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Infection systems biology: from reactive to proactive (P4) medicine

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Summary. This short review establishes the conceptual bases and discusses the principal aspects of P4—shorthand for predictive, preventive, personalized and participatory medicine—medicine, in the framework of infectious diseases. P4 medicine is a new way to approach medical care; instead of acting when the patient is sick, physicians will be able to detect early warnings of disease to take early action. Furthermore, people might even be able to adjust their lifestyles to prevent disease. P4 medicine is fuelled by systems approaches to disease, including methods for personalized genome sequencing and new computational techniques for building dynamic disease predictive networks from massive amounts of data from a variety of OMICs. An excellent example of the effectiveness of the P4 medicine approach is the change in cancer treatments. Emphasis is placed on early detection, followed by genotyping of the patient to use the most adequate treatment according to the genetic background. Cardiovascular diseases and perhaps even neurodegenerative disorders will be the next targets for P4 medicine. The application of P4 medicine to infectious diseases is still in its infancy, but is a promising field that will provide much benefit to both the patients and the health-care system. [**Int Microbiol** 2012; 15(2):55-60]

Keywords: microbial systems biology · infection biology · P4 medicine · OMICs

Introduction

The premise of P4 medicine, shorthand for predictive, preventive, personalized and participatory medicine, is that diseases result from perturbations of biological networks. Disease-perturbed networks both cause and reflect the progression of a disease. Thus, diseases can be diagnosed, treated and prevented by understanding and intervening in the networks that underlie health and illness. And this is exactly the prevailing view on infectious diseases. Infections can be considered as the result of the interplay between two complex biological networks: the host and the pathogen. The understanding of this interaction requires large-scale analysis of the host–pathogen interface. This knowledge should help to identify host pathways important for infection as well as pathogen determinants involved in disease progression. The identified host and pathogen targets may help to develop innovative therapies based on the modulation of the host–pathogen interface. This short review establishes the conceptual bases and discusses the principal aspects of P4 medicine in the framework of infectious diseases.

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Systems biology, health and disease

Due to the enormous complexity of human biology, medical research has historically followed a reductionist strategy, which attempts to explain complex phenomena by defining the functional properties of the individual elements that make up the system. Thus, research focus went progressively from the organism as a whole (anatomy), to the organs (physiology), cells (cellular biology) and, more recently, molecules (genes, proteins, lipids and metabolites, i.e., molecular biology). This reductionist strategy assumes that the world that surrounds us can be understood in terms of the properties of its constituting parts by decomposing Nature into its simplest parts and laws.

One must admit that this strategy has been extraordinarily successful. Not only has it led to the discovery of the intimate nature of the cellular and molecular structure of human biology, but it has also resulted in dramatic advances in clinical practice in all its specialties. Reductionism, however, has its limits: it is not able to explain all phenomena, especially those that involve more than one source and require the coordinated function of different structures (systems), infectious diseases being a clear example. It has become obvious that only rarely biological functions can be attributed to individual molecules. In contrast, most biological systems, in health as well as in disease, arise from complex interactions among the numerous components of cells, such as proteins, DNA, RNA and small molecules. Research in molecular biology over the last forty years has revealed the nature and profound complexity of biological systems. Such a complexity cannot be understood by studying isolated genes and proteins individually [3,4]. In fact, biological systems should be studied as an integrated whole [3,4].

An alternative to the reductionist mindset is the perspective based on the "system", interpreted as a group of individual components with emerging properties that cannot be attributed to any single element on its own. Systems biology is a new interdisciplinary field of research in which the interactions among its components—both internal and external—that influence biological processes are formulated with mathematical expressions. Systems biology was conceived to manage the complexity observed in biological systems in a quantitative and modelled manner. This holistic approach allows us to comprehend the functions of biological systems (processes) and thoroughly research how their interactions, both internally and with other systems, result in the appearance of new emerging properties. Therefore, the construction of predictive models of the networks and dynamic interactions between the biological components is essential and should be based on the integration of high-throughput OMICs data (transcriptomics, proteomics, metabolomics, lipidomics, etc.). Through iterative rounds of model development, testing, and filling in the gaps with experimental data, models are refined to provide predictions that can ultimately be used to help identify therapeutic targets and improve clinical outcomes.

