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J Immunol 2000; 165:1918-1924; doi: 10.4049/jimmunol.165.4.1918
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Helicobacter pylori Urease Binds to Class II MHC on Gastric Epithelial Cells and Induces Their Apoptosis

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Infection by Helicobacter pylori leads to injury of the gastric epithelium and a cellular infiltrate that includes CD4+ T cells. H. pylori binds to class II MHC molecules on gastric epithelial cells and induces their apoptosis. Because urease is an abundant protein expressed by H. pylori, we examined whether it had the ability to bind class II MHC and induce apoptosis in class II MHC-bearing cells. Flow cytometry revealed the binding of PE-conjugated urease to class II MHC+ gastric epithelial cell lines. The binding of urease to human gastric epithelial cells was reduced by anti-class II MHC Abs and by staphylococcal enterotoxin B. The binding of urease to class II MHC was confirmed when urease bound to HLA-DR1-transfected COS-1 (1D12) cells but not to untransfected COS-1 cells. Urease also bound to a panel of B cell lines expressing various class II MHC alleles. Recombinant urease induced apoptosis in gastric epithelial cells that express class II MHC molecules, but not in class II MHC− cells. Also, Fab from anti-class II MHC and not from isotype control Abs blocked the induction of apoptosis by urease in a concentration-dependent manner. The adhesin properties of urease might point to a novel and important role of H. pylori urease in the pathogenesis of H. pylori infection. The Journal of Immunology, 2000, 165: 1918–1924.

Helicobacter pylori is a Gram-negative, spiral-shaped bacterium that infects over half of the world’s population. Infection with H. pylori is associated with the development of chronic gastritis and peptic ulcers, and is a risk factor for the development of gastric carcinoma (reviewed in Refs. 1 and 2). A characteristic feature of the infected tissue is damage to the surface gastric epithelial cells. Although both host and bacterial factors are considered important determinants in the clinical outcomes of H. pylori infections, several studies using different strains have suggested that bacterial factors can directly mediate most of the epithelial damage.

Urease is regarded as an important virulence factor for H. pylori survival in gastric acid through the hydrolysis of urea. Ammonia, a byproduct of this process, neutralizes hydrochloric acid and thus allows H. pylori to colonize the human stomach. Urease is one of the most abundant proteins produced by H. pylori, representing ~5% of the total bacterial cell protein (3, 4). Isogenic mutant H. pylori strains showed that urease is essential for colonization because the mutant bacteria were unable to colonize gnotobiotic pigs (5). However, the urease-negative H. pylori strains also failed to colonize under hypochlorhydic conditions (6), which suggested a role for urease in colonization beyond its ability to neutralize the gastric acidity.

Adherence of H. pylori to the surface of gastric epithelial cells is undoubtedly a key step in colonization and has been shown to induce host cell protein phosphorylation and changes in the cytoskeleton (7, 8). One of the consequences of the signals initiated during H. pylori adhesion to cultured gastric epithelial cells is increased apoptosis of the epithelium, which has been noted to occur in vivo (9, 10). Thus, the bacterial structures responsible for adhesion and stimulation of host cell death may be regarded as important virulence factors. Although a number of epithelial cell molecules have been reported to contribute to H. pylori binding to the epithelium (11–17), it is not clear which cell and bacterial factors stimulate host cell apoptosis.

Class II MHC molecules are best known for their regulation of immune responses through the presentation of foreign Ags to CD4+ T cells. However, engagement of class II MHC by TCRs or bacterial superantigens also has consequences for the class II MHC-expressing cells, including apoptosis (18). We have recently shown that H. pylori uses class II MHC molecules on the surface of gastric epithelial cells as receptors (19). Following the binding of H. pylori to class II MHC, apoptosis was increased in gastric epithelial cells. The induction of gastric epithelial cell apoptosis was dependent on class II MHC expression because it was blocked by anti-class II MHC Abs, and cells deficient in class II MHC expression were refractory to apoptosis induction by H. pylori. Because urease is a major surface protein of H. pylori that facilitates the colonization of the gastric epithelium and has been shown to activate human monocyte secretion of cytokines (20, 21), we sought to examine whether urease is a bacterial receptor that binds to class II MHC molecules on gastric epithelial cells and is thus responsible for initiating apoptosis. In this report, we show that H. pylori urease binds to cells which express class II MHC and to affinity-purified class II MHC. Urease binding to solubilized membrane proteins from class II MHC+ cells is inhibited by anti-class II MHC Abs. Urease bound to a panel of human B cell lines expressing a diverse set of class II MHC alleles. Further, we show that urease induced apoptosis that depends on the expression of class II MHC molecules by the target cells. The observations presented in this report demonstrate a previously unsuspected role for urease in H. pylori pathogenesis and provide added support for the...
use of this bacterial product in the development of vaccination strategies to reduce infection and cellular injury by this important human pathogen.

