Host–microbe interactions: fungi/viruses/parasites

Editorial overview

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Abbreviations

AICD	activation-induced cell death
AID	autoimmune disease
CWP	cell wall protein
GPI	glycosyl phosphatidylinositol
IFN	interferon
IL	interleukin
LRR	leucine-rich repeat
MS	multiple sclerosis

This issue of *Current Opinion in Microbiology* deals with host–microbe interactions of fungi, viruses and parasites. Our understanding of how fungi, viruses and parasites interact with their respective hosts continues to improve as new technology becomes available. To begin to comprehend the complete picture of microbe–host interactions, we must be able to analyse and manipulate the microorganism's genome, to study the ways it exploits host cells and interacts with the host immune system. The reviews in this issue highlight some of these areas of research. In the first part of this issue, the reviews discuss the dynamics of the interactions between fungal pathogens and host cells from the perspective of both the pathogen and the host. The second group of reviews addresses the complex interactions between viruses and the host immune system. The final group of reviews considers how new and improved molecular studies of protozoan parasites have enhanced our understanding of protozoan microbiology.

Fungi

Fungal pathogens encounter an array of difficulties in colonizing their host. The pathogen must adhere to and degrade host tissue, must monitor the extracellular milieu for environmental signals and transduce these to the nucleus, responding with a program of gene expression in some cases tailored to a given host environment. The first three reviews address these issues directly. Klis and colleagues (pp 348–352) discuss the structure of the yeast cell wall and detail mechanisms of attachment of fungal proteins to the cell wall. Sundstrom (pp 353–357) then discusses a number of cell wall crosslinked adhesins in *Candida albicans*, important in host interaction. Wang and Heitman (pp 358–362) then review the current understanding of signal transduction pathways in *Cryptococcus neoformans*. The last two reviews explore two examples of the dynamic pathogen–host interaction. First, Romani (pp 363–367) reviews the T cell response to *Candida* that determines the nature of the host response and indeed resistance or susceptibility in immunocompetent mice. Then, de Wit and Joosten (pp 368–373) review the elegant molecular dance between *Cladosporium* and tomato and detail the molecular recognition between host and pathogen.

Largely from work in *Saccharomyces cerevisiae*, a comprehensive model of the structure of the cell wall is emerging (see review by Smits *et al.*). Composed largely of β1-3 glucan, the organizing principle of the cell wall is proposed to be glucan-crosslinked proteins that are linked either directly to β1-3 glucan, in the case of the Pir (proteins with internal repeats) proteins, or via a β1-6 linkage, in the case of the GPI-CWPs. Maturation of GPI-CWPs proceeds through a transient GPI-linked plasma membrane-localized form before ultimate conjugation to β1-6 glucan. Recent work emphasizes that the cell wall is a dynamic structure whose structure and protein content changes in response to stress and environmental signals. In pathogenic fungi, a dynamic cell wall is presumably critical to virulence. Indeed, clear examples of gross cell wall remodeling are found in diverse fungi; for example, in the yeast-hyphal transition in *C. albicans* and in the cell wall changes, including loss of α-(1,3) glucan, that accompany intracellular growth of *Histoplasma capsulatum* in trachea epithelial cells [1].

The GPI-CWPs mentioned above are of considerable importance in pathogenic fungi and representatives in *Candida* include pH-regulated cell wall proteins essential for survival at mucosal or systemic locations in the host, hyphal-specific proteins and adhesins. Three of the four adhesins cloned to date in *C. albicans* are, by sequence homology, GPI-CWPs (see the review by Sundstrom). *ALS1* and *ALA1* are members of the *ALS* gene family and mediate adherence to endothelial cells in culture and to components of the extracellular matrix. *HWP1*, a hyphal wall protein identified some time ago, has been recently shown to mediate a covalent attachment to epithelial cells. The intersection of adhesins with GPI-CWPs is not limited to *C. albicans*. In *C. glabrata*, we recently cloned a lectin that recognizes host carbohydrate, and, from sequence comparison, it too appears to be a GPI-type cell wall protein [2]. Most β1-6 glucan is in the outer layer of the cell wall so it is perhaps not surprising that many of

the genes critical for the initial interaction with the host should be crosslinked to the cell wall via β1-6 glucan and thus localized in the outer cell wall layers.

