BACTERIAL PATHOGENICITY

Induction of secretion of interleukin-8 from human gastric epithelial cells by heat-shock protein 60 homologue of *Helicobacter pylori*

HIROYUKI YAMAGUCHI, TAKAKO OSAKI*, NAOTO KURIHARA†, MASAKI KITAJIMA†, MASANORI KAI‡, MOTOMICHI TAKAHASHI, HARUHIKO TAGUCHI and SHIGERU KAMIYA

Department of Microbiology and *Division of Flowcytometry, Kyorin University School of Medicine, Mitaka, †Department of Surgery, Keio University School of Medicine, Shinanomachi, Shinjuku and ‡Department of Microbiology, Leprosy Research Center National Institute of Infectious Diseases, Higashimurayama-shi, Tokyo, Japan

Escherichia coli cells expressing fusion proteins consisting of β -galactosidase and bacterial heat-shock protein (HSP) 60 of *E. coli*, *Yersinia enterocolitica* or *Helicobacter pylori* were constructed, and designated as HY1, HY2 or HY3, respectively. Fusion proteins prepared from HY2 and HY3 induced secretion of interleukin-8 (IL-8) from human gastric epithelial KATO III cell cultures. On the other hand, the parent strain (*E. coli* pop2136), PEX (pop2136 transformed by vector) and fusion protein prepared from HY1 did not induce IL-8 secretion from KATO III cells. Other human gastric (MKN45) and non-gastric cell lines (Int 407 and A549) did not secrete IL-8 following treatment with these proteins. These results indicate that *H. pylori* HSP60 induces IL-8 secretion from human gastric cells and the levels of IL-8 differ among the various gastric cell lines, suggesting that HSP60 might be an important virulence factor associated with chronic gastric inflammation following *H. pylori* infection in man.

Introduction

Interleukin-8 (IL-8) is a potential mediator associated with gastric inflammation in patients with *Helicobacter pylori* infection [1, 2]. Rieder *et al.* reported that direct contact of *H. pylori* with epithelial cells was needed to induce IL-8 *in vivo* [3], indicating that a surface component of *H. pylori* might be important. An earlier study demonstrated that the heat-shock protein (HSP) 60 (Hsp B) homologue of *H. pylori* is located on the bacterial cell surface and is associated with adhesion of *H. pylori* to human gastric epithelial cells [4]. HSP60 conserved in prokaryotic cells and also eukaryotic cells is thought to facilitate the folding, unfolding and translocation of polypeptides as chaperonins and is associated with chronic inflammation [5, 6]. Therefore, HSP60 of *H. pylori* is a candidate virulence factor for chronic gastritis induced by *H. pylori*.

The present study examined whether recombinant *H.*

pylori HSP60 expressed by *Escherichia coli* induces IL-8 secretion from cultured human cell lines.

Materials and methods

Human cell lines

Human gastric carcinoma cells (KATO III, MKN45) and non-gastric cells (Int407, small intestinal epithelial cells; A549, lung cancer cells) were obtained from the Japanese Cancer Research Resources Bank (JCRB). They were grown at 37°C in RPMI1640 containing fetal calf serum 10% v/v in an atmosphere containing CO₂ 5%.

Monoclonal antibody (MAb)

MAb H9 (IgG2a), which reacted with *H. pylori* HSP60, was described previously [7]. The 60-kDa antigen derived from *H. pylori* strain TK1029 was partially purified by extraction from SDS-PAGE separating gel. BALB/c mice were immunised intraperitoneally (i.p.) with the antigen mixed with Freund's complete adjuvant (Difco Laboratories, Detroit, MI, USA), three times at an interval of 10 days. Ten days after the last i.p. injection, the mice were given the

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Corresponding author: Dr H. Yamaguchi (e-mail: hiro-ya@ta2.so-net.ne.jp).