Materials and Methods

Cell lines and hybridomas

The gastric epithelial cell lines, Kato-III, N87, and AGS were obtained from American Type Culture Collection (Manassas, VA) and were maintained in RPMI 1640 medium supplemented with 10% FCS. Additional cell lines differing in the levels of class II MHC molecules’ expression were also used. COS-1 cells were kindly provided by Dr. M. Falzon (University of Texas Medical Branch (UTMB)), Galveston, TX). COS-1 cells transfected with the genes encoding for HLA-DR1 α- and β-chains (ID12-22) were provided by Dr. M. Xu (Ag Express, Worcester, MA). COS cells were transfected with the CXCR2 receptor provided by Dr. J. Navarro (UTMB). A panel of B cell lines expressing various class II MHC alleles in a homozygous manner, kindly provided by the UTMB Tissue Ag Lab (UTMB), were also included. The B cell lines and their alleles were as follows: 9070 (DRB1*0803; DBQ1*0301), 9063 WT47 (DRB1*13; DRB3), 9064 (DRB1*14; DRB3), 9036 (DRB1*11; DRB3), and 9002 MZ070782 (DRB1*01). The hybridomas HBSS55, HB145, and HB180 (American Type Culture Collection) were used as the source of anti-human class II MHC Abs (L243, IVA12, and 9.3F10 respectively).

Purification of Fab from anti-class II MHC (IVA-12) Ab

Anti-class II MHC (IVA-12) Ab was purified through a Protein G Sepharose HR 16/5 column. One-hundred microliters of 2 mg/ml purified IgG in PBS-D (0.1 M NaCl, 0.1% Triton X-100, 0.02% sodium azide) were loaded on the column and washed with PBS-D to remove non-specifically bound proteins. The column was then eluted with 0.1 M glycine (pH 2.1) to obtain Fab. The purity of Fab was assessed by SDS-PAGE analysis.

Binding of urease to the surface of gastric epithelial cells and B cells

Recombinant H. pylori urease was obtained from Orovax (Cambridge, MA) and was biotinylated to assess its binding to the surface of epithelial cells using flow cytometry. Briefly, gastric epithelial cells (Kato-III, N87, and AGS) were cultured in the presence or absence of 100 U/ml IFN-γ for 48 h and then washed twice with PBS containing 1% BSA and 0.02% sodium azide. Gastric epithelial cell lines as well as B cell lines expressing multiple class II MHC alleles (9070, 9064, 9063 WT47, 9036, and 9002 MZ070782) were then incubated with biotinylated urease for an additional 45 min on ice. After washing with PBS/BSA/azide the cells were incubated with avidin-PE to detect the bound biotinylated urease. The cells were resuspended in 0.5 ml paraformaldehyde (0.5% in PBS) and were analyzed by flow cytometry on a FACScan (Becton Dickinson, San Jose CA). Competition studies were done by preincubating the cells with the indicated proteins at the indicated concentrations for 2 h on ice followed by incubation with biotinylated urease for an additional 2 h on ice.

Detection of class II MHC among gastric epithelial cell-derived proteins that bound to urease-coated Sepharose beads

Recombinant H. pylori urease was coupled to cyanogen bromide-Sepharose 4B beads (Sigma), according to the manufacturer’s instructions. The urease-coated beads were used to capture gastric epithelial cell-derived proteins with affinity for urease. Beads treated under the same coupling conditions in the absence of urease were used as controls. Gastric epithelial cell proteins were metabolically labeled and solubilized as described previously (19). Briefly, the cells were metabolically radiolabeled with [35S]methionine and [35S]cysteine (0.5 mCi/10^6 cells). They were then washed and lysed in ice-cold 10 mM Tris-HCl buffer (pH 8.1) containing protease inhibitors (2 mM PMSF and 10 mM N-ethylmaleimide) for 30 min on ice. Nuclei were then removed by centrifugation at 500 × g for 10 min. The supernatant was subjected to ultracentrifugation at 100,000 × g for 45 min. The membrane pellet was solubilized in the lysis buffer containing 0.5% Triton X-100 followed by a second ultracentrifugation at 100,000 × g for 45 min. The membrane proteins were pre-cleared by incubation with normal rabbit serum, which was followed by three passages over Staphylococcus aureus Cowen strain A and one passage over protein A-Sepharose beads (Sigma).

