Molecular Mimicry between *Helicobacter pylori* Antigens and H⁺,K⁺–Adenosine Triphosphatase in Human Gastric Autoimmunity

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Abstract

Autoimmune gastritis and *Helicobacter pylori*–associated gastric atrophy develop through similar mechanisms involving the proton pump H⁺,K⁺–adenosine triphosphatase as autoantigen. Here, we report that *H. pylori*–infected patients with gastric autoimmunity harbor in vivo–activated gastric CD4⁺ T cells that recognize both H⁺,K⁺–adenosine triphosphatase and *H. pylori* antigens. We characterized the submolecular specificity of such gastric T cells and identified cross-reactive epitopes from nine *H. pylori* proteins. Cross-reactive *H. pylori* peptides induced T cell proliferation and expression of T helper type 1 functions. We suggest that in genetically susceptible individuals, *H. pylori* infection can activate cross-reactive gastric T cells leading to gastric autoimmunity via molecular mimicry.

Key words: *Helicobacter pylori* • T cell epitopes • autoreactive T cells • mimicry • mucosal immunity

Introduction

Several mechanisms have been proposed for how pathogens might induce activation and critical expansion of autoreactive T cells and start autoimmune disease (1–6). Activation of resting autoreactive T cells may be achieved by viral and bacterial superantigens that bind a variety of MHC class II molecules and activate large numbers of T cells, irrespective of their specificity (7). Pathogen-induced tissue inflammation may result in local activation of APCs and enhanced processing/presentation of self antigens that causes T cell priming, followed by T cell activation and expansion of additional specificities (epitope spreading; references 8, 9). Another mechanism would imply that the inflammatory setting and the paracrine secretion of T cell growth factors induce the expansion of activated autoreactive T cells, whose small number was previously insufficient to drive an autoimmune disease. Such a mechanism is referred to as bystander activation (10). Moreover, a microbial antigen can include an epitope that is structurally similar to an autoantigen epitope, providing the basic element of the mechanism referred to as molecular mimicry (5, 6, 11–14).

Autoimmune chronic gastritis (AIG) is an organ-specific inflammatory disease leading to gastric atrophy, hypochloridria, and eventually to pernicious anemia. AIG is characterized by lymphocytic infiltrates in the gastric mucosa and by destruction of parietal cells, resulting in mucosal atrophy (15). In most AIG patients, serum anti-parietal cell autoantibodies (PCAs) are detectable. The autoantigen recognized is the gastric H⁺,K⁺–adenosine triphosphatase (ATPase), the proton pump, localized in the parietal cell canaliculi (16, 17). H⁺,K⁺–ATPase is also the target of autoreactive T cells that infiltrate the gastric mucosa of AIG patients (18).

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Abbreviations used in this paper: AIG, autoimmune chronic gastritis; ATPase, adenosine triphosphatase; BLAST, basic local alignment search tool; MI, mitogenic index; ORF, open reading frame; PCA, parietal cell autoantibody.
Helicobacter pylori infection is one of the most common bacterial infections in humans and its clinical outcomes are highly variable, including chronic gastritis, duodenal or gastric ulcers, mucosal atrophy, gastric carcinoma, or gastric lymphoma (19). In H. pylori–infected patients who develop gastric corpus atrophy, an increased incidence of positive PCAs has been reported, which significantly decreases after H. pylori eradication (20, 21). Based on the similarities between H. pylori–induced corpus atrophy and classical AIG, we inferred that in some individuals genetically predisposed to organ–specific autoimmunity due to their MHC class II haplotype, H. pylori infection plays a role in induction or exacerbation of gastric autoimmunity (20).

To test this hypothesis, we selected four women with chronic AIG and current H. pylori infection. Biopsy specimens of their gastric mucosa were cultured in IL–2–conditioned medium to expand in vivo–activated gastric T cells. Specimens were disrupted, and single T cell blasts were cloned (18). Gastric T cell clones were screened for their ability to proliferate in response to H⁺,K⁺–ATPase and/or to a H. pylori lysate. In addition to CD4⁺ T cell clones that proliferated to H. pylori lysate or to H⁺,K⁺–ATPase, we found a remarkable number of gastric T cell clones that recognized both H⁺,K⁺–ATPase and H. pylori antigens, and we identified their cross-reactive epitopes at the molecular level.

