CHLAMYDIA ANTIGENS AND USES THEREOF

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BACKGROUND

Chlamydia trachomatis (Ct) is the globally leading cause of bacterial sexually transmitted infections (STI), with an estimated 131 million new cases of genital Ct infections per year. Symptoms of acute infection include e.g. painful urination and urethral or vaginal discharge, but the majority of infections are asymptomatic. If untreated, chlamydia can give rise to chronic infection and sequelae that include pelvic inflammatory disease, chronic pelvic pain, ectopic pregnancy and tubal factor infertility. Although persistent infection with high-risk HPV types is a known prerequisite for cervical cancer (CxCa) development, Ct has been discussed as a co-factor in CxCa development, based on its biological features such as induction of inflammation, evasion of cell-mediated immunity, inhibition of apoptosis, and involvement in DNA damage and genetic instability. Additionally, large seroepidemiological studies have reported significant associations between Ct seropositivity and CxCa.

Most existing diagnostic methods only utilize a very small fraction of the almost 900 open reading frames (ORFs) encoded in the Ct genome. Protein microarrays are an excellent tool to identify disease-associated antibody reactivity patterns since they possess high density capacity and allow the simultaneous detection of antibodies to a large variety of antigens, up to an entire bacterial proteome. Wang et al. described a genome-wide Ct microarray in ELISA microtiter plates. These approaches are extremely time-consuming and resource-intensive, and require large sample volumes. In order to eliminate the need for cloning, expressing, purifying and immobilizing the proteins individually, in situ protein array production strategies have been developed allowing proteins to be synthesized directly on the microarray surface using cell-free expression systems by the multiple spotting technique (MIST), where individually cloned expression vectors or PCR products are transferred onto microarray slides in a first spotting step. Subsequently, a cell-free transcription and translation mixture is spotted directly on top of the first spot in a second spotting step. As each synthesis is performed in a few nanoliters, reagent consumption is low. Synthesis of each protein occurs in an individual droplet on the planar surface, minimizing the risk of contamination; also, no background is generated between the protein spots. Proteins binding to the solid support do not require capturing agents such as antibodies. Previous studies have shown that most proteins can be produced in full-length, and very many fold into a functionally active conformation. Therefore, it is highly desirable to develop an assay that circumvents individual cloning, expression and purification of hundreds or thousands of ORFs, in combination with the advantages of a slide-based microarray with regard to reagent and
sample consumption. Using Ct as a complex model organism, we describe a novel method to perform proteome immunoassays (PIA). Our method to produce bacterial whole-proteome microarrays is based on the combination of MIST and cell-free, on-chip protein expression based on expression constructs generated by two successive PCRs directly from bacterial genomic DNA. PIA bypasses both the generation of expression vectors, and purification and printing of proteins onto microarrays. Bacterial proteins expressed on the microarray can be recognized by serum antibodies, thereby providing an efficient method for immunoprofiling of patient samples which allows the de novo identification of disease-related antigens.

SOLUTION

The technology can be used for diagnosing cervical carcinoma or for diagnosing a Chlamydia spp. infection in a patient comprising

1. contacting an antibody-containing sample of said subject with at least one Chlamydia trachomatis polypeptide or epitope fragment thereof;
2. detecting the presence of at least one antibody specifically binding to at least one Chlamydia trachomatis polypeptide or epitope fragment thereof; and
3. thereby providing the diagnosis; and to devices and uses related thereto.

ADVANTAGES
TECHNOLOGY OFFER

- Immunoprofiling of patient serum
- Diagnostic assay

SCOPE OF APPLICATION

- Immunoprofiling
- Diagnostic assay