The solubilized membrane proteins were incubated with urease-coated beads for 16 h at 4°C. After incubation, unbound proteins were washed with PBS until the cmr of the wash reached background levels. The proteins bound to the urease-coated beads were eluted with 0.1 M glycine buffer (pH 2.2), and the corresponding cpm were measured by liquid scintillation counting to assess the level of binding. The eluted proteins were separated on 12% SDS-PAGE and were analyzed by autoradiography, as described previously (23).

To determine the extent of class II MHC binding to the urease-coated beads in relation to other proteins that could bind, the Kato-III and N87 membrane proteins were incubated with a mixture of the anti-MHC II Abs IVA12, L243, and 9.3F10 or an isotype control. Subsequently, urease-coated beads were incubated with the membrane proteins that were pre-treated with anti-class II MHC Abs.

Urease binds to purified class II MHC from gastric epithelial cells

Wells in 96-well microtiter plates were coated with recombinant urease (400, 100, and 25 µg/ml), BSA, the anti-HLA-DR Ab 9.3F10, or with borate buffered saline (BBS) used as the binding buffer. Affinity-purified [35S]methionine-labeled class II MHC (19) were added to the wells and were incubated for 2 h at room temperature. After incubation, unbound proteins were washed with PBS from the wells. The bound class II MHC molecules were eluted with SDS sample buffer, run on 12% SDS-PAGE gel, and detected by autoradiography.

Cross-linking of surface class II MHC on gastric epithelial cell lines

Cells (2.5 × 10^6) were allowed to attach overnight in a 25-cm² flask. The cells were exposed to varying concentrations of urease or to anti-class II MHC Ab (RFD1, IgM isotype at 10 µg/ml; Serotec, Raleigh, NC) for 12, 24, 48, and 72 h. At the end of incubation, the cells were harvested and the DNA fragmentation, as an indicator of apoptosis, was assayed as described below.

Detection of DNA fragmentation

DNA fragmentation was measured using a commercially available ELISA kit (Boehringer Mannheim, Indianapolis, IN), as previously described (19). After stimulating the cells with urease, low m.w. nucleosome fragments in the cytoplasm of the cells were collected as described previously (19). Wells in an ELISA plate were coated with anti-histone Ab. The nucleosome-containing samples were added to the wells and trapped by the Ab. After incubation, the unbound DNA was washed off and quantities of histone-associated DNA were estimated using an anti-DNA and an enzymatic detection system. The absorbance was measured at 405 nm by Titertek Multiskan MCC/340 (Irvine, CA) and compared with a substrate solution as a blank. The apoptotic index was calculated according to the manufacturer’s instructions by dividing the absorbance of stimulated cells by the absorbance for control cells.

Statistical analysis

Results are expressed as the mean ± SEM. Data were compared using a two-tailed Student’s t test and the differences were considered significant if p values were <0.05.

Results

H. pylori urease binds to the surface of gastric epithelial cells

We have previously shown that class II MHC on human gastric epithelial cells act as receptors for H. pylori (19). Initial efforts to identify H. pylori proteins responsible for bacterial binding to class II MHC and induction of apoptosis focused on outer membrane proteins from H. pylori. The extracted outer membrane proteins were found to induce apoptosis. Urease was detected among the extracted proteins using an ELISA assay and Western blots (not shown). Because urease is an abundant protein expressed on the surface of H. pylori (21) and has been shown to stimulate cytokine secretion in monocytes (20), we examined whether urease could...
contribute to bacterial binding to the epithelium. To examine urease binding we treated three human gastric epithelial cell lines with either medium or IFN-γ, since we have previously shown that IFN-γ increases both the expression of class II MHC and the binding of *H. pylori* to gastric epithelial cells (19). The cell lines were selected based on the level of class II MHC molecules expressed on their surface. As previously shown, both Kato-III and N87 express class II MHC, and the expression is higher in the latter cell line (19). The variant of the AGS cell line used in these studies does not express class II MHC, even after IFN-γ stimulation. The extent to which urease bound to the surface of the gastric epithelial cell lines correlated with the level of class II MHC expression (Fig. 1). IFN-γ increased the amount of urease binding only to the cell lines which express class II MHC molecules.