Materials and Methods

Generation of Gastric T Cell Clones. Four women (mean age, 45; range, 29–53 yr) with chronic AIG and thyroiditis provided their informed consent for this work. Their MHC haplotypes were as follows: HLA–A2, A11, B35, B41, DRB1*0701, and DRB1*1303 in patient 1; HLA–A1, A24, B15, B35, DRB1*0404, and DRB1*0803 in patient 2; HLA–A23, A32, B27, B35, DRB1*0403, and DRB1*1303 in patient 3; and HLA–A2, A3, B18, B50, DRB1*0301, and DRB1*1104 in patient 4. All patients had serum PCAs and thyroid peroxidase autoantibodies, but not intrinsic factor autoantibodies or hematologic abnormalities. All the patients were receiving levothyroxine for hypothyroidism. Patient 4 also suffered from hematologic abnormalities. All the patients were receiving autoantibodies, but not intrinsic factor autoantibodies or H. pylori infection. Positive curea breath test was found consistent with medium, 5 μg/ml of porcine albu- min, 0.5 μg/ml of porcine gastric H⁺,K⁺–ATPase (22, 23), and H. pylori lysate (10 μg/ml aqueous extract of NCTC11637 strain) by measuring [³H]TdR uptake after 60 h (18).

Analysis of TCR Vβ Chain Repertoire of Gastric T Cell Clones. The repertoire of the TCR Vβ chain of H⁺,K⁺–ATPase–specific Th clones was analyzed with a panel of 22 mAbs specific to the following: VB1, VB2, VB4, VB7, VB9, VB11, VB14, VB16, VB18, VB20, VB21.3, VB22, and VB23 (Beckman Coulter); and VB3.1, VB5.1, VB5.2, VB5.3, VB6.7, VB8, VB12, VB13, and VB17 (AMS Biotechnology GmbH). Iso- type-matched nonspecific Ig were used as negative control. Data acquisition was performed in a FACS caliber™ flow cytometer using the CELL Quest™ software program (Becton Dickinson). From each T cell clone, mRNA was extracted by mRNA direct isolation kit (Qiagen). For cDNA synthesis, the same amount of mRNA (50 ng) was used, and cDNA was synthesized by Moloney murine leukemia virus–reverse transcriptase (New England Biolabs, Inc.) and oligo–(dT) primers according to the manufacturer’s protocol. cDNA mix of all samples was amplified under equal conditions by a 30-cycle PCR using Vβ T cell receptor typing amplifier kit for VB10, VB15, and VB19 (CLONTECH Laboratories, Inc.) according to the manufacturer’s instructions.

Generation of H⁺,K⁺–ATPase Overlapping Peptides and Prediction of Candidate Cross-reactive H. pylori Peptides. To span the 1,034-amino acid α chain and the 270–amino acid B chain of porcine H⁺,K⁺–ATPase, 205 and 56 overlapping 15-mer peptides with a 10–amino acid overlap, respectively, were prepared by automated simultaneous multiple peptide synthesis, as described previously (24). Amino acid sequences of H⁺,K⁺–ATPase epitopes recognized by the 13 cross-reactive T cell clones were in silico to identify H. pylori peptides that might be candidates for cross-reactivity. Homologies between the 13 H⁺,K⁺–ATPase epitopes and peptides present in both genomes of H. pylori J99 and 26695 strains were screened by using the basic local alignment search tool (BLAST) server of the National Center for Biotechnology Information. Standard BLAST search parameters were used with the following adaptations: word size, 2; Expect 100000; Matrix, Blosum 45 with existence 19 and extension 1 penalty settings. Amino acid sequences in H. pylori J99 and 26695 open reading frames (ORFs) with sufficient homology (top 100 of the BLAST results) to the relevant H⁺,K⁺–ATPase epitopes and with a minimal length of nine amino acids were studied for the presence of motifs that would predict binding to patient MHC class II alleles by using the ProPred MHC class II Binding Peptide Prediction Server (25). The threshold of prediction was set at 3%. Identified H. pylori peptides were adjusted to a 15–amino acid length based on the appropriate H. pylori ORFs.