In the dimorphic yeast *C. neoformans*, the signal transduction pathway controlling filamentation and expression of a number of virulence traits has been significantly elucidated (see the review by Wang and Heitman). There are two separate pathways, possibly headed by two distinct heterotrimeric G-protein-coupled receptors that respond to very different signals. One, responsive to pheromones, signals a MAP kinase cascade that controls mating and filamentation and the other, which senses starvation, regulates a cAMP-dependent signaling cascade that controls mating and expression of such virulence attributes as capsule formation and melanin production. Many of the same players in the MAP kinase cascade that control mating in *S. cerevisiae* and filamentation in *S. cerevisiae* and *C. albicans* are important in *C. neoformans*. Indeed a *Ste12* homolog in *C. neoformans* is implicated in control of filamentation as in *S. cerevisiae* and *C. albicans*. *C. neoformans Ste12* is, interestingly, not required for mating as in *S. cerevisiae*, making the point that different components of the signaling pathways are recruited for different tasks depending on the organism. The functional roles of even highly conserved members of signal transduction pathways must therefore be established in the individual organisms.

Most individuals possess immune systems capable of severely limiting proliferation of *C. albicans* on mucosal surfaces and preventing any dissemination whatsoever. In mice, exploration of the immunological events leading to *Candida* resistance or sensitivity in mice has made clear that at the core of systemic immunity is the action of phagocytic cells — primarily neutrophils. T cells play an important adjunct role in fungal immunity, and the nature of the T cell response in mice can largely determine resistance or susceptibility to disseminated candidiasis. Thus, cytokines that favor a Th1 response are required for resistance to *Candida* infection, whereas cytokines that favor a Th2 response are dispensable. The review by Romani points out that there are complications to this simplistic view. Particularly interesting is the fact that interleukin (IL)-4 deficient mice, while able to fight a primary infection as well as wild-type mice, are not immune to subsequent challenge. Thus, a 'Th2' cytokine, neutral or even detrimental in fighting a primary infection is apparently essential for long-term immunity. One target of IL4 appears to be neutrophils themselves. Neutrophils are important sources of IL12, placing them squarely in a regulatory as well as an effector role. IL4 stimulates neutrophil IL12 production, perhaps explaining the IL4 requirement in long-term immunity. The complexity of the host network for controlling fungal infection, limiting the immune response, and developing long-term immune memory is a major challenge in thinking about immunotherapeutic strategies for fungal infections.

Finally, the plant–pathogen interaction of *Cladosporium fulvum* and tomato is a well elaborated example of the gene-for-gene relationship that shapes recognition between plants and fungal (as well as bacterial) pathogens (see the review by de Wit and Joosten). Different serovars of plants possess receptors for an array of fungal gene products called elicitors and encoded by avirulence genes. Recognition of the fungal 'elicitor' results in a so called hypersensitive response characterized by release of reactive oxygen, localized necrosis and a non-productive infection. In the absence of recognition, either because the elicitor is missing from the fungal strain or the receptor from the plant, a productive infection results. In *Cladosporium*, a tomato pathogen, the elicitor proteins are secreted, and many of them, though not all, are essential for virulence on susceptible plants. The resistance loci encode cell surface receptors characterized by leucine-rich repeats (LRR) and a single transmembrane domain, with no cytoplasmic domain to speak of. The simplest model has the elicitor binding to its cognate receptor and thereby signaling a hypersensitive response via a kinase cascade. However, for the avirulence gene *avr9*, there is an as yet unidentified high affinity receptor on both resistant and sensitive serovars of tomato. The working model is that a heterodimeric receptor composed of the resistance gene Cf9 and the high affinity receptor binds the avr9 protein and this trimeric complex signals the hypersensitive response.

Intriguingly, for bacterial plant pathogens, resistance loci tend also to encode LRR proteins; however, the recognition of the elicitor probably occurs in the plant cytoplasm after delivery of the bacterial protein into the plant cell via a type III secretion apparatus [3]. Given that many bacterial elicitors are themselves required for virulence and given the connection of type III secretion to bacterial virulence in animal pathogens, a model is suggested in which avirulence genes originally evolved as classic virulence factors, only becoming avirulence factors when recognized by an evolved resistance gene. In the *Cladosporium*–tomato interaction, plant recognition of the fungal protein is almost certainly extracellular (see review by de Wit and Joosten). However, a number of fungal elicitors are also required for virulence on susceptible plants. Perhaps in plant fungal pathogens as well, avirulence genes originally evolved as classic virulence factors with extracellular roles in pathogenicity.

