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Post-Genomic Research on Leishmaniasis:

a critical self appraisal

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Abstract

Scientific conferences, a major feature of academic life, rarely provide the opportunity for self appraisal of a research field. During a recent meeting on leishmaniasis research in the post-genomic era, ~60 researchers participated in group discussions that aimed to provide a critical self-appraisal of the state of the field and to highlight major roadblocks likely to prevent translation of new research into tools for leishmaniasis control. These discussions demonstrated a surprising concordance of views and highlighted a number of critical areas for future development.

Leishmaniasis - a major public health problem

Leishmaniasis remains one of the major burdens on human health in developing countries, with the WHO recently classifying leishmaniasis as a Category I: Emerging or uncontrolled disease [1,2]. Leishmaniasis is distributed world-wide, in 88 countries, and an estimated 1.5-2.0 million children and adults develop clinical leishmaniasis every year, though many more subclinical infections go un-recorded. 75% of clinical cases affect the skin (cutaneous leishmaniasis; CL) and the remaining 25% represent systemic and potentially fatal visceral leishmaniasis (VL, also known as Kala-azar). 90% of visceral leishmaniasis cases occur in India, Bangladesh, Nepal, Sudan and Brazil, with 70,000 or more deaths reported annually [1,2]. It is widely recognized that this figure is a gross under-estimate and might represent only one fifth of the true death toll. Among parasitic infections, only malaria kills more people. In addition, leishmaniasis is in the top ten parasitic diseases for its impact on socio-economic development, with a burden of 2.4 million DALYs (disability adjusted life years; http://www.who.int/whr/2002/en/whr02_en.pdf). Increasing overlap with the spread of AIDS has heightened the threat of HIV / Leishmania co-infections, particularly in India and East Africa [3].

Genomes, proteomes, microarray analysis, single nucleotide polymorphisms (SNPs) and an increasing ability to manipulate both parasite and experimental hosts genetically all promise to revolutionize the next decade of research on leishmaniasis. However, translating the output from this new scientific utopia into meaningful measures for disease control will be one of the greatest challenges for the research community. During a recent international conference on leishmaniasis*, an attempt was made at critical self-appraisal of the

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molecular, cellular and genetic aspects of current leishmaniasis research, to obtain a grass-roots impression of the potential roadblocks affecting the development of new drugs and immune-based interventions. Evaluation was made using SWOT analysis (Box 1) and the results are presented in Figure 1. This article represents an expansion of some of the major points raised. We hope that it will focus the attention of funders to some of these issues and also may provide impetus for those working in other areas of parasitology to undertake a similar analysis of their own field of research.

Data sharing and resource management

The publication of the comparative genome sequences of *Leishmania* species responsible for cutaneous, mucocutaneous and visceral leishmaniasis [4], together with that of other trypanosomatids [5-8], represented the beginnings of an information explosion unparalleled in the history of leishmaniasis research. The sequencing of 26 additional kinetoplastid genomes is planned by the National Human Genome Research Initiative (<http://www.genome.gov/>). An extensive genetic toolkit [9], and the future prospects of RNAi [10] and methods for regulated gene expression [11], allows an almost unlimited capacity to generate novel transgenic lines of *Leishmania*, each meriting their own phenotypic and genetic analysis.

The first priority faced in this new research environment is the establishment of centralized data management and integrated tools for bioinformatics, e.g. by extending and improving the existing Wellcome Trust Sanger Institute's GeneDB database (<http://www.genedb.org/>) and by increased cooperation with other pathogen databases, e.g. the NIH-funded ApiDB / EuPathDB (<http://eupathdb.org/eupathdb/>), and the WHO / TDR drug target prioritization database (www.TDRtargets.org). These linkages are planned or in place, as is an initiative to determine SNP variation across a large number of fresh field isolates, and to incorporate these data into GeneDB. The merging of these different kinds of data repositories, underpinned by different architectures, will also facilitate seamless public domain access to data sets emerging from microarray, proteomic and metabolomic analysis, as well as future inclusion of phenotypic data, ranging from cell biological analysis to immune response parameters. Integration of vector genomes will provide further opportunity for exploitation.

