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Principles of Diagnosis of Infectious Diseases

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Glossary

Contagion The ability of a disease to spread by exposure of a person to an infected patient.

Cytopathic effect The microscopic observation of damage to a cell in culture after infection by a pathogenic agent, usually a virus.

Differential diagnoses A list of potential diagnoses in a patient who develops a particular set of clinical manifestations.

Immunohistochemistry A method by which antigens such as those of a particular agent are detected in a tissue section

by microscopy by reaction with an antibody and subsequent colorimetric or fluorescent signal-generating methods.

In situ hybridization A method for the detection of a genetic sequence in tissue by annealing with a complementary nucleic acid probe coupled with colorimetric, fluorescent, or radiographic detection.

Opportunistic infection Infection with a normally nonpathogenic organism that occurs in an immunocompromised host.

Prior to the work of Koch and Pasteur demonstrating in 1876 that anthrax is caused by the microscopic bacterium *Bacillus anthracis*, the concept of infectious diseases did not exist. Indeed, at that point, there was only one curiosity that could be called an infectious disease, namely, anthrax. Robert Koch's teacher, Jacob Henle, had published in 1840 a theoretical treatise, 'On Miasmata and Contagia,' in which he put forth the criteria that one would have to meet to establish that a microorganism caused disease. These criteria now known as Koch's postulates are considered the fundamental steps by which an agent is proven to cause a particular infectious disease (Table 1).

Three centuries earlier, Hieronymus Fracastoro had described clearly the principles of contagion, that is, that exposure of a person to a specific disease such as measles could result in the individual's development of measles, not smallpox, and exposure to smallpox resulted in cases of smallpox, not measles.

The scientific identification of viruses, bacteria, fungi, algae, protozoa, and helminths as etiologic pathogens of specific diseases then exploded after elucidation of the etiology of anthrax from the late nineteenth century to today. Progress employed available tools such as microscopy, experimental animals, and microbial cultivation techniques and advanced more rapidly with the knowledge of the nature of viruses, immunologic methods, electron microscopy, and molecular methods.

The Clinical Context

When a patient seeks medical attention because of illness, the physician searches for evidence to establish a diagnosis that leads to treatment that will cure or ameliorate the illness. Prior to the medical encounter, the diagnostic possibilities are enormous ranging from medical and surgical diseases to the field of psychiatry. Infectious diseases in theory offer the greatest opportunities for specific

nonsurgical treatment of illness. The key to a favorable outcome is an astute clinician who seeks appropriate clinical, occupational, social, and travel history; considers wisely the potential epidemiological exposures; and gathers incisive data from the physical examination, radiological studies, and laboratory testing. The intermediate result is a prioritized list of differential diagnoses with focus on identifying diseases with the highest clinical consequences and those that are amenable to treatment.

Specific diagnoses require isolation of the agent in culture, microscopic visualization of the pathogen in tissue lesions, and/or detection of a specific host immune response to the organism. These tasks are accomplished by clinical microbiology and immunology laboratories and pathologists.

Isolation of the Agent in Culture

Bacteria comprise the group of agents of the greatest volume and therapeutic consequence that are cultivated in the microbiology laboratory. The culture results must be interpreted with knowledge of the pathogenic potential of the bacterial species isolated and the normal microbial flora at the anatomical site that was the source of the cultured sample. The recognition of contaminants and opportunistic pathogens requires specific knowledge and judgment. *Escherichia coli* could be the cause of septic shock or enterocolitis or merely normal flora or a contaminant. On the other hand, *Mycobacterium tuberculosis* is always a pathologically significant isolate.

There are numerous approaches to the isolation of bacteria that are designed to recover particular pathogens that involve the appropriate media containing particular nutrients, salts, inhibitors of other organisms, and growth conditions (e.g., temperature, pH, and anaerobic atmosphere). Selection of culture media (nonselective, selective, and differential) for a particular specimen is mostly based on the clinical history and the specimen source (blood, feces, CSF, and sputum). Automation of blood culture systems has

Table 1 Henle–Koch postulates for the demonstration that a microorganism causes a disease

1. The organism occurs in every case of the disease in question and under circumstances that can account for the pathological changes and clinical course of the disease
2. It occurs in no other disease as a fortuitous and nonpathogenic parasite
3. After being fully isolated from the body and repeatedly grown in pure culture, it can induce the disease anew

Based on Rivers' translation (1937).

greatly expedited and improved the isolation of infectious agents from blood. It is necessary to specify whether the culture requested is routine, anaerobic, or other specific microbial groups (e.g., *Mycobacteria*) or a particular agent (e.g., *Brucella*). Some bacteria grow slowly requiring that cultures be requested to be held longer if certain organisms are suspected. Some bacteria have yet to be cultivated (e.g., *Treponema pallidum*).