Over the past two decades, work in a number of labs established critical genetic tools and indeed a genetic framework for the molecular study of fungal pathogens. These are now being exploited on an unparalleled scale to examine the host interaction of a variety of fungi. Our understanding of fungal virulence remains relatively sketchy, although as these reviews make clear that the questions have been framed. Look at these pages in the future for the answers.

Purging ourselves of troublesome virus infections?

Mankind has benefited greatly from vaccines that protect against many virus infections. Nevertheless, far more agents are not contained currently by vaccines. Included amongst the uncontrolled are all of the eight human herpes viruses, most viruses that cause diarrhea or respiratory disease in infants, the majority of hepatitis viruses, the agents that cause the common cold, and, of course, the AIDS virus. Added to the list of diseases clearly caused by viruses are many syndromes where viral agents are suspected to participate in the aetiology; examples include numerous autoimmune diseases, certain neoplasias, asthma, and perhaps even Alzheimer's disease and chronic fatigue syndrome. Indeed one can find enthusiastics who advocate a role for one or more virus infections in almost any chronic disease syndrome. One expects, however, that our viral troubles will diminish as we apply our expanding knowledge of fundamental virology, genetics and immunology to the design of vaccines and therapeutic agents.

This issue compiles reviews by six leading groups of immunologists working in the field of viral pathogenesis. In the first review, Christine Biron (pp 374–381) unravels the subtle interactions that occur at the onset of infection between the virus and the host that set the scene for subsequent immune events. As is pointed out, during the initial phase of infection, certain structural components of the virus may interact with intracellular signaling pathways to induce cell products that in turn shape the nature and efficacy of subsequent innate and acquired immune defenses. The field is still very much in its infancy, but some provocative examples support such pattern etching ideas. Two key events are the extent of interferon (IFN)-$\alpha\beta$ and interleukin (IL)-12 production both of which act to influence the inherent plasticity of subsequent innate and adaptive immune responses. Curiously, IFN$\alpha\beta$ may negatively regulate IL-12 expression and hence the downstream innate and adaptive immune events that IL-12 itself regulates. Other examples by which viral induced factors that shape the nature and effectiveness of immunity are discussed.

The review by Raymond Welsh and James McNally (pp 382–387) discusses our current understanding of the events that occur when effector T cell responses are shut down and controlled following viral infection. Biochemical details are still lacking, but the outcome, which involves apoptosis and growth factor deprivation, is influenced significantly by the level of activation T cells receive. When highly activated cells are triggered through their T cell receptor (TCR), activation-induced cell death (AICD) occurs. During AICD, transient immune deficiency is present and nonviral specific T cell function is also inhibited. The mechanism of innocent bystander turn off is not understood, but it is likely to represent a combination of TCR triggering, exposure to cytokines and some stimulus from temporarily defective antigen presenting cells (APCs). A crucial event remaining after silencing is the maintenance of memory cells, but it is unclear what factors determine those destined to die and those rescued from such a fate. Under conditions of overwhelming T cell activation, the stage is set for clonal exhaustion. Functionally the end result is the blunting of both effector cell function and memory. However, the process seems to advance through an anergy phase prior to clonal elimination. Clonal exhaustion also involves apoptosis and the absence of rescue factors. Clonal exhaustion is a rare event in natural infection but could be occurring in the late phase of AIDS and HBV hepatitis.

Luca Guidotti and Francis Chisari (pp 388–391) discuss a fascinating mechanism by which $CD8^+$ T cells effect immunity seemingly without damaging virus infected target cells. Characteristically, cytotoxic T cells (CTLs) are considered homocidal and destroy infected targets by either an apoptotic or lytic mechanism. In the purging mechanism, however, CTLs deliver a signal likely to involve one or more cytokines, which induces target cells to selectively discard species of nucleic acids which include those derived from the infecting virus as well as viral capsid particles. The authors speculate how purging might operate and point out the virtues of such a process when viruses infect irreplaceable cell types. Biochemical details of the purging mechanism are still to be worked out, but they include the proteolytic cleavage of a cellular protein (SSB/La) that normally stabilizes viral RNA. A purging process has also been noted for some other viruses (herpes, pox and arena) and could account in part for cytokine mediated innate immunity. Inducing cells to purge on demand represents a valuable antiviral therapeutic strategy.