Infection as a result of host–pathogen interplay

Infection can be viewed as a consequence of specific interactions between microbial pathogens and host cells. Critical host cell components include receptors that allow viral and bacterial attachment to, and eventually entry into, a target cell. Host cell receptors may also be crucial in sensing bacterial and viral molecules or patterns to activate early (i.e., innate) host cell responses [22,23,32]. While investigations of virulence-associated factors on the pathogen side have progressed over the years, few comprehensive studies have analyzed the host–pathogen interplay. It is already accepted that host cell factors are essential in infectious processes. In addition, it has been shown, in many cases, both in vitro and in living animals carrying specific genetic defects, that the absence of critical host cell functions can either prevent or enhance infection.

Pathogens rely on the coordinated action of virulence factors to colonize the host and to evade or subvert the subsequent immune response [12,17,19,29]. Classical examples using intestinal microorganisms demonstrate that bacteria trigger cytoskeletal changes at the host cell plasma membrane to promote invasion and in most cases this is due to modulation of the activation status of small GTPases [9,10]. Actin cytoskeleton dynamics are important not only during virus assembly but also for virus internalization via endocytosis. During infection, microbes also encounter locally resident as well as recruited phagocytic cells, including neutrophils, macrophages and dendritic cells [12,17,28,29]. These cells are equipped with a variety of receptors that detect the presence of invading microbes which may lead to phagocyte maturation, synthesis of antimicrobial molecules and phagocytosis [12,13,14,17,29]. Consequently, pathogens use different strategies to counteract phagocytes [8,12–14,17,28].

To avoid detection by pathogen recognition receptors (PRRs), microorganisms have evolved ways of altering the

molecules recognized by these receptors [24]. A key strategy employed by viruses is to inhibit PRR signalling to prevent interferon induction. For example, respiratory syncytial virus encodes proteins blocking "toll-like" receptors (TLR) 3, 7 and 9-dependent signalling [30]. Many pathogens, either viruses or bacteria, also control the cell death pathway. This is crucial for the outcome of infection, not only in terms of the ability of the pathogen to avoid destruction but also with respect to how the infected cell communicates to the immune system. In general, viruses either accelerate or inhibit the cell death pathways, depending on the biology of the specific virus [6]. As viruses do, obligate intracellular pathogens generally suppress apoptotic death. Because this death is less inflammatory than cytotoxic death, many non-obligate intracellular bacteria use this strategy to neutralize host cells.

The invasion of host cells by microbial pathogens is generally associated with the activation of NF-κB, mitogen-activated protein kinases (MAPKs) and interferon regulatory factor (IRF) signalling pathways inducing the transcription of genes coding for a variety of inflammatory mediators and interferons. For many microorganisms, activation of these responses leads to clearance of the infection. Not surprisingly, these responses are normally counteracted by pathogens by, for example, the injection of proteins into the cell cytosol to quench them [12,17,29]. Also many viral pathogens subvert the activation of inflammatory signalling pathways by blocking the activation of the intracellular receptors sensing nucleic acids [15,31]. Finally, evidence indicates that microbial pathogens may also modulate chromatin structure to control the activation of host responses. In some cases, causing chromatin injury can be a strategy to take control of major cellular functions including the cell cycle. In other cases, manipulation of chromatin structure at specific genomic locations by modulating epigenetic information provides a way for the microbe to impose its own transcriptional signature onto cells [2,16,26,27]. This is an emerging field largely unexplored.

Systems biology of microbial infections

Several strategies combining bioinformatics with transcriptomics, chemical genetics and functional genomics have been used to identify host factors essential for pathogen entry, survival, and replication. Nevertheless, the application of systems biology to microbial infections is still in its infancy and data mostly pertain to a few human infections. However, the technologies, computational methods, and genome information needed to successfully implement this approach already exist. Technologies such as next-generation sequencing have opened the door to sequencing total transcriptomes. Proteomic technologies are evolving rapidly, with throughput and sensitivity approaching that of microarrays. Metabolomics, glycomics, lipidomics, and phosphoproteomics are comparatively underdeveloped, but improvements are certainly expected. In this context, it is not surprising that most of the studies have focused on transcriptomics and functional genomics.