Class II MHC molecules are involved in the binding of *H. pylori* urease to gastric epithelial cells

Because *H. pylori* uses class II MHC on gastric epithelial cells as receptors (19) and the extent of urease binding coincided with the level of class II MHC expression by the three different cell lines, we examined whether class II MHC molecules were responsible for urease binding. To that end, urease-coated beads were used to identify which gastric epithelial cell membrane proteins bound to urease. Solubilized membrane proteins from gastric epithelial cell lines (Kato-III, N87, and AGS) metabolically labeled with [35S]methionine and [35S]cysteine were incubated with urease-coated beads or uncoated beads. The extent to which epithelial proteins bound to urease was quantified by measuring the amount of radioactivity in the proteins that were eluted from the beads (Fig. 2A). The results showed that radioactive protein binding to the urease beads was high when class II MHC expression was high, particularly after IFN-γ treatment (Fig. 2A). The proteins obtained from AGS cells, which do not express class II MHC, demonstrated minimal binding to the urease-coated beads compared with the binding to control beads. When the proteins that bound to urease-beads were analyzed by SDS-PAGE, bands that coincided in size with class II MHC α- and β-chains were detected (Fig. 2B). Samples with low cpm did not show any bands by autoradiography even after 3 wk of exposure. The identity of the bands was confirmed by western blotting with a mixture of anti-class II MHC Abs (not shown).

To assess the contribution of class II MHC in the overall binding of urease independently of other gastric epithelial cell-specific proteins to which urease might bind, a nongastric epithelial cell line transfected with the HLA-DR1 class II MHC allele was used as the source of proteins. The untransfected cell line was used as a control. Again, the binding of proteins correlated with the expression of class II MHC (Fig. 2, C and D). Because proteins other than class II MHC were detected in the autoradiographs, we sought to confirm whether they might represent proteins that are coexpressed with class II MHC and also bind urease or proteins that coprecipitate with class II MHC. To that end, we preincubated the gastric epithelial proteins with an anti-class II MHC Ab mixture (IVA12, L243, and 9.3F10) or an isotype control before they were incubated with urease-coated beads. Blocking of class II MHC decreased the binding to the urease-coated beads to background levels comparable to those obtained with control beads (Fig. 2E). The high m.w. proteins were absent in all conditions where class II MHC were absent. These proteins may represent proteins recruited by class II MHC when they are cross-linked by urease and which may be involved in the signaling processes leading to apoptosis.

Urease binds to affinity-purified class II MHC from gastric epithelial cells

As an independent approach to confirm that urease adheres to class II MHC molecules on gastric epithelial cells, 96-well microtiter plates were coated with various concentration of either urease, BSA, anti-HLA-DR, or BBS (binding buffer). Then, metabolically labeled and purified class II MHC molecules were added to each well. Unbound class II MHC molecules were washed off, and bound class II MHC were eluted and separated on a 12% SDS-PAGE gel (Fig. 3). The gel revealed that class II MHC chains were recovered from the wells that were coated with urease (Fig. 3) whereas no class II MHC molecules were recovered from the wells coated with either BBS or BSA. Affinity-purified class II MHC molecules captured by the anti-HLA-DR Ab were run on the last lane as a positive control. These data confirm that urease binds to class II MHC molecules.

**Binding of urease to a panel of B cell lines expressing various HLA-DR and -DQ alleles**

Given that urease binds directly to class II MHC molecules, it was important to determine whether polymorphic differences in class II MHC could influence the binding of urease. To that end, we assessed the binding of urease to a panel of human B cells homozygous in HLA-DR or HLA-DQ (9070, 9064, 9063 WT47, 9036, and 9002 MZ070782). Urease bound to the various alleles examined. The only apparent differences in binding were due to differences in the level of class II MHC expression by each of the cell lines (Fig. 4). The binding to multiple class II MHC alleles is a property shared by bacterial proteins referred to as superantigens. To determine whether a superantigen that is known to bind to class II MHC could influence the interaction of urease with cells expressing class II MHC, the cells were preincubated with either staphylococcal enterotoxin B (SEB) or urease before incubation with biotinylated urease. The binding of biotinylated urease to the cells was detected with PE-avidin as described in Materials and Methods. Using this approach, it was noted that SEB, as well as unbiotinylated urease, significantly reduced the level of urease binding (Fig. 5). A mixture containing the anti-class II MHC Abs L243, IVA12, and 9.3F10 also reduced the level of urease binding.
Urease-induced apoptosis of gastric epithelial cells that express class II MHC

