MINI-CONFERENCES

[November, 2006-11-06 - 18h00 - ROOM A]

MC01 - Leishmania vaccines: Where are we?

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Leishmaniasis causes significant morbidity and mortality worldwide and is an important public health problem. Several species of *Leishmania* cause human diseases that range from self-healing cutaneous lesions to fatal visceral leishmaniasis, mucosal leishmaniasis and diffuse cutaneous leishmaniasis. In some cases, the only possible cure for this disease is drug treatment, and prolonged use of such drugs has lead to parasite drug resistance and toxicities associated with chemotherapy. From the immunological features associated with resistance to *Leishmania* infection, there is a general agreement that the establishment of a protective anti-*Leishmania* immune response in mice requires the presentation of appropriate antigens by antigen-presenting-cells, the induction and expansion of CD4+ T helper 1 (Th1) lymphocytes, and the activation of macrophages for efficient killing of the parasites. In humans, there is a good correlation between Th1 responses and resistance to cutaneous leishmaniasis. Therefore, it is accepted that effective vaccination against *Leishmania* infection depends on the generation of parasite-specific, long-lesting memory T cells that, following exposure to infecting parasites, rapidly expand as effector Th1 T cells for production of IFN-g. In this way, most of the recent research in screening and development of antileishmanial vaccines has addressed the elicitation of such favorable cytokine phenotypes in *in vivo* models. Despite all this knowledge, to date, there is no effective vaccine against *Leishmania* in routine use anywhere in the world. Several vaccine preparations are in more or less advanced stages of development and testing. Many vaccine strategies have been pursued, including the use of the whole cell lysate, killed, avirulent/attenuated, or irradiated parasites. Recent advances in the ability to manipulate the *Leishmania* genome by introducing or eliminating genes has the potential to make live-attenuated vaccines much more feasible. Additionally, DNA vaccines and purified recombinant parasite antigens with different adjuvants have also been tested. Most of these strategies have shown some degree of effectiveness in animal models but little or no protection in humans. However, some *Leishmania* antigens have been identified and characterized that might be potential vaccine candidates, called second-generation vaccines. The most recent alternative is the vector-directed vaccine approach. The saliva of blood-sucking sand fly, vector of *Leishmania*, contains a varied repertoire of molecules that modulates their hosts′ hemostatic, inflammatory and immune responses. In animal models, these molecules seem to exacerbate *Leishmania* infection and may, in fact be mandatory for establishment of the parasite in the vertebrate host. . Thus, high-throughput analyses of salivary components have shown specific peptides that could be used for vaccine development. Humans, hamsters and mice pre-exposed to sand fly saliva or purified proteins developed a DTH response at the site of the insect bite and antibody production that can be useful as an epidemiological marker. The hosts′ salivary exposition also decreased *Leishmania* infection in experimental models, but this not necessarily confers immunity to the parasite itself or prevent subsequent disease progression. Finally, development of an anti-Leishmania vaccine has proven to be a difficult endeavor. Use of several antigens, selected through diverse approaches, did not achieve an acceptable level of protection. It is possible that insufficient knowledge of *Leishmania* pathogenesis and of reliable surrogate markers of protection represents the main root of repeated failures. A concerned effort in these fields is urgent and probably could offer some, albeit cautious, optimism about eradication of leishmaniasis worldwide.

[November, 2006-11-06 - 18h30 - ROOM A]