The second priority, with its own set of inherent difficulties, is to create lasting, carefully-curated parasite strain repositories. Studies with established culture-adapted lines, new mutants and new clinical isolates will all benefit from the ability for independent cross-laboratory verification of phenotypes, and from the potential to assess parasites across multiple laboratory-specific analytical platforms. Limited *Leishmania* repositories do exist in London (debbie.nolder@lshtm.ac.uk), Montpellier (parasito@univ-montp1.fr) and Rio de Janeiro (<http://www.fiocruz.br>), but these might require substantive funding to meet an increasing scientific demand. Quality control will be high on the agenda for any successful cell line repository, as will be ease of strain distribution across international boundaries. Parasite typing is becoming increasingly complex. Typing of glycoconjugates is still in its infancy, and glycome initiatives would be a welcome addition to the molecular armament (<http://www.glycoarrays.org.uk/>). Biological contamination of lines may range from *in vitro* acquired mycoplasma to the contamination of *in vivo* passaged lines with adventitious pathogens of rodents. Thus, to make any future expansion of repositories a meaningful proposition, a rigorous policy for submission will need to be adopted, akin to that used by other established banks such as the American Type Culture Collection (ATCC).

* 'Dormy House II: Integrated functional genomics on the road to leishmaniasis control' was held at the Dormy House Hotel, Broadway, UK, 24-27 September 2007.

Along with data and resource sharing, there is likely to be an increased need for standardization among the research community. Although it is well-recognized that laboratories retain local 'tricks of the trade', clear bench marks need to be established to assist in the development and implementation of drug screening programs and vaccine identification. As yet, no clear guidelines exist as to the conditions under which a drug should be validated or vaccine efficacy evaluated. Depending upon source or culture conditions, macrophages used to establish *in vitro* amastigote drug screening assays may vary widely in their activation status, a key parameter for the action of some anti-leishmanials [12]. Given the limited global resource and the high costs associated with drug and vaccine development, true candidature as a potential target should rest on fulfilling certain minimal criteria. Examination of previous attempts to independently evaluate vaccine candidates exemplifies some of the problems that otherwise occur (<http://www.who.int/tdr/publications/tdrnews/news65/leishmaniasis.htm>)

Disease Models

Murine models have underpinned many key discoveries on the pathogenesis of leishmaniasis. Studies on natural resistance genes in mice led to the discovery of genes controlling the outcome of human VL [13-15], common immune effector mechanisms have been identified and a detailed analysis of the cellular immune response to *Leishmania* in mice has helped shape analysis of the human immune response [16]; parasite genes involved in tissue tropism have been discovered [17]; and new drugs have been identified that are now in clinical use [12].

Nevertheless, justifiable concerns exist on the appropriateness of models to predict the response to drugs and vaccines, in humans and the relevant animal reservoirs of disease. Vaccines targeting solely T helper cell type 1 (Th1) immunity, as initially championed on the basis of murine studies, have proved far from effective at inducing protection in non-human primates and in clinical trials [18] (although arguably this failure also reflects a generic difficulty in attaining sufficient levels of immunogenicity in man with the available adjuvants and priming regimes). Early models of leishmaniasis poorly reflected the natural dose and site of infection (and thus obtained a distorted view of the major immune system players [19]), and even less so the contribution that sandfly products [20], or parasite products co-inoculated during transmission [21], might play in assisting parasite establishment and immune modulation. Murine models of VL are limited in not reaching a fatal conclusion, whereas the hamster fails to recapitulate the host protective response made by most individuals infected with visceralising *Leishmania* [18, 22]. For some important forms of leishmaniasis, including mucocutaneous leishmaniasis and post Kala-azar dermal leishmaniasis, no models are currently available.

How might this change in the future? Analysis of the early immune response clearly needs to be fully inclusive of the biology of transmission - though our understanding of this remains incomplete. Filamentous proteophosphoglycan (fPPG) [21] and salivary components [20] may contribute to disease establishment in some vector-parasite-host combinations, but the generality of these effects is far from clear [23]. The activity of some components may be negated by naturally acquired immunity (although conversely such immunity may point to potential targets for vaccinations [24]). Extending these arguments to suggest that all experimental vaccine studies require sandfly challenge would currently pose severe constraints on the number of laboratories involved in such research, unless a centralized resource was available. At the end of the day, models are models, and there may be dangers of over accentuating traits perhaps only associated with a limited range of endemic foci.

Although there is scope for assessing the human immune response to sandfly products and sandfly bite in normal volunteers, formal challenge experiments pose difficulties. Hence, a question faced here (and by no means uniquely for researchers of leishmaniasis) is whether the static *in vitro* 'model' involving human peripheral blood mononuclear cells is more relevant than the physiologically intact and dynamic model that rodents provide? A similar argument may also apply to VL, where immune responses of consequence occur within systemic organs [25], in specialized microenvironments that are currently hard to mimic *ex vivo*. Murine models and *in vitro* culture systems can and should be improved, but the more important issue is based on what premise? Here, a clear emphasis needs to be on using clinical data to refine these models, rather than allowing refined models to set a new and potentially inappropriate clinical agenda.