H. pylori binding to class II MHC on gastric epithelial cells triggers apoptosis of the epithelial cells (19). Ligation of HLA-DR by the bacterial superantigens staphylococcal enterotoxin A and SEB has been shown to stimulate apoptosis (18, 24, 25). Thus, to confirm whether urease acts as a superantigen in its ability to ligate class II MHC molecules and whether it represents the bacterial coreceptor that initiates apoptosis of gastric epithelial cells, we examined its ability to trigger apoptosis on epithelial cells. IgM Abs to class II MHC were used as controls in these studies. After cross-linking with the anti-class II MHC Ab, there was a significant increase in the apoptotic index in cells expressing class II MHC molecules (Kato-III), but not in cells lacking class II MHC molecules (AGS) as evaluated by ELISA (3.95 ± 0.3, p , 0.05; Fig. 6A). No apoptosis was detected by ELISA when the cells were cultured either in medium alone or with isotype control Ab. When Kato-III and AGS cells were exposed to various concentrations of urease (0.1, 1, and 10 mg/ml) for 48 h, there was a dose-dependent induction of apoptosis by urease in class II MHC+ Kato-III cells, but not in class II MHC− AGS cells (Fig. 6B).

The noted induction of apoptosis by urease in class II MHC+ Kato-III cells but not in class II MHC− AGS cells at 48 h was not due to different kinetics of apoptosis in the cell lines, because an analysis at 12-h intervals up to 72 h showed a progressive increase in apoptotic index up to 48 h in class II MHC+ Kato-III cells (exposed to urease). However, there was no cell death in class II MHC− AGS cells at any of the time points examined (not shown). To determine the induction of apoptosis by urease binding to class II MHC in the absence of other gastric epithelial cell-specific proteins, class II MHC-transfected COS-1 cells (1D12) were cultured with urease. Controls were untransfected COS-1 cells or cells transfected with a plasmid control. Urease induced

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apoptosis only in the COS-1 cells that expressed class II MHC (Fig. 7A). The role of class II MHC on gastric epithelial cells in the binding of urease and subsequent induction of apoptosis was further confirmed when Fab from an anti-class II MHC Ab inhibited apoptosis in a dose-dependent manner (Fig. 7B).

**Discussion**

The ability of bacteria to adhere to the host cells is important for their successful colonization. The bacterial adhesins that mediate those initial interactions with the host cells are thus important virulence factors. Although *H. pylori* is known to colonize the gastric epithelial surface, the mechanisms of adherence and pathogenesis are unclear. Different adhesins and/or receptors have been reported to contribute to the colonization of the human stomach by bacteria. However, the relative importance of each in colonization and/or pathogenesis has not been established. Among the host cell structures to which *H. pylori* have been shown to bind include sialic acids, fucose-containing blood group Ags, membrane lipids, mucin, and several basement membrane constituents (11–17). However, most of the studies to date appear to be complicated by differences in the model systems, strains used, and some apparent contradictions.

In addition to contributing to colonization of the target tissue, adhesion of the bacteria to the target cells is believed to be important in pathogenesis as it allows for a direct effect of the bacteria on the host cells.
bacterial toxins on the epithelium. Also, the release of IL-8 by gastric epithelial cells appears to be enhanced by direct contact of the bacteria with the host cells (26–29). The release of IL-8 by gastric epithelial cells influences the recruitment of neutrophils, which together with other recruited inflammatory cells, mediate some of the epithelial damage. Attachment of the bacteria to the epithelial cells also induces actin polymerization and the phosphorylation of cellular proteins (7, 8), which may be reflective of some of the epithelial damage. Urease is present on the outer membrane surface of H. pylori and may play a role in the compensatory increase in cellular proliferation by urease, some cell populations may be stimulated in an interleukin-dependent manner.