partially purified antigen intravenously. Then 3 days after the last immunisation, spleens were removed for cell fusion with mouse myeloma cells (P3-X63-Ag8-U1). The hybridoma cells producing MAb that reacted with the affinity-purified *H. pylori* HSP60 and the sonicated MKN45 cells (human gastric cancer cell line) in ELISA were collected. The hybridoma cells with apparent specific antibody production were cloned by limiting dilution. Cells 10^6 were inoculated i.p. into a BALB/c mouse pretreated i.p. with pristane (Wako Pure Chemical) 0.5 ml, 4 days before the inoculation of the cells and ascites fluids were obtained from the mouse c. 2 weeks later. The immunoglobulins in the ascites fluids were purified with the Immunoglobulin-Easy-Separation kit (Pharmacia Biotechnology, Tokyo, Japan). The purified MAb was used for affinity chromatography and immunoblotting analysis.

Affinity-purified *H. pylori* HSP60

HSP60 was affinity-purified with specific MAb recognising *H. pylori* HSP60 from *H. pylori* strain TK1029 (*cagA* and *vacA* positive) isolated from a patient with gastric ulcer as described above [8].

Construction of *E. coli* expressing *H. pylori* HSP60

DNA fragments encoding *E. coli* GroEL, *Yersinia enterocolitica* HSP60 or *H. pylori* HSP60 were amplified from bacterial strains by PCR as indicated in Table 1. The primer sets used were as follows: upstream 5'-GAATTCATGGCAGCTAAAGACGTAAG-3', downstream 5'-GAATTCCTTACATCATGCCG-CCCTAGC-3' (*E. coli* and *Y. enterocolitica*); upstream 5'-GAATTCATGGCAAAAGAAATCAAATT-3', downstream 5'-GAATTCCTTACATCATGCCGCCCTAGC (*H. pylori*). The recognition sequences GAATTC with *EcoRI* restriction endonuclease were added on both sides of 5'-upstream and 3'-downstream for subcloning the resultant cDNA into a plasmid pEX. The resultant cDNA was integrated into a plasmid pEX capable of producing a fused protein with β -galactosidase [9]. The

constructed plasmids were transformed into *E. coli* pop2136. The bacteria were cultivated at 30°C and then shifted to 42°C for 2 h to induce the expression of the recombinant protein (fusion protein). Bacterial pellets were stored at -80°C until their use in SDS-PAGE. Fusion proteins expressed by pEX vector in *E. coli* pop2136 containing the *clts857* repressor have been shown to account for >30% of the total SDS-extracted bacterial proteins [10]. All sequences of the amplified cDNA inserted into plasmid pEX were confirmed by direct sequencing. The bacteria were cultivated at 30°C and then shifted to 42°C for 2 h to induce the expression of the recombinant protein (fusion protein). Bacterial pellets were stored at -80°C until use in SDS-PAGE.

Preparation of fusion proteins

The fusion proteins were prepared as described previously [10]. Each *E. coli* strain (HY1, HY2 or HY3) harbouring pHY1, pHY2 or pHY3 encoding *hsp60* of *E. coli*, *Y. enterocolitica* or *H. pylori*, respectively, was sonicated with a Sonifer 250 (Branson, NY, USA) and then insoluble fractions were collected by centrifugation at 10 000 g. The pellet (insoluble fraction containing fusion protein) was incubated in 50 mM Tris-HCl, pH 8.0, containing 1 mM EDTA, 1 mM DTT and 8 M urea for 1 h at 37°C. After the solutions containing fusion protein had been dialysed against PBS and sterilised by filtration, they were used for induction of IL-8 from cultured cell lines KATO III (human gastric cancer cells), MKN45 (human gastric cancer cells), Int407 (human small intestinal cells) and A549 (human lung cancer cells). Parental strains of *E. coli* pop2136 and PEX (pop2136 harbouring pEX) treated as described above were used as negative controls.