MC02 - The Changing Link between Mitosis and Cytokinesis in Trypanosome

Wang, C.C *(University of California, San Francisco)*

When cell cycle progression in Trypanosoma brucei is blocked by knocking down the expression of cyclins or cyclin-dependent kinases, a substantial percentage of the cells in procyclic form continues with cytokinesis and cell division, resulting in formation of annucleate cells (zoids). But in the bloodstream form, a mitotic arrest is accompanied by a complete blockade of cytokinesis without zoid formation, albeit leaving an un-inhibited progression of the kinetoplast cycle. The arrested mitosis in bloodstream form does not totally stop the nucleus from entering a new G1 phase, which leads to the formation of a large nuclear aggregate. Among the protein kinases known to coordinate mitosis with cytokinesis in the eukaryotes, polo-like kinase plays little role in controlling mitosis in trypanosome. It regulates primarily cytokinesis and localizes to the flagellum attachment zones, which could form the cleavage furrow during cell division. Aurora B kinase regulates mitotic spindle formation, chromosome segregation and initiates cytokinesis in both forms of trypanosome. A knockdown of its expression in the procyclic form inhibits mitotic spindle formation, blocks mitosis and cytokinesis but maintains two widely segregated kinetoplasts suggesting an arrest at the stage of cytokinetic initiation. But in the bloodstream form, depletion of aurora B kinase results in a large nuclear aggregate with multiple kinetoplasts, basal bodies and flagella, while the cell body swells quickly to a round shape filled with microtubules. Apparently, the mechanisms in coordinating mitosis with cytokinesis differ significantly between the two developmental stages of the same organism, T. brucei. It provides not only a rare opportunity for dissecting these distinctive mechanisms but also a chance for anti-African sleeping sickness chemotherapy.

[November, 2006-11-07 - 11h00 - ROOM A]

MC03 - Exploration and Exploitation of Parasite Genomes: Integrating Computational and Wet-Lab Biology

Roos, D.S. *(University of Pensylvania)*

With the emergence of large-scale datasets – from genome and transcript sequences, to RNA and protein expression profiles, to interactome and metabolic pathway maps, to population polymorphisms and comparative genomic analysis – computational tools have joined the spectrum of experimental techniques available to the research biologist. It is increasingly possible for *in silico* research to complement studies at the lab bench, and vice-versa.

The rise of computational biology poses several challenges, however. First, how to effectively capture, maintain, update, annotate, integrate, and query these resources? Genome database development is a challenge for any organism, but the difficulty is in some ways less acute for parasites, because the driving motivation is often to identify prominent targets that distinguish the pathogen from the host. Second, how to ensure effective access to genomic-scale datasets? Unless the underlying information is electronically-accessible, these data cannot be considered as truly published. How to provide prompt release, in archival formats, while ensuring appropriate citation and credit, is a continuing challenge. Third, how do we ensure that biologists – often with limited background in mathematical, statistical, and computational methods – are able to effectively exploit genomic-scale datasets? Computational approaches are increasingly central to biomedical research, offer an unusually level playing field for researchers worldwide, and are particularly important for new investigators.

Contrasting parasites with each other and their hosts, tracing parasite evolution, and identifying targets for diagnostic, drug, or vaccine design, are fundamentally questions of comparative genomics. The emergence of genomic-scale datasets for many phylogenetically-related parasite and vector species (apicomplexans, kinetoplastida, several helminth and insect groups) opens many new avenues for research. For example, analysis of apicomplexan parasites (*Babesia, Cryptosporidium, Eimeria, Neospora, Plasmodium, Sarcocystis, Theileria, Toxoplasma,* etc) reveals metabolic pathways, surface antigens, cellular components, and other features that are unique to individual species, highlighting unique areas of each organisms′ biology. Features that are unique to parasite groups provide targets for therapeutic development, while features that are shared with other species, including horizontal gene transfers, providing insights into organismal evolution.

[November, 2006-11-07 - 11h00 - ROOM B]

MC04 - The immune response in resistance to *Toxoplasma gondii* infection in humans

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Toxoplasma gondii is an obligate intracellular parasite that infects a wide range of warm-blooded vertebrates and causes disease in agricultural animals and humans. Somo individuals infected with *Toxoplasma gondii* develop ocular lesions. In order to study immunologic parameters in the response to *T. gondii* in infected persons with and without ocular lesions and in noninfected controls, subjects were divided into groups on the basis of presence of serum antibodies to *T. gondii*, presence of ocular lesions, and clinical history. Production of interleukin-2 and interferon-γ by peripheral blood mononuclear cells from patients with probable congenital toxoplasmosis was decreased, compared with that in persons with presumed acquired infection. Cell proliferation and delayed-type skin reaction induced by soluble toxoplasma tachyzoite antigen followed the same pattern. Asymptomatic persons showed high levels of interleukin-12 and interferon-γ, whereas persons with ocular lesions had high interleukin-1 and tumor necrosis factor-α responses toward soluble toxoplasma tachyzoite antigen. These data suggest that patients with ocular disease due to congenital infection show tolerance toward the parasite. Furthermore, susceptibility to ocular lesions after acquired toxoplasmosis is associated with high levels of interleukin-1 and tumor necrosis factor-a, whereas resistance is associated with high levels of interleukin-12 and interferon-γ.