Transcriptomics. cDNA microarrays have been used to determine the effects of pathogens on host-cell gene expression or in tissues upon animal infection. In many cases, the responses induced by wild type strains and isogenic mutants lacking known virulence factors have been compared. These studies have addressed questions such as which are the gene expression programmes induced by pathogens, how virulence factors modify these programmes and the contribution of the PRRs to the induction of the programmes. One of the main conclusions of all these studies is that there is a common host response associated to infection, the so-called "alarm signal" [18]. Data show that many of the gene clusters targeted by microbial pathogens belong to the "alarm signal" [15,28]. To understand the full complexity of the host–pathogen interaction it is necessary the use of arrays containing probes to both human (animal, plants) and microbial genes to monitor simultaneously gene expression of both host and pathogen.

Functional genomics. With the advent of the RNA interference (RNAi) technology and its improvement over the last decade, large-scale reverse genetic screens have become feasible in model organisms and now also in human cells [5]. RNAi allows to downregulate (or silence) expression of specific genes by introducing double-stranded RNA (dsRNA) with sequence-complementarity to the target-mRNA. To avoid induction of interferon- or other unspecific responses to long dsRNA, small interfering dsRNAs (siRNAs) directly or small hairpin RNAs (shRNAs) are used for mammalian cells. In the context of host–pathogen interplay, loss-of-function screens using RNAi may uncover host factors that are restrictive (host resistance factors, HRFs; e.g., silencing these factors enhances pathogen replication), or permissive host factors (host susceptibility factors, HSFs; e.g., when these factors are silenced pathogen replication is reduced). In general, siRNA screens are prone to yield false

positives due to off-target siRNA effects [11]. Therefore, hits from primary screens should be validated using additional siRNAs. The size of the primary hit list selected for this validation depends on what stringency is chosen to filter the raw screening data. For several recent arrayed genome-scale siRNA-screens in human and in fruit fly cells (e.g., between 10,000 and >20,000 targets), the validated hit-list comprised ca. 300 host factors [1,7,20,21,34]. Given the large number of hits, basically all of the current host–pathogen interaction studies originating from larger siRNA-screens resemble proof-of-concept studies in that only a small subset of the hits is usually selected for detailed functional characterization.

A major issue affecting the application of systems biology to microbial infections is the lack of integration of the information obtained from a variety of omics which, in turn, prevents the construction of predictive models. Moreover, several rounds of biological perturbations (i.e., the use of mutant pathogens, cellular siRNA knockdowns or knockout mice) are required to produce a predictive model that could be effectively utilized by the general infectious disease community. In fact, in most cases, researchers do not attempt to validate the information obtained in vitro using suitable infection models.

P4 infectious diseases medicine

The term "P4 medicine" (personalized, predictive, preventive and participatory medicine) was coined by David Galas and Leroy Hood from the Institute for Systems Biology (ISB) in Seattle. The ISB studies biological complexity based on three fundamental premises: (i) there are two types of biological information, i.e., digital genome information and environmental information, outside the genome, that modifies the above-mentioned digital information; (ii) biological information is captured, processed, integrated and transferred by means of biological networks (RNA, proteins, controlling regions of the genes and small molecules) to the molecular systems that execute vital functions; and (iii) biological information is codified in a multi-scale hierarchy: DNA, RNA, proteins, interactions, biological networks, tissues and organs, individuals and, finally, ecologies. It is important to highlight that the environment affects each level of this hierarchy and modulates the reception of the digital information from the genome.

Let us consider the four "P" of P4 medicine in the context of infectious diseases:

Personalized. It is said that P4 medicine will be "personalized" because it will be based on the genetic information of each individual including the infecting microbial pathogen. However, this would not be of much help without functional studies to validate the genomic information. A very elegant example is the study by Lalita Ramakrishnan and co-workers reporting a locus associated to differential production of an anti-inflammatory product leading to hypersusceptibility to tuberculosis and leprosy [33]. On the other hand, it is technologically feasible to analyze host responses to different infections ex vivo by challenging blood cells in a test tube. These data could be compared to those obtained from infected patients and even connected to different genotypes. Altogether, this information will help to predict susceptibility to certain infections or whether the defence response will be enough to clear the pathogen. The pathogen side is easier to tackle. There are platforms allowing the identification of the pathogen without culturing, the detection of virulence factors associated to bad prognosis, and even the characterization of antibiotic-resistance markers.