SDS-PAGE and immunoblotting

SDS-PAGE with acrylamide 8% w/v gels was performed as described by Laemmli [11]. Each bacterial pellet stored at -80°C (1 ml of cultured

Table 1. Plasmids and bacterial strains used

Plasmid or bacterial strain	Relevant characteristics or purpose for use
Plasmid	
pEX	Protein expression vector
pHY1	<i>groEL</i> of <i>E. coli</i> cloned into pEX
pHY2	<i>hsp60</i> of <i>Y. enterocolitica</i> cloned into pEX
pHY3	<i>hsp60</i> of <i>H. pylori</i> cloned into pEX
Bacterial strain	
<i>H. pylori</i> TK1029 (genotype: <i>cagA</i> +, <i>vacA</i> +)	Used for amplification of <i>H. pylori</i> HSP60-encoding gene by PCR
<i>Y. enterocolitica</i> ZM20 0:3	Used for purification of <i>H. pylori</i> HSP60 by affinity column with MAb* H20
<i>E. coli</i> pop2136	Used for amplification of <i>Y. enterocolitica</i> HSP60-encoding gene by PCR
	Used for amplification of <i>E. coli</i> GroEL-encoding gene by PCR
	Parent strain for transformation
PEX	<i>E. coli</i> harbouring pEX
HY1	<i>E. coli</i> harbouring pHY1
HY2	<i>E. coli</i> harbouring pHY2
HY3	<i>E. coli</i> harbouring pHY3

*MAb recognising *H. pylori* HSP60.

bacterial cells expressing fusion proteins) was suspended in 300 μ l of PBS. The bacterial solutions were mixed with 300 μ l of sample buffer (pH 6.8; 0.012 M Tris, glycerol 20% v/v, 0.015 M SDS and 0.4 mM 2-mercaptoethanol). The solutions were heated for 10 min at 100°C. Finally, 20 μ l of the bacterial lysates were loaded into each lane of a separating 8% gel. Immunoblot analysis was done as described by Towbin *et al.* [12]. After electrophoresis, the separated proteins were transferred to nitrocellulose membranes (Schleier and Schuell, Dassel, Germany) at 0.25A overnight. After blocking with phosphate-buffered saline (PBS; pH 7.4) containing skimmed milk 1% w/v (Yukijirushi Nyugyo, Sapporo, Japan) (PBS-S) for 1 h at room temperature, the membranes were treated for 1 h with MAb H9 diluted to 1 μ g/ml with PBS-S. They were then incubated for 1 h with goat anti-mouse IgC peroxidase conjugate diluted 1 in 500 with PBS-S. Immunoblots were developed with 50 mM Tris-HCl buffer (pH 7.4) containing H₂O₂ 0.12% v/v and 1 mM 3,3'-diaminobenzidine tetrahydrochloride (Dojinkagaku, Kumamoto, Japan).

Detection of IL-8 secretion

Established cultured human cells (3×10^4) were incubated in RPMI1640 containing fetal bovine serum 10% v/v with fusion proteins or control cell lysate (1.25–150 μ g/ml) for 24–96 h at 37°C. After cultivation, the amount of IL-8 in each culture supernate was measured by a sandwich-ELISA, with a commercially available ELISA kit (Cytoscreen Human IL-8; Bio Source International, CA, USA).

Statistical analysis

The statistical significance of the differences was assessed by the Friedman test.

Results

Profiles of the prepared fusion proteins

The profiles of the prepared fusion proteins were examined by SDS-PAGE and immunoblotting with MAb H9 directed to bacterial HSP60 [8]. As shown in Fig. 1, each *E. coli* (HY1, lane 4; HY2, lane 5; HY3, lane 6) expressing bacterial HSP60 was confirmed by SDS-PAGE as being constructed. All fusion proteins with mol. wts of *c.* 180 kDa (lanes 9–11) were recognised by MAb H9 directed to bacterial HSP60 [7]. The band corresponding to a 60-kDa molecule in all samples recognised by MAb H9 was thought to be native GroEL from the parent *E. coli* strain, and especially in lanes loaded with the fusion proteins, the intensity of bands was stronger than that in the other lanes. This phenomenon could be explained by the fact that the overexpression of foreign antigen such as the fusion protein in *E. coli* enhances production of native GroEL to eliminate the foreign protein [10].