FIGURE 7. The induction of apoptosis by urease depends on its interaction with class II MHC. A, Various cell lines that differ in their expression of class II MHC molecules were compared for their susceptibility to apoptosis induction by H. pylori-urease. The COS-1 cells untransfected or transfected with HLA-DR1 (1D12) or the chemokine receptor CXCR2 were exposed to urease (0.1, 1, 10 μg/ml) for 48 h. DNA fragmentation was assayed by ELISA. Recombinant urease induced apoptosis in class II MHC+ cell lines (1D12) and not in the other cells (p < 0.05). B, Fab of anti-class II MHC Ab can block urease-induced apoptosis in gastric epithelial cells. Kato-III cells were treated with IFN-γ (100 U/ml) for 48 h in the presence of Fab from the anti-class II MHC Ab IVA12 or from control Ab during the last 16 h of culture. Then the cells were exposed to 10 μg/ml recombinant urease for an additional 48 h, and the apoptotic index was measured. Fab decreased the induction of apoptosis by urease in a concentration-dependent manner.

H. pylori-induced apoptosis of cells expressing class II MHC either constitutively or following gene transfection, and apoptosis induction by H. pylori was blocked by Abs to class II MHC. However, the bacterial proteins that attached to class II MHC molecules on gastric epithelial cells and transduce apoptotic signals were unknown.

Based on the observations that H. pylori can bind to class II MHC on gastric epithelial cells, the expression of proteins with the ability to bind human class II MHC by these bacteria was suspected. Our initial analysis by ELISA and Western blot of extracted outer membrane proteins revealed the presence of urease in extracts that were able to elicit apoptosis of human gastric epithelial cells. Urease is present on the outer membrane surface of H. pylori following adsorption of urease from other H. pylori bacteria undergoing autolysis (21). Thus, it was not surprising to find urease among the proteins in the extract, however, it was surprising to find that H. pylori urease was capable of binding to human class II MHC and inducing apoptosis.

The beads coated with urease only immunoprecipitated cellular proteins when class II MHC were present or in the absence of anti-class II MHC Abs. However, when class II MHC were precipitated by urease-coated beads, there were also unknown additional proteins of a higher m.w. that coprecipitated. These additional proteins may represent proteins that have an affinity for class II MHC and which could be involved in the intracellular signals delivered by cross-linked class II MHC. Alternatively, they may reflect proteins that normally do not have affinity for class II MHC, but which together with other recruited inflammatory cells, mediate some of the epithelial damage. Attachment of the bacteria to the epithelial cells also induces actin polymerization and the phosphorylation of cellular proteins (7, 8), which may be reflective of some of the epithelial damage. Urease is present on the outer membrane surface of H. pylori and may play a role in the compensatory increase in cellular proliferation by urease, some cell populations may be stimulated in an interleukin-dependent manner.

The observation that urease binds to class II MHC on gastric epithelial cells may help explain why urease-negative mutants of H. pylori failed to colonize in an animal model of infection even under hypochlorhydric conditions (6). In other studies with urease-negative mutants, it was speculated that the failure to colonize was due to a requirement of the enzymatic activity to provide a more hospitable environment for H. pylori to grow (5, 36). Interestingly, one of those mutants that fails to form the enzyme complex was found to adhere to gastric epithelial cells in vitro (37). Because the recombinant urease used in these studies lacks enzymatic activity, but bound to and induced apoptosis of class II MHC+ cells, it may be inferred that the binding of urease to class II MHC may be mediated by a domain that is present in one of the individual subunits, regardless of its association as an enzymatic complex.

While inducing apoptosis of gastric epithelium, H. pylori urease may play a role in the compensatory increase in cellular proliferation and could thus contribute to an increase in DNA mutations which may give rise to gastric cancers. Although our studies with established cell lines have not shown the stimulation of cell proliferation by urease, some cell populations may be stimulated in an interleukin-dependent manner.
vivo. This possibility parallels observations with the intact bacterium.

In summary, H. pylori urease may represent an important target for immunization for reasons other than what has been previously suspected. The development of Abs that would block the binding of urease to class II MHC may effectively prevent bacterial binding to the target tissue and colonization, and reduce the cellular injury. Thus, efforts are currently directed at mapping the sites of urease contact with human class II MHC molecules.

Acknowledgments
We thank Kim Falkowetz for her technical assistance with flow cytometry.

References
8. Segal, E. D., S. Falkow, and L. S. Tompkins. 1996. Helicobacter pylori attach-
mental to gastric cells induces cytoskeletal rearrangements and tyrosine phosphor-