Although ocular lesions are frequent in individuals infected with *Toxoplasma gondii*, disease intensity varies greatly between patients. Autoimmunity has been suggested as a possible component to retinal destruction. When we studied the peripheral blood mononuclear cells from patients with mild disease responded to one or more retinal antigens witha significantly higher frequency than patients without disease or with severe disease. Interestingly, the cytokines produced by the proliferating mononuclear cells did not follow any specific patterns, except for the fact that IL-4 and IL-5 were seldom detected. Our results suggest that although the presence of an immune response towards autoantigens is not protective against the development of ocular lesions by the *T. gondii*, it may protect against the development of severe disease.

However, all these results must be interpreted taking into account that the genotypes of *T. gondii* strains isolated from Brazil are highly divergent when compared to the previously described clonal lineages. Several new predominant genotypes were identified from different regions of Brazil by us and therefore the heterogeneous genetic makeup of Brazilian strains may have an influence in the patters of immune response observed by us.

[November, 2006-11-07 - 11h30 - ROOM A]

MC05 - Genome diversity and evolution histories of *Plasmodium vivax* and *P. falciparum*.

Xin-zhuan Su, . *(National Institutes of Health, Bethesda, Maryland, USA)*

Studies of genome diversity and evolutionary histories of malaria parasites are important for developing disease control measures. A highly polymorphic genome may provide the genetic background that can generate drug resistance genotypes at an accelerated rate; and a complex genome may make it more difficult to develop an effective vaccine to control the parasite. Great progresses have been made in our understanding of malaria parasite genome diversity and evolution histories in recent years, however, many questions remained unanswered or being debated. In this presentation, genome-wide polymorphism (microastellite and single nucleotide polymorphism) from both *Plasmodium vixav* and *Plasmodium falciparum* will be compared and discussed with reference to current debates on parasite evolutionary history and population structure, and their implication on genetic mapping, particularly genome-wide association studies.line
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[November, 2006-11-07 - 11h30 - ROOM B]

MC06 - CNS Infection by the Opportunistic Pathogen *Acanthamoeba* in the Immune Compromised Host