Detection of IL-8 secretion from established human cell lines by recombinant *H. pylori* HSP60

As shown in Fig. 2, IL-8 secretion was induced from human gastric KATO III cells by HY3 (fusion protein of *H. pylori* HSP60). The amounts of IL-8 detected after cultivation with 150 μ g of fusion protein/ml for 24, 48, 72 and 96 h, were 2862, 2884, 3068 and

Fig. 1. Profiles of SDS-PAGE (lanes 1–6) and immunoblotting with MAb H9 recognising bacterial HSP60 (7–11) of fusion proteins prepared by treatment with 8 M urea. The prepared antigens were loaded as follows: lanes 2 and 7, control (pop2136); 3 and 8, PEX; 4 and 9, HY1; 5 and 10, HY2; 6 and 11, HY3.

Fig. 2. IL-8 secretion from human gastric carcinoma cell line (KATO III) induced by fusion protein PEX, HY1, HY2 and HY3. Concentrations ($\mu\text{g/ml}$) of the prepared antigens used were 1.25 (a), 6 (b), 30 (c) and 150 (d) and IL-8 secretion was assayed after incubation for 24 (■), 48 (■), 72 (■) and 96 (□) h.

4321 pg/ml, respectively. Similarly, HY2 (fusion protein of *Y. enterocolitica* HSP60) also induced a level of secretion of IL-8 as high as that induced by HY3. Furthermore, a significant dose-response was observed when HY2 and HY3 were used in IL-8 induction experiments ($p < 0.01$). Neither HY1 (fusion protein of *E. coli* GroEL), PEX nor *E. coli* pop2136 induced production of significant amounts of IL-8. In contrast, HY3 did not induce IL-8 secretion from other cell lines such as MKN45, Int407 and A549 cells (Table 2). These results indicate that recombinant *H. pylori* HSP60 induces IL-8 secretion from KATO III cells, but not from other cell lines such as MKN45, Int407 and A549.

Discussion

The induction of IL-8 secretion from KATO III cells with fusion proteins containing *H. pylori* or *Y. enterocolitica* HSP60, but not with *E. coli* GroEL was observed in the present study. Amino-acid sequences of *H. pylori* HSP60, *Y. enterocolitica* HSP60 and *E. coli* GroEL have been reported previously [13–15]. The similarities of the amino-acid sequences among these junior proteins were as follows: 55.4% for *H. pylori* HSP60 versus *Y. enterocolitica* HSP60; 55.9% for *H. pylori* HSP60 versus *E. coli* GroEL; and 88.2% for *Y. enterocolitica* HSP60 versus *E. coli* GroEL. As shown in Fig. 3, the amino-acid regions that include an identical amino-acid position in *H. pylori* HSP60 and *Y. enterocolitica* HSP60 but not in *E. coli* GroEL might be associated with IL-8 induction from KATO III cells.

The induction of IL-8 secretion from KATO III cells but not from another gastric cell line (MKN45) by *H. pylori* HSP60 was observed. Aihara *et al.* [16] have reported that the secretion of IL-8 by live *H. pylori* was different in various human gastric cancer cell lines (MKN45, MKN1, MKN28, MKN74 and KATO III). A previous study reported that *H. pylori* HSP60 was associated with adhesion of *H. pylori* to human gastric

epithelial cells [4]. Huesca *et al.* reported that surface HSPs 60 and 70 of *H. pylori* are associated with attachment to human gastric epithelial cells by binding to the receptor, sulphated glycolipid [17]. These reports suggest that the different amounts of sulphated glycolipid on the various epithelial cells might also be associated with differences in IL-8 induction.

