPG in attachment has not been investigated in the same detail as in the *L. major*/*P.papatasi* system. LPG has been found on the surface of all *Leishmania* species, including *Viannia*, and has functions in addition to midgut attachment, for example mediating complement resistance of metacyclic promastigotes. However, recent work has indicated that LPG may not be involved in attachment in certain parasite vector-combinations, possibly the majority, in particular the permissive vectors that can act as experimental hosts for a range of *Leishmania* species. We have begun to investigate this alternative mechanism by developing a new sand fly gut binding assay using fluorescently labelled parasites. This has shown that binding is stage-specific, with procyclic promastigotes and metacyclic promastigotes unable to bind to the epithelium, whereas nectomonad and leptomonad promastigotes can bind. A knock-out strategy is currently being employed to identify the parasite receptor responsible for binding in these vector hosts. Understanding this mechanism will be of fundamental importance in explaining vector specificity, but also in practical terms as permissive vectors have the potential to establish parasites in new epidemiological situations. This appears to have already happened in at least one very important case: the establishment of visceral leishmaniasis in Latin America.

Transmission cycles of cutaneous leishmaniasis in the Galilee, Northern Israel

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The ecology and transmission of cutaneous leishmaniasis (CL) caused by *Leishmania tropica* were studied extensively in two adjacent foci in northern Israel. The sand fly fauna in the two foci was not identical, *Phlebotomus sergenti* comprising over 90% in the southern focus (Sf) on the outskirts of Tiberias and only 30% in the more northerly focus (Nf) comprising the villages of Amnun, Karkon and Korazim. *P. arabicus* and *P. simici*, two prominent species in the Nf were absent from the Sf. Infections with *L. tropica* were detected in *P. arabicus* (7%) and *P. sergenti* (1%) from the Nf and only *P. sergenti* (10%) from the Sf. Laboratory studies showed that *P. arabicus* is a more competent vector of *L. tropica* and that *P. sergenti* is totally refractory to infection with *L. tropica* from the Nf. High susceptibility of *P. arabicus* is probably mediated by abundant corresponding specific glycosylation of proteins on the surface of its midgut epithelial cells. Rock hyraxes were incriminated as reservoir hosts with similar infection rates in both foci (~10%). Other mammals studied were not infected. *L. tropica* from sand flies, humans and hyraxes from the Nf were antigenically like many strains of *L. major*, whereas strains from the Sf were antigenically closer to all the other strains of *L. tropica* studied until now. The adjacent foci differ with respect to the circulating parasite and vector but burgeoning peri-domestic hyrax populations seem responsible for the emergence of CL in both.

An outbreak of the cutaneous leishmaniasis in Tiberias – An epidemiological approach for planning and evaluation of the intervention toward control of the disease

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In the years 2000 -2003 there was an outbreak of cutaneous leishmaniasis in Tiberias, northern Israel. The disease incidence rose from 0.5-0.7 per 100,000 in the 1990's to 60.0 per 100,000 in 2003. A geographical mapping of the disease indicated two foci at the periphery of the city. In cooperation with various regional services an intervention plan was put into action to control the disease outbreak. This program included three essential components: 1) Reduction of the population of hyrax, which were identified as disease reservoir, together with the destruction of their habitats; 2) A spraying campaign against the sand fly (the disease vector); 3) A campaign to educate the public and the health workers concerning the disease. Following introduction of the program, there was a significant morbidity

reduction. The disease incidence rate in the effected neighborhoods fell from 133.0 per 100,000 in 2003 to 44.4 per 100,000 in 2004. In the conference the epidemiological analysis of the outbreak will be presented together with presentation of control measures, as developed by Ministries of Environment and Health, the Local Authority and Nature and Parks Authority. Similarly, evaluative components of the plan will be presented including: specific disease incidences, the GIS maps and the results of the survey used to measure the level of satisfaction, knowledge and attitudes amongst community pupils, trained as local area health agents.

Perspectives of sand fly control in the Judean Desert

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Phlebotomine sand flies (Diptera: Psychodidae) are vectors of leishmaniases and also pose a nuisance biting burden. There is no prophylactic vaccine available for leishmaniasis. Personal Protection Measures fail for a number of reasons including user acceptability and proper product deployment. Treatments of wild reservoir animals might induce severe environmental implications and must be assessed. In the Judean Desert, transmission of *Leishmania tropica* involves the vector *Phlebotomus sergenti*, which is found in large numbers in the vicinity of houses but only seldom inside homes. Under these circumstances, outdoor sand fly vector control must be employed for preventing disease transmission. Outdoor sand fly control in Israel involves spraying large quantities of residual insecticide on house walls and adjacent surfaces. The aim is to create a barrier zone that prevents sand flies from reaching the vicinity of man. The efficacy this method, depends on the length of time and the dose to which the flies are exposed. High summer temperatures, UV radiation and blowing dust reduce insecticidal efficacy. Coverage of large areas and repeated applications should theoretically compensate for the low residual concentrations that remain. On the other hand achieving adequate coverage or long-lasting residual efficacy is difficult, expensive and endangers people, non target animals and the environment. Since 2005 the Ma'ale Adummim municipality has been employing residual insecticide spraying on the circumference garden rockeries and walls for sand fly control. In small controlled field experiments we tested the potential residual efficacy of several insecticides. The results of both large scale treatments and controlled experiments will be presented. The results include insights into sand fly behavior that affect efficacy. Specifically sand fly behavior in relation to treated surfaces, frequency and duration of contact, and possible avoidance of exposure to lethal doses. In order to improve the efficacy of outdoor residual sand fly control we intend to employ poisonous sugar baits combined with surface spraying. The combined control methods are designed for simple, quick and easy implementation bringing about immediate protection against biting sand flies and long term effective reduction in sand fly populations. Ultimately the combined methods will minimize the area dimensions that require residual insecticide spraying,

leading to a reduction in overall cost as well as a reduction in the potential harm to the environment.

Spatial analyses to improve understanding of infectious disease epidemiology

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Infectious diseases are non-randomly distributed in space and time because transmission is influenced by variation in patterns of contact, environment, susceptibility, seasonality, and other factors. Analyses of space-time patterns of infection and disease are increasingly beings used to develop insights into risk factors and foci for intervention. Tools such as GIS, cluster analysis, and space-time statistics are being regularly applied to epidemiologic studies of infectious diseases. The ways in which these tools can be used to develop and test hypotheses or design treatment and prevention will be discussed. Examples of specific studies will be presented to illustrate the opportunities and challenges that these analyses offer. The implications for future research design and inference will be addressed, as well as what this might imply for research strategies and policy.