NEW AND CONVENTIONAL METHODS OF DIAGNOSIS OF RICKETTSIAL DISEASES

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Introduction

Rickettsias are obligate intracellular organisms usually seen microscopically as pleomorphic coccobacilli. Each of the rickettsial pathogenic for humans is capable of multiplying in one or more species of arthropodes as well as in animals and humans; Indeed the majority of the rickettsias are maintained in nature by a cycle which involves an insect vector and an animal reservoir, **[Theodore, 1991]**.

Rickettsial diseases of humans consists of a variety of clinical entities caused by microorganisms of the family Rickettsiacea. It could be classified into five major groups namely typhus fevers, spotted fevers, Q fever, scrub typhus and trench fever, **[Richard, 1985]**.

Rickettsial diseases are characterized by sudden onset of fever, headache, myalgias and arthralgias. Rash frequently accompanies all rickettsiosis except Q fever. The type of rash and its distribution help to distinguish the various diseases. pulmonary and gastrointestinal symptoms are common in most rickettsiosis, **[Joseph and Daniel, 1988]**.

A study by **Botros et al., [1989]** was done to determine the prevalence of rickettsial infections among humans residing in the Nile Delta, Suez canal area and Nile Valley of Egypt. The antibody prevalence rate for R. typhi among patients with febrile illness ranged from 25% to 41% and from 2% to 15% for R. conorii. These findings suggested that Rickettsiae are of considerable importance as a cause of human infection in Egypt, and R. typhi and R. conorii are the most common causes of human rickettsial diseases in Egypt, **[Botros et al., 1989]**.

In addition to the conventional methods of laboratory diagnosis, recently there are new diagnostic techniques as ; isolation of rickettsia by centrifugation shell-vial technique, detection of rickettsia using polymerase chain reaction and western immunoblotting, **[Teysseire and Raoult, 1992]**.

The Aim of the work:

The aim of this work is to present and evaluate a review on the different methods of diagnosis of rickettsial diseases as an important cause of pyrexia of unknown aetiology.

MICROBILOGY

Definition and morphology:

Rickettsiae are small prokaryotic cells, they are gram negative organisms. Rickettsiae are pleomorphic, short, rod-shaped or coccobacillary organisms, ranging from 0.8 to 2um long and 0.3 to 0.5um wide. However, C. burnetii ranging in size from 0.4 to 1.0um long and 0.2 to 0.4um wide, **[Silverman and Wisseman, 1978]**.

Characteristically, they vary in diameter, members of the spotted fever group are the largest, Coxiella burnetii is the smallest, and members of the typhus are intermediate, **[Ormsbee, 1989]**.

Rickettsiae occur singly, in pairs, in short chains or in filaments. The cells may be associated with binary fission, and often in dense irregular masses within affected cells, particularly those of mesothelial origin which line the serous cavities. Some species are found only in the cytoplasm, but others, such as those of the spotted fever group, occur in the nucleus as well. C. burnetii appears as a microcolony in a cytoplasmic vacule with many scores of organisms, individual organisms may be difficult to visualize, the entire contents of the vacule appearing to be a granular mass, **[Ormsbee, 1989]**.

Rickettsiae do not have flagella, they are non-motile and do not show obvious capsules, **[Burrows, 1973]**.

Staining properties:

The organisms stain gram negative, but this stain is of no diagnostic value, and they need special stains for good viewing by light microcopy, [Wisseman, 1990]. Except for R. tsutsugamushi, rickettsias stain a brilliant red with Gimenez stain purple with Giemsa stain, [Ormsbee, 1989], also stained red against a blue background with MacChiavello's stain and a light blue against a pink background with Castaneda's stain. They may also be stained with Wright's stain in tissue sections to give a blue nucleus and pink cytoplasm, The coccoid and coccibacillary forms stain uniformly, but bipolar staining of the bacillary forms is not uncommon, [Burrows, 1973].

R. tsutsugamushi stains reddish-black with a modified Gimenez stain, [Wisseman, 1990].

Ultrastructure

Ultrastructurally. rickettsiae resemble gram negative bacteria. The cell wall and the cytoplasmic membrane are trilayered structures, [Barron, 1983].

Rickettsial cell walls are made up of peptidoglycans containing muramic acid and diaminopimelic acid, [Jawetz et al., 1991]. Peptidoglycan layer lies between the outer membrane (OM) and the inner membrane (IM). A five-layered cell wall has been described for R. prowazekii, and electron microscopy has also revealed a capsular like structure for it, [Barron, 1983].

Lipopolysaccharide (LPS) has been demonstrated in many rickettsias, but in R. tsutsugamushi it is absent. Observation of various species of rickettsia also have revealed the presence of chromosomal structure and ribosome like structures, [Barron, 1983].