Enhancement of clinical research activity

Expansion of clinical research activity is therefore widely regarded as fundamental on a number of levels. Single cytokine analysis has failed to deliver a correlate of immunity [26] and the proposition that poly-functional T cell responses will serve better [27] needs to be validated. Tissue remodeling accompanies all forms of clinical leishmaniasis and underlies the curative process. Indeed, some of the most encouraging results in experimental vaccination have been obtained using adjuvant combinations that induce granulomatous nodules at the site of vaccination [28]. Such observations suggest that further examination of local lesion pathology may provide new clues to correlates of infection not demonstrable in peripheral blood [29, 30]. How can we move forward to share clinical data and resources across the research community? Pathology services are being networked across the UK and elsewhere and electronic image capture and web-based information systems could likewise be applied to link leishmaniasis research centers in developing and developed countries, overcoming the current limitations on the transport of clinical material. Such an enterprise might cement clinical and non-clinical links, drive model development and, with appropriate engagement of local pathologists, allow valuable clinical material to be examined by a broad range of investigators.

'Public' awareness

Two areas of 'public' engagement raised concern for the future. First, the need was identified to further engage young scientists and aspiring academic clinicians in research on leishmaniasis, and thus to counter the inevitable attraction of higher profile diseases such as tuberculosis, malaria and HIV. It is difficult to quantify precisely the effect that disproportionate funding may have on a field of research, but there is no doubt that leishmaniasis, along with the other major neglected diseases, receive a minor slice of the funding available for infectious disease research. The promise of high-profile, high investment science is a strong attraction and with many new initiatives targeting the global 'big three' (HIV, malaria and TB), there is the perception that opportunities as an independent investigator will be more limited outside rather than within the fold.

The second issue identified was how to sell leishmaniasis research as being full of opportunities. International agencies may play a significant role here, but there is also a degree of self-promotion required by the research community that has perhaps been lacking in the past. Limited data on the economic importance of leishmaniasis are available [31], yet leishmaniasis is recognized as being one of the top ten diseases associated with impaired economic development [1]. Additional studies, in addition to further cost-benefit analysis of new interventions [32,33] are required to convince of the future benefits of leishmaniasis control. Additional benefits for Pharma's engagement in leishmaniasis research could be highlighted. Immuno-chemotherapy trials for leishmaniasis might be conducted in a fraction of the time taken for other diseases, and might allow proof of principle for drugs under

investigation for other diseases such as cancer. Similarly, therapeutic vaccine trials may give increased opportunities to explore the potential of novel vaccine delivery systems, again with relatively rapid results.

The way forward

Here we have focused principally on the broad issues that emerged at the Dormy House meeting, particularly as discussed in the SWOT sessions. Intriguingly, many of the key issues discussed were remarkably close to the core issues that arose at the first Dormy House meeting held in 1985 [34]. The difference now is how those issues can be addressed with all the additional resources of the post-genomic era. Many exciting novel research data were presented at the 2007 meeting which are not reported here as participants were encouraged to share their unpublished data. However, there was a sense that the ‘-omics’ were already making their mark on leishmaniasis research, and that the research community is now better-placed than ever before to achieve the ever-elusive targets of a new drug or a vaccine being delivered ‘in the next 5-10 years’.

Box 1 SWOT analysis

SWOT stands for strengths, weaknesses, opportunities and threats. SWOT analysis is a common management tool used to perform a subjective analysis of an organization (in this case, leishmaniasis research activity) and its relationship to the environment (here, loosely viewed as the objective of reducing burden of disease). The outcome of a SWOT analysis can be used to formulate future objectives. Three SWOT analyses were conducted under the headings: ‘parasite biology’, ‘host-pathogen interactions’ and ‘intervention. For performing the SWOT analysis, the 60 conference delegates, ranging from young investigators to leaders in the field (see Supplementary Table 1) were allocated to 5 groups, each with a chair and rapporteur. Allocation to groups was based on main research interests (experimental immunology, pathogen molecular biology, parasite biochemistry and cell biology, genetics and epidemiology, and vaccination and other interventions). Given the broad expertise of many delegates, these allocations were of necessity somewhat contrived! By subject specific allocation in this way, we hoped to identify any bias of opinion resulting from self-interest. Happily we can report that little was evident!

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

SWOT analysis on current leishmaniasis research. Discussion groups split their time discussing leishmaniasis research in the context of (a) pathogen biology, (b) host-pathogen interactions and (c) interventions. Abbreviations: IR, immune response