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Opportunistic pathogens of the genus *Acanthamoeba* are causative agents of Granulomatous Amoebic Encephalitis (GAE) and cutaneous lesions in immune suppressed individuals including those with human immunodeficiency virus (HIV) infection or with acquired immune deficiency syndrome (AIDS). Many of these individuals use marijuana illicitly or therapeutically for treatment of nausea, loss of appetite, or chronic pain. However, marijuana and its principle psychoactive cannabinoid delta-9-tetrahydrocannabinol (THC) are immune suppressive and modulate inflammatory responses. These immunomodulatory effects have been shown to be effected by receptor as well as non-receptor modes. However, at concentrations approaching nanomolar levels, THC acts through the activation of one of two Gi/o protein-coupled receptors: CB_1 that is localized primarily in the brain and CB_2 that is found in cells of the immune system. We have developed in vitro and in vivo models to study the immune response to *Acanthamoeba*. In these systems, macrophages and macrophage-like cells play a central, if not principal, effector role against amoebae. Using (B6C3)F1 mice as the host, intranasal instillation of *Acanthamoeba* was followed within a 4-day period by the appearance of granulomas within the rostral area of the brain. These granulomas were populated principally by cells that had morphological and phenotypic expression marker characteristics of microglia, the resident macrophages of the brain. Administration of THC (25 mg/kg -80 mg/kg) intraperitoneally to mice followed by instillation of *Acanthamoeba* resulted in higher mortalities as compared to animals similarly infected and receiving vehicle. Histopathological analysis indicated that brains of THC treated mice, in contrast to those of vehicle controls, lacked granulomas. To garner insight as to the mode of action through which THC affected granuloma formation and macrophage-like cell accumulation around amoebae, in vitro studies were performed using murine thioglycollate-elicited peritoneal macrophages, microglia isolated from Sprague-Dawley neonatal rat cerebral cortices, or murine microglial-like cells (EOC-20). THC inhibited in a dose-related fashion the chemotactic response of peritoneal macrophages to amoeba-conditioned medium. A comparable dose-related inhibition in chemotaxis was obtained when peritoneal macrophages were treated with the CB_2-selective agonist O2137 (1R, 3R)-1-[4-(1,1-Dimethylheptyl)-2,6-dimethoxyphenyl]-3-methylcyclohexanol) but no inhibition was obtained when the CB_1-selective agonist ACEA (N-(2-Chloroethyl)-5Z,8Z,11Z,14Z-icosatetraenamide) was used. These results were replicated when mice were exposed to THC (25 and 50 mg/kg) and their peritoneal macrophages assayed in vitro for a chemotactic response. A comparable THC-mediated inhibition in the chemotactic response to amoeba-conditioned medium was obtained when neonatal rat cerebral cortex microglia were used. Again, the CB_1 agonist ACEA had no effect on chemotaxis while the CB_2 agonist O2137 exerted a robust inhibition. Finally, *Acanthamoeba* induced a robust production of pro-inflammatory cytokines (IL1β, TNFα, IL6) and chemokines including macrophage chemotactic proteins (MCP1, MCP5) by the three cell types. THC inhibited this induction of proinflammatory factors that play a role in recruiting inflammatory cells to focal sites of infection. Collectively, these results suggest that THC mediates inhibition of microglia and macrophage-like cell migration toward focal sites of amoebae and that this inhibition is linked functionally to the CB_2. Furthermore, THC-mediated inhibition of pro-inflammatory factors may articulate a mode through which this cannabinoid inhibits focal aggregation of inflammatory cells at sites containing *Acanthamoeba* thereby allowing their dissemination and increasing mortality in infected hosts. Supported in part by National Institutes of Health awards DA005832, DA05274, DA015608, and T32DA07027.

[November, 2006-11-08 - 11h00 - ROOM A]

MC07 - EARLY HOST- PARASITE INTERACTIONS LEADING TO THE DEVELOPMENT OF CD8+ T CELL RESPONSES AGAINST MALARIA LIVER STAGES.

Zavala, F *(Johns Hopkins University)*

Immunization with malaria sporozoites induces a strong CD8+ T cell response which is dependent on CD11c+ dendritic cells. This response reaches a peak 4-5 days after immunization and then undergoes a severe contraction phase that eliminate 90 % or more of the activated CD8+ T cells. After day 10 and until days 20-30 different memory cell subsets are developed in different organs. CD4+ T cells and IL-4 play a critical role in the development of memory cells belonging to the effector/peripheral subset that resides in the liver and other non-lymphoid organs, and is responsible for the inhibition of liver stage parasites. The initiation of this CD8+ T cell response immune response was thought to occur in the liver or liver-draining lymph nodes (LN) by dendritic cells which pick up antigen from infected hepatocytes. However, our recent studies indicate that this CD8+ T cell response begin in extra-hepatic compartments. Mice were subjected to P.yoelii-infected mosquito bites or direct sporozoite-injection in the ear, and the numbers of antigen-experienced CD8+ T cells specific to the epitope of the circumsporozoite protein (SYVPSAEQI), were compared in various lymphoid tissues and the liver. Two days after immunization through the ear, the earliest-activated IFN f^x-secreting CD8+ T cells, were found in the draining auricular LN, not in the contralateral auricular LN, not in liver or not liver draining LN (coeliac), although they later spread to these sites. The induction of this response requires metabolically active sporozoites a heat inactivated sporozoites are not capable of inducing detectable effector responses, indicating that an active interaction between parasite and dendritic cells is required. In addition, treatment of mice with TLR ligands before immunization with parasites, severely inhibit the CD8+ T cell responses thus, suggesting that mechanisms related to cross-presentation are involved in dendritic cell acquisition and presentation of parasite antigen. When the draining auricular LN was ablated prior to immunization, the total numbers of IFNf^x-secreting CD8+ T cells in the liver fell to less than half, thus underlining its importance in overall generation of effector CD8+ T cells. Furthermore, protective immunity to intravenous sporozoite challenge was drastically reduced, if the draining LN and spleen were ablated before immunizing through the ear. This study highlights for the first time, the critical importance of skin-draining lymph nodes in initiating an immune response to Plasmodium sporozoites entering through their natural route, and provides compelling evidence that extra-hepatic sites are necessary for development of sporozoite-induced protective immunity against malaria.