Although a few fungi grow on some standard bacterial media, most fungi are recovered in the mycology laboratory on media designed especially for their propagation. Fungi that are widely disseminated as spores in the environment and may contaminate cultures or normal yeast flora of some body sites require judgment to initiate or withhold antifungal treatment. Identification of fungi has been accelerated greatly in microbiology laboratories by performing either hybridization tests or polymerase chain reaction (PCR) on media growing a fungus that is not identifiable by conventional morphological techniques such as blood culture bottles that contain yeast growth.

Identification of *Mycobacteria* has also been greatly improved by the availability of DNA-based tests on specimens inoculated into liquid medium. Once mycobacterial growth is flagged by the automated system, molecular identification is possible, shortening the time until appropriate treatment is initiated.

Viral infections can be diagnosed by propagation of viruses in cell culture. The presence of viral replication in monolayers of cells in the virology laboratory is classically recognized by injury to the cells caused by the viral infection, known as cytopathic effect. Many viruses do not cause microscopically detectable injury to the cell monolayer, and there is no cell type that is universally susceptible to all families of viruses. Moreover, there are viruses that have yet to be recovered in cell culture. It is wise to provide the name of the patient's condition (e.g., meningoencephalitis or pneumonia) to the virology laboratory and to indicate the particular viruses that are suspected in order that appropriate cells and procedures will be employed.

Viruses that are recovered in cell culture can be identified by immunologic and molecular methods. Direct detection by such methods has replaced a large portion of viral isolation efforts.

Some viruses (e.g., the herpes viruses including herpes simplex virus types 1 and 2, cytomegalovirus, varicella–zoster virus, and Epstein–Barr virus) cause infections that persist long after the acute illness. Recovery of any of these viruses other than in the classic acute infection may reflect asymptomatic viral shedding or an opportunistic reactivation causing

clinical illness. The viral quantities are greater in clinically significant conditions.

Detection of a Specific Host Immune Response

The immune system, which evolved to control infectious diseases, also provides an approach to determine the diagnosis of an infectious disease. The adaptive immune response recognizes antigens of the infecting agent by production of antibodies and generation of antigen-specific T lymphocyte-mediated cellular immunity. The antigens that are recognized range from epitopes that are present on only one species, subspecies, or strain to antigens that are shared by agents in a genus, family, or larger group. The patient's antibodies to particular antigens that are detected and measured in the serology laboratory frequently identify the agent of the infection. Various serological tests employ the methods of immunofluorescence, enzyme immunoassay, agglutination, Western immunoblot, or others.

The drawbacks and pitfalls of the serological diagnostic approach include the frequent absence of antibodies in serum at the time of the patient's presentation for medical diagnosis early in the course of illness prior to their production, the occurrence of cross-reactive antibodies stimulated at an earlier time by other organisms that may be misinterpreted as diagnostic of the current illness, the occurrence of persistent specific antibodies stimulated by a previously resolved infection, and misinterpretation of the reported results owing to lack of knowledge of the levels of antibodies that are generally considered diagnostic.

Visualization of the Infectious Agent in Tissue Sections

Although it is sometimes possible to observe infectious organisms by direct examination of a clinical specimen macroscopically (e.g., worms passed in feces) or microscopically (e.g., *T. pallidum* by dark-field microscopy of a genital lesion of suspected syphilis), diagnostic evaluation usually begins with a stained smear of sputum, cerebrospinal fluid, urine, wound exudate, or other specimen. The pathologist examines microscopic sections stained routinely with hematoxylin and eosin (H&E). Large organisms such as helminths are readily observed and can be identified by their specific morphological characteristics by one with particular expertise.

Routinely stained sections reveal brown-pigmented fungi, which stand out from the pink and blue of eosin and hematoxylin.

Some eukaryotic protozoa and fungi can be detected and identified by the presence of pigment (e.g., some malarial parasites), subcellular structures (e.g., kinetoplasts of *Leishmania*), or particular size, reproductive process, and cell wall structure (e.g., broad-based budding and refractile cell wall of *Blastomyces dermatitidis* and large sporangiothecae containing endospores of *Coccidioides immitis*).

Some viruses cause pathological changes in the infected cells such as the formation of syncytial multinucleated giant cells and accumulations of proteins often of viral origin that are stained with eosin or hematoxylin (viral inclusions) in the nucleus or cytoplasm. Recognition of the cytopathic effects and eosinophilic or basophilic cytoplasmic or intranuclear inclusions can lead to a specific diagnosis such as rabies or cytomegalovirus infection or point to a group of viruses that cause similar pathological changes such as herpes simplex viruses 1 and 2 and varicella-zoster virus.

More often, infectious organisms are detected in smears of clinical samples or histological sections that have been stained by methods such as the gram stain that color gram-positive bacteria dark blue and gram-negative bacteria pink. The morphology of the bacteria as cocci or bacilli further characterizes the agent. The acid-fast stain is particularly useful for detecting *Mycobacteria* and diagnosing tuberculosis. Bacteria that stain poorly or not at all by the gram method can be visualized after silver impregnation staining (e.g., the Warthin–Starry method), which can detect *Treponema*, *Legionella*, *Leptospira*, and indeed nearly all bacteria. A separate silver-staining technique (e.g., Gomori’s method) is very useful for detecting fungal yeast and hyphae. The only pathogenic fungus with a mucin capsule, *Cryptococcus*, is identified by a stain such as mucicarmine.