It has been reported that direct contact of *H. pylori* with epithelial cells was associated with the induction of IL-8 secretion [3]. Censini *et al.* reported an association of various Cag proteins encoded by the *cag* pathogenicity island of *H. pylori* with IL-8 secretion from gastric epithelial cells [18]. Huang *et al.* [19] have shown that water-soluble extract containing bacterial surface components such as urease, FlaAB, HSPs, membrane proteins and lipopolysaccharide (LPS) induced IL-8 secretion from gastric epithelial cell lines, AGS and KATO III. They also indicated that a urease-negative mutant and a non-motile mutant did not differ from the parent *H. pylori* strain with respect to IL-8 induction [19]. Furthermore, it has been reported recently that *H. pylori* LPS did not induce IL-8 production from AGS cells [20]. It is possible that porin protein is involved in the induction of IL-8 production from gastric epithelial cells, as *H. pylori* porins have been reported to stimulate human monocytes and lymphocytes to release various cytokines such as TNF- α , IL-6 and IL-8 [21]. Recently, Galdero *et al.* [22] reported that GroEL and DnaK of *E. coli* induced both secretion of cytokines (TNF- α , IL-6 and IL-8) from human monocytes and lymphocytes and expression of adhesion molecule in endothelial cells.

The present study demonstrated for the first time that HSP60 or *H. pylori* induced IL-8 secretion from gastric epithelial cells, suggesting that HSP 60 might be an important virulence factor associated with chronic gastric inflammation following *H. pylori* infection. The mechanism by which IL-8 was induced by *H. pylori* HSP60 and the reason why the induction level of IL-8 was different depending on the gastric cells used are not yet understood.

Table 2. Induction of IL-8 secretion from gastric and non-gastric epithelial cells by prepared antigens*

Prepared antigen added to cultures (150 μ g/ml) [†]	Mean IL-8 secretion (pg/ml)* from											
	Gastric epithelial cell line				Non-gastric epithelial cell lines							
	MKN45				Int407				A549			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
pop2136	364	375	615	NT	37	34	86	NT	1335	1421	1661	NT
PEX	440	680	653	1112	12	17	59	147	1159	1260	1230	1789
HY1	309	380	444	793	13	16	36	108	997	1159	1466	1841
HY2	472	618	820	934	13	20	56	162	1381	1442	1688	1954
HY3	342	417	529	966	20	26	67	203	1158	1228	1339	1589
Medium	48	70	89	144	24	43	94	158	543	633	751	877

NT, not tested.

*The results shown are the average of duplicate assays.

[†]The antigens were prepared as described in the text.

HpHSP60 AA 1:M-AKEIKFSDSARNLLFEGRQLHDVAVKVTMGPRGNVLIQSYGAPSIITKDGVSVAKIEI 59
YeHSP60 AA 1:MAAKDVKFNGDARIKMLRGVNLADAVKVTLGPKGRNVLDKSFGSPTITKDGVSVAREI 60
EcGroEL AA 1:MAAKDVKFNGDARVKMLRGVNVLDADAVKVTLGPKGRNVLDKSFGAPTITKDGVSVAREI 60

* * *

HpHSP60 AA 60:ELSCPVANMGAQLVKEVASKTADAAGDGTTTATVLAYSIFKEGLRNITAGANPIEVKGRM 119
YeHSP60 AA 61:ELEDKFENMGAQMVKEVASKANDAAGDGTTTATVLAQSIIITEGLKAVAAGMNPMDLKRG I 120
EcGroEL AA 61:ELEDKFENMGAQMVKEVASKANDAAGDGTTTATVLAQAIIITEGLKAVAAGMNPMDLKRG I 120

** ***** *

HpHSP60 AA 120:DKAAEAIINELKKASKKVGGKEETQVATISANS DHNIGKLIADAMEKVGKG DVI TVEEA 179
YeHSP60 AA 121:DKAVIAAVEELKKLYVPCSDSKAIAQVGTISANS DSTVGELIAQAMEKVGKEGVI TVEEG 180
EcGroEL AA 121:DKAVTAAVEELKALSVPCSDSKAIAQVGTISANSE TVGKLIAEAMDKVGKEGVI TVEDG 180