The morphology of R. tsutsugamushi is distinct among the rickettsia in that its outer leaflet is much thicker and its inner leaflet is much thinner than the corresponding leaflets in other rickettsial species, [Joseph and Daniel, 1988].

Antigenic properties:

A group antigen is shared by members of each group in which there are a number of different species causing different diseases, as spotted fever and typhus fevers. On the other hand there in only one organism, C. burnetii, associated with Q fever. Group antigens are ether soluble and have been widely used in the complement fixation test. Species specific antigens have also been prepared from purified rickettsiae after removal of group antigens, which permits identification of specific organisms (species) within a group, **[Barron, 1983]**.

The rickettsiae contain both somatic and capsular antigens, and a more complicated antigenic situation has been found in C. burnetii in that phase variation has been demonstrated. This is analogous to the S--R (smooth to rough) variation described for bacterial colonies. Freshly isolated strains exist in phase I; after passage, usually in chicken embryos, phase II organisms emerge.

Phase I organisms have a polysaccharide material that is lost on passage, **[Burrows, 1973]**.

Serologic cross-reaction occurs between some members of pathogenic rickettsia and OX-2, OX-19 and OX-K strains of proteus vulgaris.

Thus, as rickettsiae and proteus organisms share certain antigens, so during the course of rickettsial infections, patients develop anti rickettsial antibodies that cross-react with strains of proteus, [Wisseman, 1990].

Metabolism:

Purified rickettsiae contain both RNA and DNA in a ratio of 3.5:1. They contain various enzymes concerned with metabolism. They possess both synthetic and energy-yielding enzyme systems. Thus they oxidize intermediate metabolites like pyruvic, succinic, and glutamic acids and can convert glutamic acid into aspartic acid, **[Jawetz et al., 1991]**.

ATP can be produced from glutamate (but not from glucose) via the citric acid cycle, additional ATP may be imported by an ATP - ADP translocator mechanism. A limited amount of protein synthesis, but no multiplication, may occur in R. prowazekii outside the host cell under special conditions, **[Winkler, 1976]**.

Folic acid is present and a possible function is suggested by the synthesis of serine from glycine and formaldehyde in the presence of tetrahydrofolic acid by the Q fever rickettsia, **[Burrows, 1973]**.

Although members of the genus Rickettsia grow only in the presence of eukaryotic host cells, they possess considerable synthetic capabilities and generate their own ATP via the tricarboxylic acid cycle, **[Weiss and Moulder, 1984]**.

Resistance:

Rickettsiae lose their biologic activities when they are stored at $0^{o}C$ this is due to the progressive loss of nicotinamide adenine dinucleotide (NAD).

These properties can be restored by subsequent incubation with NAD. They may also lose their biologic activity if they are starved by incubation for several hours at $36^{o}C$. This loss can be prevented by the addition of glutamate, pyruvate, or adenosine triphosphate (ATP). Subsequent incubation of the starved organism with glutamate at $30^{o}C$ leads to recovery of activity. Although rickettsiae are usually killed by storage at room temperature, dried feces of infected lice may remain infective for months at room temperature, **[Weisseman and Waddell, 1975]**.

In general, rickettsiae are quickly destroyed by heat, drying and bactericidal chemicals. The organisms of Q-fever (C.burnetii) is the rickettsial agent most resistant to drying. This organism may survive pasteurization at $60^{o}C$ for 30 min. and can survive for months in dried feces or milk. This may be due to the formation of endospore like structures by C. burnetii.

So, effective disinfection can be achieved with 2% formaldehyde, 1% Lysol, ethyl ether and 5% hydrogen peroxide, **[Jawetz et al., 1991]**.

Growth and cultural characteristics:

With the exception of Rochalimea quintana, which grows slowly on a modified blood agar medium, rickettsiae are obligate intracellular prokaryotes, [Ormsbee, 1989].

Rickettsiae grow readily in the yolk sac of the embryonated egg. Many rickettsial strains also grow in various kinds of tissue cultures. However, rate of growth is relatively slow, they multiply by binary transverse fission and the generation time is as long as 8-16 hours, [Jawetz et al., 1991].

It has been suggested that rickettsiae grow best when the metabolism of the host cells is low.

Thus, their growth is enhanced when the temperature of infected chick embryos is lowered to 32°C.

If the embryos are held at 40°C, rickettsial multiplication is poor. Conditions that influence the metabolism of the host can alter its susceptibility to rickettsial infection.

Rickettsiae grow in different parts of the cell. The typhus group are usually found in the cytoplasm, and the spotted fever group, in the nucleus. Coxiellae grow only in cytoplasmic vacuoles, [Jawetz et al., 1991].

Growth in the cytoplasm: Rickettsia attach to the host cell