Immunohistochemistry

The most powerful tool for specific microscopic diagnosis of infectious diseases is immunohistochemistry. Antibodies to antigens of particular bacteria, viruses, fungi, and protozoa have been produced, standardized, and made commercially available to react with the agents after appropriate processing of the tissue sections. After incubation of the antibody with the appropriately prepared microscopic section, the unbound antibody is washed away. Only antibody bound to the infectious agent’s antigens remains bound to the tissue section. The bound antibody is reacted with a series of reagents that deposit a colored precipitate on the antigen, allowing the detection, anatomical localization, and morphological analysis that has the ability to identify the agent and establish the diagnosis. The location of the agent is often very particular, and the expected size and shape of an organism add weight to confidence in the diagnosis.

In Situ Hybridization

Each infectious species contains a unique genome, DNA for bacteria, fungi, protozoa, and some viruses and RNA for the other viruses. Transcription of the genetic DNA into messenger RNA results in many copies of some RNA sequences. *In situ* hybridization is the method of detecting specific DNA or

RNA sequences by annealing complementary DNA or RNA containing a label that is used to generate a visible product at the location of the target nucleic acid sequence in infected tissues. Those powerful techniques have not been exploited for the diagnosis of infectious diseases as extensively as immunohistochemistry. However, they are very effective and can be used to distinguish among even closely related organisms.

Detection of DNA or RNA with Nucleic Acid Sequence of a Specific Infectious Agent

Powerful methods for the amplification of the nucleic acid sequences of few genomes of a bacterium, virus, or other agent to produce and detect millions of copies of the targeted sequence have provided a sensitive approach to the diagnosis of infections. The key to the technique is polymerase chain reaction (PCR), a heat-stable DNA polymerase from organisms that reside in thermal springs. Thirty or more thermal cycles of DNA synthesis initiated by primers with specific DNA sequences yield logarithmic increase in the particular DNA fragment. For RNA genomes such as those of RNA viruses, a step of enzymatic conversion of RNA to DNA is performed using reverse transcriptase.

Discovery of the Etiologic Agents of Emerging Infectious Diseases

Identification of a novel, causative agent of a previously unknown infectious disease is a special challenge. In 1967, the US Surgeon General stated, “It’s time to close the book on infectious diseases. The war on infectious diseases is over, and we have won.” It was believed for the next quarter of a century that vaccines and antibiotics had controlled infectious diseases. In reality, approximately 70 previously unknown agents of infectious diseases have been identified beginning in 1967 with Marburg virus, the original agent of an African viral hemorrhagic fever. Because it is clear that the emergence of infectious diseases will be a continuing occurrence, medical personnel should understand how these mysteries are solved.

There are four steps in the discovery of emerging pathogens: (1) awareness of the presence of an unknown disease, (2) detection of an infectious agent in association with the disease, (3) determination that the agent causes the disease (Koch’s postulates revisited), and (4) determination that the etiologic agent is novel. Awareness of an unknown disease may occur because of the abrupt onset of a cluster of cases of a severe illness or recognition of distinctive gross or microscopic pathological lesions. Severe acute respiratory syndrome caused by a novel coronavirus is a recent example.

In contrast, a syndrome of unknown etiology may have been well defined clinically for many years prior to discovery of an infectious etiology. Gastric and duodenal ulcers caused by *Helicobacter pylori* and cat scratch disease caused by *Bartonella henselae* are examples of such diseases for which a microbial agent was unsuspected or long elusive, respectively. The methods for the initial detection of a previously unknown agent have included microscopy, bacterial culture, cell culture, animal inoculation, electron microscopy, cross-reaction of

antigens in serological tests, immunohistochemistry, and PCR amplification followed by DNA sequencing of the products. These are the same methods used to diagnose infection experimentally and characterize the agents. The methods used to support the etiologic role of the pathogen have included experimental animal infection, self-experimentation, laboratory accident, and demonstration of the development of a specific immune response to the agent. Demonstration that the pathogen is novel includes classical biochemical phenotyping, antigenic analyses, DNA or RNA gene sequencing, unique ultrastructural morphology, and identification of a novel toxin.

The field of emerging infectious diseases is where diagnostic infectious diseases and basic science meet to solve current biomedical infectious challenges. The roles of the astute clinician, pathologist, and laboratory technologist have been important. Knowledge of microbiology, virology, immunology, molecular biology, and both cutting-edge and classic technologies has been critical to effective application to the discovery of novel pathogens. Often, identification of the genus of the agent allows the selection of effective

antimicrobial treatment that would have been previously unknown. The payoff of knowledge is noteworthy.

Further Reading

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