[November, 2006-11-08 - 11h00 - ROOM B]

MC08 - Draft Genome Sequence and Annotation of Aedes aegypti, Vector for Dengue and Yellow Fever Viruses

Haas, B.J.H. *(The Institute for Genomic Research)*

We have recently completed the initial sequencing and annotation of the mosquito Aedes aegypti, vector for the dengue and yellow fever viruses. The genome totals 1.31 billion base pairs divided among 4,758 scaffolds. Genome assembly and subsequent annotation efforts were challenged by the high repeat content; approximately 68% of the sequenced genome was found to consist of repetitive sequences. Preliminary annotation, performed collaboratively between The Institute for Genomic Research (TIGR), VectorBase, and The Broad Institute, identified 15,419 protein-coding genes, 992 of which were found alternatively spliced. Comparisons of the draft Aedes aegypti genome to that of Anopheles gambiae, the vector for malaria, reveals genome expansion in Aedes resulting from transposable element insertions. The resulting sequence and annotation for Aedes aegypti represent a major milestone in our efforts to better understand disease vector biology. The methods used to annotate the genome and our resulting discoveries will be described.

[November, 2006-11-08 - 11h30 - ROOM A]

MC09 - Unraveling the Intimate Life Cycle of *Plasmodium*. Part I. Vessel Invasion.

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Malaria, a parasitic disease caused by *Plasmodium spp*, is responsible for more than one million of human deaths per year. Laveran discovered its etiologic agent in 1880, when he first observed the parasite in the blood of a patient suffering from malarial fever. About twenty years later, Ross and Grassi showed that mosquitoes are the vectors of the disease, transmitting the parasite by biting the vertebrate host. It is commonly said that parasite transmission to the mammalian host occurs when an infected mosquito injects its saliva together with the parasites directly in the blood circulation. However, since the work of Boyd and Kitchen in the late 1930′s, evidence has accumulated showing that most of the transmitted parasites are released in the dermis during the mosquito bite. By imaging a rodent fluorescent parasite *P. berghei*, we observed the fate of these parasites *in vivo* in the mouse dermis after natural transmission. The parasites injected in the dermis were highly motile and invaded both

blood and lymph vessels. Those that invaded lymph vessels were found in the first draining lymph node (LN), a few were found in the second draining LN (< 0.5% compared to the first LN) and none in the collector lymph duct. The parasites in the first draining LN lost their original shape over time and were found inside or in close association with dendritic cells. Unexpectedly, a small proportion of parasites started to transform inside LN but did not fully complete their development. Therefore, our data confirm that the dermal parasites get access to the liver by invading blood vessels and reveal that they can also invade the lymphatic system where they apparently cannot survive, but can nonetheless deliver antigens.