*** * ****

HpHSP60 AA 180:KGIEDL DVVEGMQFDRGYLSPYFVTNAEKMTAQLDNAYILLTDKKISSMKDI LPLEKT 239
YeHSP60 AA 181:PGLQDEL DVVEGMQFDRGYLSPYFINKPETGSIELESPFI LLADKKISNIREMLPVLEAV 240
EcGroEL AA 181:TGLQDEL DVVEGMQFDRGYLSPYFINKPETGA VELESPFI LLADKKISNIREMLPVLEAV 240

* ***** *

HpHSP60 AA 240:MKEGKPLLI IAEDIEGEALTTLVVNKL RGVNLIAAVKAPGF GDRRKEMLKDI AILTGGQV 299
YeHSP60 AA 241:AKAGKPLLI IAEDVEGEALATLVVNTMRGI VKVA AVKAPGF GDRRKAMLQDI ATLT VGT V 300
EcGroEL AA 241:AKAGKPLLI IAEDVEGEALATAVNTIRGI VKVA AVKAPGF GDRRKAMLQDI ATLT VGT V 300

* ***** *

HpHSP60 AA 300:I SEELGLE SLENAEVEFLGKAGRIVIDKDN TTIVDGKGHSDDVKDRVAQIKTQIASTTS DY 359
YeHSP60 AA 301:I SEEIGLELEKT TL EDLGQAKRVVIN KDTTII IDGVGDEAAIQGRVTQIRQQIEATS DY 360
EcGroEL AA 301:I SEEIGMELEKAT LEDLGQAKRVVIN KDTTII IDGVGEAAIQGRVAQIRQQIEATS DY 360

**** * ** *

HpHSP60 AA 360:DKEKLQERLAKLSGGVAVIKVGAASEVEMKEKKDRVD DAL SATKA AVEEGIVIGGGAALI 419
YeHSP60 AA 361:DKEKLQERVAKLAGGVAVIKVGAATEVEMKEKKARVEDALHATRAAVEEGV VAGGGVALI 420
EcGroEL AA 361:DREKLQERVAKLAGGVAVIKVGAATEVEMKEKKARVEDALHATRAAVEEGV VAGGGVALI 420

* ***** *

HpHSP60 AA 420:R-AA-QK-VHL-NLHDDEKVGEIIMRAIKAPLAQIAINAGYDGGVVVNEVEKH EGHFGF 475
YeHSP60 AA 421:RAASAITAAGLKGDNEDQNVG IKVALRAMESPLRQIVVNAGEEASVIANNVKAGSGSYGY 480
EcGroEL AA 421:RVASKL--ADLRGQNEDQNVG IKVALRAMEAPLRQIVLNCGEEPSVVANTVKGGDGNYGY 478

* * *

HpHSP60 AA 476:NASNGKYVDMFKEGIIDPLKVERIALQNAVSVSSLLL TTEATVHEIKEEKATPAMPDMGG 535
YeHSP60 AA 481:NAYSEEYGDMIAMGILDPTKVTRSALQYAASIAGLMITTECMITDLP RDDKADMG-AGG 539
EcGroEL AA 479:NAATEEYGNMIDMGILDP TKVTRSALQYASVAGLMITTECMVTDL PKND-AADLGAAGG 537

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Fig. 3. Homology of amino-acid sequences among *H. pylori* HSP60 (HpHSP60 AA), *Y. enterocolitica* HSP60 (YeHSP60 AA) and *E. coli* GroEL (EcGroEL AA). Homologous sequences are indicated by an asterisk. The positions of amino acids which are homologous between *H. pylori* HSP60 and *Y. enterocolitica* HSP60 but not *E. coli* GroEL are shown as boxes.