The fact that viruses might be involved in the causation of autoimmunity received a sober review from Lindsay Whitton and Robert Fujinami (pp 392–397). They critically discussed ways in which viruses could misdirect the immune system to result in autoimmunity and caution that some assumed autoimmune lesions may in fact be virus mediated. The case is well founded for viruses as a cause of some animal models of autoimmunity. In a few instances, the seductive molecular mimicry hypothesis even seems to find support. This hypothesis that autoimmune disease (AID) results from antigen sharing between virus and the host is best shown when hosts are provided by transgene technology with a new self protein derived from the virus itself. More frequently, bystander mechanisms are involved in AID pathogenesis. These include viral damage and release of host proteins and the overproduction of cytokines, which cause a breakdown in immune homeostasis. In such instances, a progressive involvement of additional host autoantigens often occurs, so-called determinant spreading. Whereas viruses may cause AID in some animal models, linking viruses to human AID is tenuous and at best indirect. With the AID multiple sclerosis (MS), almost all human and some animal viruses have been advocated at times as its cause and currently HHV6

is the favorite candidate. Careful scrutiny will ultimately resolve if the candidate of this virus is sustained.

The issue of neurodegenerative disease, as is MS, and the role of known viruses is discussed by Michael Buchmeier and Thomas Lane (pp 398–402). They describe the cell and molecular mechanism by which two well-studied murine viruses, mouse hepatitis virus (MHV) and Theiler's murine encephalomyelitis virus (TMEV), cause lesions akin to MS in mouse brains. Of particular interest is the observation that MHV, an RNA virus, can persist without replication. Furthermore, this persistent viral RNA appears responsible for the chronic neurodegeneration. Could a similar process be occurring in MS with causative virus (smoking gun) long since departed?

Samuel Speck and Herbert Virgin (pp 403–409) analyze in detail the interactions between host and virus genetic events that occur during infection of mice with their natural gamma herpesvirus pathogen γHV68. This model is revealing how the host contains acute infection, latency and reactivation. Moreover since human gamma herpesviruses are involved in neoplasia, the γHV68 model might reveal clues about how latent or productive infection interacts with immunodeficiency to slide into lymphoma. So far with γHV68, a single oncogene has been identified. In addition to providing a mechanistic update of host and viral encoded events involved in γHV68 pathogenesis, the review discusses important methodological issues that in the past have confused the interpretation of some previous observations made with the model.

The last review by Tracy Hussell and Peter Openshaw (pp 410–414) discusses the pathogenesis of respiratory syncytial virus (RSV), an extremely important pathogen of children and the elderly which currently is uncontrolled by vaccines. The main stumbling blocks to vaccine development are that prior immunity can enhance the severity of natural infection and that the latter may fail to protect from reinfection even against homologous viral strains. An added problem in the RSV field is the absence of an ideal animal model to study pathogenesis and vaccine assessment. Fortunately, molecular biology has recently provided the reverse genetic tools that promise the re-engineering of the RSV genome and the definition of viral components involved in protection and pathology. The disease augmentation events appear associated with the secreted form of the attachment protein (G), which induces an immune response dominated by $CD4^+$ Th2 cells. Vaccines that emphasize $CD4^+$ Th1 and $CD8^+$ responses are showing promise and a number of candidate approaches are under consideration for human trials. Other avenues to control this important pathogen, such as the use of a human's monoclonal antibody and some new antivirals, are also discussed in this lucid review.

In conclusion, viral pathogenesis is a fast-moving field that is reaping benefits from fundamental studies in medical biology. The outcome will be a full appreciation of how viruses cause disease and which steps are vulnerable to control by the host. Lets hope that more viruses will be purged from the troublesome list, some perhaps joining smallpox in the dodo file.

The emergence of protozoan microbiology

Since 1989 we have witnessed a series of mini-revolutions in the molecular study of protozoan parasites. These stem from developments in our ability to genetically modify key lineages of the major parasitic genera (*Trypanosoma*, *Leishmania*, *Toxoplasma*, *Entamoeba*, *Plasmodium*, *Giardia* and *Trichomonas*), and the introduction of functional approaches including targeted gene knockouts and genetic rescue of mutants. Parasite genome projects have been initiated and are now progressing rapidly. Combined with new or improved experimental systems for culturing parasites in their infective stages, we are now poised to understand how these organisms manage to cause disease, and persist in the host for periods of many years. Although the genetic methodological repertoire is still evolving and has some distance yet to equal that available to our prokaryotic or fungal colleagues, there is no doubt that the genetic barrier has been resoundingly smashed. Conceptually, protozoan parasites can now be studied with tools and approaches familiar to all microbiologists.

Genes and genomics

The Apicomplexan parasites *Plasmodium* and *Toxoplasma* illustrate the fruits of the molecular genetic revolution in protozoan microbiology. Both of these parasites are obligate intracellular parasites, have a well-described sexual cycle, and propagate with a haploid chromosomal complement.

