Helicobacter pylori – interactions with phagocytes

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INTRODUCTION

Helicobacter pylori possess the remarkable ability to persist in the human stomach for long periods, probably for life. For such prolonged colonization this pathogen must have acquired sophisticated mechanisms allowing it to escape both innate and acquired cellular immunity. The most important accessory cells directly involved in the eradication of bacteria are phagocytic cells of the monocytic and myelocytic lineages and, in particular, the macrophages and polymorphonuclear neutrophils. Bacteria which are directly recognized, ingested and destroyed by phagocytes are not pathogenic in immunocompetent individuals. In contrast, some bacterial pathogens have evolved various mechanisms allowing them to resist direct engulfment by phagocytes. These pathogens usually exploit easily attainable host constituents which are pivotal in physiological functions. H. pylori express numerous surface ligands: sialic acid-specific haemagglutinins, heparan sulphate-binding proteins, extracellular matrix-binding compounds, Lewis X and Lewis Y determinants, which interact with specific receptors on various host cells including phagocytes. In this study we try to establish the role of those ligand–receptor interactions in the engulfment of H. pylori reference and clinical strains by phagocytes.

METHODS

Patients

Twelve children and young adolescents (aged 8–18 years) and nine adults (aged 19–70 years) with chronic gastritis or gastroduodenitis were investigated. From each patient an H. pylori strain was isolated. Five healthy subjects (aged 24–50 years) were included in the study. In the sera from all investigated subjects, IgG antibodies to surface bacterial antigens, present in a glycine acid extract of H. pylori CCUG 17874, were estimated by enzyme-linked immunosorbent assay (ELISA).
INTERACTION WITH PHAGOCYTES

Bacteria

The reference *H. pylori* strains CCUG 17874 and 17875 from Culture Collection University of Gothenburg, G 33 strain obtained from N. Figura, strain 25 from an adenocarcinoma case (Lund University, Sweden), and several clinical isolates from dyspeptic patients in Poland, were used for the study. The strains were classified on the basis of the specificity of their haemagglutini5,8,9, the heparin/heparan sulphate binding activity7, and the expression of Lewis X determinants5,10. The bacteria were grown on 5% blood agar for 36 h at 37°C under microaerophilic conditions. Before using in the phagocytosis assay the bacteria were washed in phosphate buffered saline (PBS), pH 7.2, heat-killed (60°C for 30 min), and labelled with fluorescein isothiocyanate (FITC)10 or stained for 20 min at room temperature with crystalline violet (0.5% solution in PBS/20% methanol) and then washed with PBS.

Modulation of bacterial surface

The *H. pylori* cells (200 μl, 2 × 10^9 cells/ml in RPMI-1640 medium, Difco, Detroit, US) were mixed with 200 μl of: fetuin, asialofetuin, heparin (H), heparan sulphate (HS), hyaluronic acid (1000 μg/ml in RPMI-1640, Sigma, St Louis, US), vitronectin (100 μg/ml in RPMI-1640, Polyclone, Lask, Poland) alone or vitronectin with fresh or heat-inactivated (56°C, 30 min) normal rabbit serum or rabbit antiserum against surface sialic-acid specific haemagglutinin – sHA (1:10)11, human sera from *H. pylori*-infected patients and healthy donors (1:5), or with 100 ng/ml of anti-Lewis X antibodies (mouse monoclonal IgM, Monocarb, Lund, Sweden). Bacterial suspensions were agitated for 1 h at 37°C and then distributed (100 μl containing 1 × 10^7 bacterial cells) into the wells of microplates containing phagocytes.

Phagocytes

Four days culture of macrophages (J 774, ATCC, Rockville, US) in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 1 mmol l-glutamine and 50 μg/ml gentamicin was used12. The macrophages (1 × 10^5 in 100 μl RPMI-1640 medium with FCS, were seeded in the wells of microplates for 18 h (37°C, 5% CO2). Before the assay the monolayers were washed with RPMI-1640 medium. The mouse peritoneal macrophages were obtained, by peritoneal lavage with worm RPMI-1640 medium containing 5% FCS, from female Balb/c mice (BBR Centre, Ry, Denmark), injected intraperitoneally (3 days earlier) with peptone proteose (Difco). The macrophages were immobilized on the microplates, and the number of monolayer-forming cells was established13. In some experiments the macrophage monolayers were incubated with *Flavobacterium heparinum* heparinase: (I) H-2519, (II) H-6512, (III) H-8891 (Sigma), for cleavage of heparin/heparan sulphate or with *Clostridium perfringens* type IV neuraminidase (Sigma) for cleavage of terminal sialic acid14. Polymorphonuclear (PMN) cells were separated by gradient centrifugation15 from the blood of healthy donors seronegative for anti-*H. pylori*