References

- Gionchetti P, Vaira D, Campieri M *et al.* Enhanced mucosal interleukin-6 and -8 in *Helicobacter pylori*-positive dyspeptic patients. *Am J Gastroenterol* 1994; **89**: 883–887.
- Yoshida N, Granger DN, Evans DJ *et al.* Mechanisms involved in *Helicobacter pylori*-induced inflammation. *Gastroenterology* 1993; **105**: 1431–1440.
- Rieder G, Hatz RA, Moran AP, Walz A, Stolte M, Enders G. Role of adherence in Interleukin-8 induction in *Helicobacter pylori*-associated gastritis. *Infect Immun* 1997; **65**: 3622–3630.
- Yamaguchi H, Osaki T, Kurihara N *et al.* Heat-shock protein 60 homologue of *Helicobacter pylori* is associated with adhesion of *H. pylori* to human gastric epithelial cells. *J Med Microbiol* 1997; **46**: 825–831.
- Ellis RJ. The molecular chaperone concept. *Semin Cell Biol* 1990; **1**: 1–9.
- Young DB. Chaperonins and the immune response. *Semin Cell Biol* 1990; **1**: 27–35.
- Yamaguchi H, Osaki T, Taguchi H, Hanawa T, Yamamoto T, Kamiga S. Production and characterisation of monoclonal antibodies to heat-shock protein 60 of *Helicobacter pylori*. *J Med Microbiol* 1997; **46**: 819–824.
- Yamaguchi H, Osaki T, Taguchi H *et al.* Growth inhibition of *Helicobacter pylori* by monoclonal antibody to heat-shock protein 60. *Microbiol Immunol* 1997; **41**: 909–916.
- Stanley KK, Luzio JP. Construction of a new family of high efficiency bacterial expression vectors: identification of cDNA clones coding for human liver proteins. *EMBO J* 1984; **3**: 1429–1434.
- Amrein KE, Takacs B, Stieger M, Molnos J, Flint NA, Burn P. Purification and characterization of recombinant human p50csk protein-tyrosine kinase from an *Escherichia coli* expression system overexpressing the bacterial chaperones GroES and GroEL. *Proc Natl Acad Sci USA* 1995; **92**: 1048–1052.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680–685.
- Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979; **76**: 4350–4354.
- Hemmingsen SM, Woolford C, van der Vies SM *et al.* Homologous plant and bacterial proteins chaperone oligomeric protein assembly. *Nature* 1988; **33**: 330–334.
- Macchia G, Massone A, Burrone D, Covacci A, Censini S, Rappuoli R. The Hsp60 protein of *Helicobacter pylori*: structure and immune response in patients with gastroduodenal diseases. *Mol Microbiol* 1993; **9**: 645–652.
- Yamamoto T, Miura H, Ohsumi K *et al.* Cloning and nucleotide sequence analysis of immunodominant heat-shock protein of *Yersinia enterocolitica*. *Res Microbiol* 1993; **144**: 691–701.
- Aihara M, Tsuchimoto D, Takizawa H *et al.* Mechanisms involved in *Helicobacter pylori*-induced interleukin-8 production by a gastric cancer cell line, MKN45. *Infect Immun* 1997; **65**: 3218–3224.
- Huesca M, Borgia S, Hoffman P, Lingwood CA. Acidic pH changes receptor binding of *Helicobacter pylori*: a binary adhesion model in which surface heat-shock (stress) proteins mediate sulfatide recognition in gastric colonization. *Infect Immun* 1996; **64**: 2643–2648.
- Censini S, Lange C, Xiang Z *et al.* *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci USA* 1996; **93**: 14648–14653.
- Huang J, O'Toole PW, Doig P, Trust TJ. Stimulation of interleukin-8 production in epithelial cell lines by *Helicobacter pylori*. *Infect Immun* 1995; **63**: 1732–1738.
- Sharma SA, Tummuru MKR, Miller GG, Blaser MJ. Interleukin-8 response of gastric epithelial cell lines to *Helicobacter pylori* stimulation in vitro. *Infect Immun* 1995; **63**: 1681–1687.
- Tufano MA, Rossano F, Catalanotti P *et al.* Immunobiological activities of *Helicobacter pylori* porins. *Infect Immun* 1994; **62**: 1392–1399.
- Galdiero M, Cipollaro de l'Ero G, Marcatili A. Cytokine and adhesion molecule expression in human monocytes and endothelial cells stimulated with bacterial heat shock proteins. *Infect Immun* 1997; **65**: 699–707.