Predictive. Medicine will be "predictive" because this personalized information will allow to determine the risk for certain diseases in each individual. For example, certain gene deficiencies can predispose to recurrent infections (for a review see [25]). These are extreme cases but it is obvious that not everybody is equally susceptible to infections. On average, each human differs from another by less than one percent of their genetic makeup. But these genetic differences give rise to our physical differences, including our potential predisposition to various diseases. Health might be the combination of two types of defence mechanisms against infections: resistance and tolerance. Immunology has largely focused on the identification of mechanisms of resistance, but the molecular bases of tolerance are largely unknown. Nevertheless, plotting health (cytokine levels, biomarkers, fever, etc.) versus pathogen loads over the course of an infection may help to predict recovery and point out bifurcations (failure of treatment). The slope of this plot defines the tolerance of the individual.

Preventive. Medicine will be "preventive" because, given the prediction of risk, prophylactic measures (lifestyle or therapeutic) will be able to decrease risk. In this context, it is widely accepted that vaccination is the most efficient approach to prevent infectious disease progression and may even lead to eradication of the infection.

Table 1. Benefits of P4 infectious diseases medicine

Evidence-based medicine	P4 medicine
Symptoms based	Based on pre-symptomatic markers
Disease-treatment system	Wellness-maintenance system
Few measurements	Many measurements, including a variety of OMICs
Disease-centric	Individual-centric
No large-scale diffusion of medical information	Social networking of patients to enhanced shared experiences and diffusion

Participatory. It will be "participatory" because many of these prophylactic interventions will require the participation of the patient. This includes a variety of aspects of participation such as sharing data, educating patients and physicians and engaging patients in personal choices related to illness and well-being. The increasing uses of social networks by patients, as well as the activities exerted by patients' associations are examples of participatory actions. However, there are still technical problems for mining, comparing and analyzing data sets from thousands of millions of individuals. Issues related to data ownership (by scientists and by institutions) and a reluctance to believe in open-source and open-data policies will have to be overcome if we are to mine the incredible potential of the exploding opportunities of patient data accumulation.

Theoretically, P4 infectious diseases medicine should provide much benefit to both the patient and the health-care system (Table 1). Among its advantages are: (i) the possibility of acquiring and processing data for each particular host–pathogen interaction; (ii) the compilation and analysis of longitudinal information for each individual, which would enable early disease detection and monitor the therapeutic effectiveness of established treatments; (iii) the stratification of patients into disease groups to better defined clinical phenotypes hence leading to the development of alternative therapies specifically directed at those phenotypes, thus achieving greater success rates; and (iv) the facilitation of the entire drug development process by identifying new therapeutic target hubs, reducing adverse reactions to medication and reducing time, cost and failure rate of therapeutic assays.

However, new advances are still necessary for P4 infectious disease medicine to become a reality, including: (i) microfluidic techniques, analysis of individual cells and molecular imaging; (ii) identification and validation of organ-specific protein, micro RNA and other molecular biomarkers; and (iii) new mathematical and computational methods such as dynamic networks enabling the study of the perturbations caused by treatments in biological networks. Finally, the entire healthcare industry (from pharmaceutical companies to healthcare providers, insurance companies and medical diagnostic laboratories) will have also to carry out major changes in the years to come, possibly favoring the creation of global strategic alliances between academics, industry and administrations in order to facilitate and catalyze the arrival and development of P4 medicine.

Conclusions and future perspectives

The scientific advances and technological breakthroughs of the last few decades, together with the birth of the new science of complex systems and networks, have prepared the ground for the birth and development of a new way to handle infectious diseases: P4 medicine. This short review justifies this possibility, while discussing its main advantages and current limitations. For those sceptical readers who consider that this is still far off and is confined, or nearly so, to the realm of science fiction, I would like to remind them that just seventeen years ago sequencing a bacterial genome was a daunting task and its achievement deserved the front page of *Science*. Nowadays the same result does not merit even a full length paper in a specialized journal. On the whole, despite the fascinating technical problems ahead, P4 medicine shows great promises to maximize wellness for each individual rather than simply to treat infections. The future is just around the corner!

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