As discussed by Wellems *et al.* (pp 415–419), the international malaria genome project is progressing rapidly, with a target date for completion in the year 2000. Following the well-established philosophy of genome scientists in speeding distribution of information and reagents, this project is already having a major impact. Typically, a plethora of new genes whose functions can be deduced from database searches have emerged with implications to different aspects of malaria biology and control. Perhaps more relevant to the complex life cycle of *Plasmodium*, around 40% of all predicted open reading frames (ORFS) show no homologs in the databases. With the availability of genetic tools for expression and disruption, as well as a well-defined sexual cycle amenable to position cloning approaches, the prospects for systematic identification of genes relevant to the infectious cycle are bright in this deadly pathogen.

The impact of genome analysis on virulence is clearly illustrated in the study of antigenic variation in *Plasmodium falciparum* (see the review by Newbold, pp 420–425). As part of the erythrocytic infectious cycle, the malaria parasite resides within the peripheral circulation, thereby avoiding clearance in the spleen. The protein mediating

this behavior (PfEMP-1) was shown to be encoded by members of the large *var* gene family. In a given parasite, only a single *var* gene is expressed, and the encoded protein greatly determines the ability of the parasite to bind different physiological substrates, and to be recognized by immune sera. Thus this family is implicated in two key features essential for parasite survival: adherence and immune evasion. How the parasite manages to control and switch expression of different *var* genes when placed under selective pressures is under intensive study; thus far, it appears to differ from the mechanisms seen in other pathogens such as trypanosomes.

The molecular genetics of *Toxoplasma gondii* are exceedingly powerful and well developed. As discussed by Roos *et al.* (pp 426–432), these parasites provide a highly amenable system for probing basic mechanisms common to all Apicomplexan parasites, such as the unusual plastid-like organelle, the apicoplast. This organelle is apparently related to the chloroplast of higher algae and plants, and has probably arisen by a secondary endosymbiotic event. Although the metabolic role of the apicoplast has not been determined, pharmacological inhibition studies have established it as a target essential for parasite growth. Studies of the apicoplast have benefited greatly from the *Toxoplasma* EST project as well as the malaria genome project. Database 'mining' yielded many candidates related to proteins localized to the plastids of other species, suggesting many potential plastid functions in *Toxoplasma*. Many of these predict proteins bear potential bipartite plastid targeting signals, whose activity was confirmed by their ability to target green fluorescent protein fusions to the *Toxoplasma* plastid. This study nicely illustrates the value of *Toxoplasma* as a model system for other Apicomplexans, since the *P. falciparum* plastid targeting sequences were similarly functional when expressed in *Toxoplasma*.

Two sides of protozoal virulence

One of the major goals of microbiologists studying infectious diseases is the identification of pathogen genes critical to this process. As discussed by Gilchrist and Petri (pp 433–437), a number of candidate 'virulence genes' have emerged in studies of *Entamoeba histolytica*, the agent of amoebic dysentery. Definitive tests of such loci are complicated by the fact that technology for gene knockouts is still under development, and by the ploidy of these organisms. However, an evolutionary 'null mutant' approach has come to the rescue. Earlier investigations concerning the properties of pathogenic versus non-pathogenic strains led to the realization that in fact, two distinct species were involved: the pathogenic *E. histolytica* and the non-pathogenic commensal species *E. dispar*. Comparisons between these species suggest differences potentially related to pathogenesis, which are being systematically tested and evaluated by genetic approaches. Already, the list of confirmed virulence products is high, and promises to grow rapidly in the future.

Equally important to the infectious cycle is the host response. A fundamental paradigm of molecular pathogenesis is that amelioration and redirection of the host response is essential to survival. In the case of *Leishmania*, the ability to survive within the acidified phagolysosomal vacuole of macrophages is just the first step. As important, the parasite must evade activation of subsequent responses, including activation of nitric oxide (NO) synthase and the triggering of protective immune Th1 responses. Thus far numerous host-signaling pathways have been implicated as targets for parasite interference, as reviewed in this issue by McDowell and Sacks (pp 438–443). Perhaps chief amongst these is the ability of the parasite to abrogate IL-12 expression, as absence of this cytokine is associated with progressive leishmaniasis. Although a number of routes toward inhibition of IL-12 are suggested, perhaps the most exciting is the idea that ligation of one or more macrophage surface receptors during infection is the key step.

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