Once in the liver, the parasite leaves the blood after traversing the sinusoidal barrier. Inside of the liver parenchyma, the parasite invades a hepatocyte and develops into a schizont. The mature schizont generates thousands of intracellular merozoites, which need to return to the blood circulation in order to initiate the erythrocytic infection. To elucidate how these merozoites reach the blood circulation, we followed this process using 4-D intravital confocal microscopy. *In vivo* imaging of infected livers showed that the parasitized hepatocytes generated cellular buds filled with merozoites, which we called "merosomes". These merosomes crossed the endothelial barrier and protruded towards the lumen of hepatic vessels where they detached from the host cell and became individualized structures. Similar to the "Trojan horse", these detached merosomes transported a few to thousands of merozoites surrounded and protected by the host cell membrane, in the blood circulation. Besides manipulating the infected hepatocyte at a cellular level, the parasite also manipulates the molecular structure of the host cell/merosome membrane by inhibiting the translocation of phosphatidyl serine to its outer layer, which normaly acts as an eat-me signal to phagoctyes . This strategy of camouflage and host cell manipulation allows a safe parasite release in the blood circulation, preventing phagocytosis of merozoites. In conclusion, *in vivo* imaging has started to reveal the complexity of the pre-erythrocytic phase of *P. berghei* life cycle and some original means to escape the host immune defense.

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[November, 2006-11-08 - 11h30 - ROOM B]

MC10 - Immunology of Non-Curing Forms of Leishmaniasis in Mice and Humans

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It is by now well established that endogenous IL-10 is a central mediator of immune homeostasis, necessary to keep in check the strong inflammatory reactions that can accompany the expression of anti-microbial immunity in local tissues. A consequence of the balance struck between host immunity and pathology can be chronic or persistent infection, and the absence of IL-10 has been shown to result in more efficient clearance of a variety of pathogens. In Leishmaniasis, aside from the Th2 polarizing conditions that underlie the extreme susceptibility of BALB/c mice to cutaneous strains of Leishmania, chronic forms of cutaneous or visceral disease in humans and in other mouse models are better explained by the presence of ongoing Th1 responses that are compromised in intensity or function by IL-10. As many innate cells and lymphocyte subsets can produce IL-10, the relative contribution of these cells to the anti-inflammatory/immunosuppressive cascade in sites of chronic infection has not been carefully defined. We have analyzed the dominant source of IL-10 dependent immune suppression induced by a strain of L. major that produces non-healing dermal lesions in the face of a strong Th1 response in C57BL/6 mice. The infection induced localized recruitment and production of IL-10 from innate cells, as well as from CD4+CD25+Foxp3+ natural Treg and CD4+CD25-Foxp3- inducible Treg. The latter cells appear to be closely linked to the Th1 effector response, in that they also produced high amounts of IFN-g. In Rag-/- reconstituted, infected mice, the IL-10 producing Th1 cells were generated in the absence of either natural Treg or IL-10 from innate sources, and most importantly, were found to play a necessary and sufficient role in the suppression of protective immunity in the skin. The data are consistent with a regulatory pathway that relies not on a committed lineage of cells or on sub-optimal conditions of immune activation, but on a strong pro-inflammatory environment that drives Th1 cells through a program of development that includes IL-10 secretion as a mechanism of feedback control. IL-10 has also been implicated in the suppression of antigen-specific T cell responses in human VL based on the elevated levels of IL-10 observed in plasma and lesional tissue, and its role in preventing clearance of L. donovani in murine models of VL. In order to identify the cellular source of IL-10 in human VL, and determine if CD4+CD25+Foxp3+ natural Treg are associated with active disease, we analyzed surface marker and gene expression in PBMC and splenic aspirates from Indian VL patients pre- and 3-4 weeks post-treatment with Amphotericin B. The results did not point to an important role for natural Treg cells in human VL: they did not accumulate in and were not a major source of IL-10 in the spleen, and their removal did not rescue antigen-specific IFNg responses. By contrast, splenic T cells depleted of CD25+ cells expressed the highest levels of IL-10 mRNA, and were the predominant lymphocyte population in the VL spleen. The data implicate IL-10 producing, inducible Treg in immune suppression associated with human VL. Thus non-curing forms of leishmaniasis in both mice and humans are associated with a population of Ag-driven, CD4+CD25-Foxp3- Treg that produce IL-10 and render the effector response inadequate for clinical cure.