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Helicobacter pylori FliD protein is a highly sensitive and specific marker for serologic diagnosis of H. pylori infection

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abstract

Screening for H. pylori in large populations continues to be a challenging task, since available tests have limited sensitivity and specificity, which, in population-based approaches, leads to significant numbers of false positive and false negative results. Various H. pylori proteins associated with virulence are highly immunogenic and therefore candidates to detect the infection. There are currently no defined markers that are recognized in all H. pylori infected patients and that do not show cross-reactivity with other bacterial proteins.

We identified the H. pylori "hook-associated protein 2 homologue", FliD (UniProtKB/Swiss-Prot: P96786.4) as a novel marker of infection for serological analysis. The H. pylori FliD protein is an essential element in the assembly of the functional flagella. However, this virulence factor has not yet been tested as a diagnostic marker in serology. For this purpose FliD was recombinantly expressed in E. coli, purified by affinity chromatography and gel filtration and used to coat ELISA plates or immobilized on nitrocellulose stripes. To evaluate its antigenicity we screened a defined panel of patient sera. The recombinant H. pylori FliD protein reacted with a high percentage of human sera. Among 318 samples reported positive by histology, 310 (97.4%) were tested positive by FliD Line assay, and 165 out of 170 samples were tested positive by ELISA (97%). We could also reconfirm 297 out of 300 (99%) negative sera by Line assay and 73 from 76 (96%) by ELISA. Taken together, application of FliD in serological diagnosis of H. pylori infection presents a high specificity of up to 99% and a sensitivity of up to 97%. This makes especially the FliD ELISA a simple, cost effective and highly efficient tool to detect H. pylori infection in developing countries where prevalence is high and other screening methods are either not available or are unaffordable.

Introduction

Helicobacter pylori (H. pylori), a microaerophilic, Gram-negative and spiral bacterium is colonizing approximately half of the world population and considered to be a human-specific gastric pathogen (Michetti et al., 1999). Most infected individuals show asymptomatic chronic gastritis. However, in some subjects the infection causes chronic active gastritis, peptic ulceration and atrophy, and plays an important role in the development of mucosa-associated lymphoid tissue (MALT) lymphoma, gastric adenocarcinoma and primary gastric non-Hodgkin's lymphoma (Suganuma et al., 2001).

The World Health Organization has categorized H. pylori as a class I carcinogen (Goto et al., 1999), and direct evidence of car- cinogenesis has been demonstrated in animal models (Honda et al., 1998; Watanabe et al., 1998). Eradication of H. pylori can prevent gastric cancer in humans (Uemura et al., 2001). Test and treat strategies have been considered in populations with high gastric cancer risk (Yamaoka et al., 1998). However, such approach is hampered by the lack of efficient and affordable screening systems especially for countries of lower socioeconomic status. In these countries only

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