Submolecular Specificity of Gastric T Cell Clones Reactive to H⁺,K⁺–ATPase or to Both H⁺,K⁺–ATPase and H. pylori Lysate. Equal amounts of each of the 261 overlapping peptides of H⁺,K⁺–ATPase were pooled to have 20 pools. 4 × 10⁴ T cell blasts from each clone were cultured in triplicate for 3 d together with 1.5 × 10⁵ irradiated autologous mononuclear cells in the presence of medium, 5 μg/ml of porcine albumin, 0.5 μg/ml H⁺,K⁺–ATPase, 10 μg/ml H. pylori lysate or equal aliquots from each of the 20 pools in which each peptide component was present at a 10 μg/ml final concentration. After 60 h, [³H]TdR uptake was measured. Mitogen index (MI) was calculated as the ratio between counts in stimulated cultures and those in unstimulated cultures. Each clone was tested for proliferation to H⁺,K⁺–ATPase, H. pylori lysate, and the appropriate H⁺,K⁺–ATPase
peptide (as positive controls); the couple of flanking H\textsuperscript{+},K\textsuperscript{−}-ATPase peptides (negative controls) and the series of \textit{H. pylori} peptides were identified as possible candidates for cross-reactivity to the appropriate H\textsuperscript{+},K\textsuperscript{−}-ATPase epitope.

In some experiments, the effect of 5 \textmu g/ml anti–HLA-DR (clone G46–6) or anti–HLA-DQ (clone TU169; BD Biosciences) monoclonal antibodies or their isotype control (mouse IgG2a) on \textit{T} cell clone proliferation induced by the appropriate H\textsuperscript{+},K\textsuperscript{−}-ATPase and \textit{H. pylori} cross-reactive peptides was assessed.

**Cytokine Production Induced by Peptides.** \textit{T} cell blasts of each cross-reactive clone (5 \times 10\textasciicircum{5}) were cocultured in triplicate tubes for 48 h in 0.5 ml medium with 5 \times 10\textasciicircum{6} irradiated autologous APCs in the presence of medium, 0.5 \textmu g/ml H\textsuperscript{+},K\textsuperscript{−}-ATPase, 10 \textmu g/ml \textit{H. pylori} lysate, the appropriate H\textsuperscript{+},K\textsuperscript{−}-ATPase peptide, and the \textit{H. pylori} cross-reactive peptide that induced proliferation (10 \textmu g/ml), as well as control peptides that failed to induce proliferation. Duplicate samples of each supernatant were assayed for IL-4, IL-5, and IFN-\gamma content by ELISA assays (18).

**Perforin-mediated Cytolytic Activity and Fas–Fas Ligand-mediated Apoptotic Killing.** The ability of \textit{T} cell clones to express perforin-mediated cytotoxicity was assessed in a lectin-dependent assay against \textsuperscript{51}Cr-labeled P815 murine mastocytoma cells as described previously (26).

The ability of gastric T cell clones to induce Fas–Fas ligand-mediated apoptosis was assessed using Fas\textsuperscript{+} Jurkat cells as target and the anti–Fas antagonistic mAb M3 (Immunex), as described previously (18, 27).

**Results**

**Submolecular Specificity of Gastric T Cell Clones Reactive to H\textsuperscript{+},K\textsuperscript{−}-ATPase.** A total of 154 CD4\textsuperscript{+} and 49 CD8\textsuperscript{+} T cell clones were obtained from the gastric biopsies of four \textit{H. pylori}–infected AIG patients. All gastric clones were screened for proliferation to \textit{H. pylori} lysate, H\textsuperscript{+},K\textsuperscript{−}-ATPase, or porcine albumin (control antigen). No proliferation was detected in any of the CD8\textsuperscript{+} clones and in 108 CD4\textsuperscript{+} T cell clones, although they all proliferated to IL-2. In contrast, 18 CD4\textsuperscript{+} gastric clones (donor 1, five; donor 2, two; donor 3, four; and donor 4, seven) showed significant proliferation (MI range, 38–212) to \textit{H. pylori} lysate, but not to H\textsuperscript{+},K\textsuperscript{−}-ATPase or porcine albumin, and 15 CD4\textsuperscript{+} clones (donor 1, three; donor 2, five; donor 3, two; and donor 4, five) proliferated to H\textsuperscript{+},K\textsuperscript{−}-ATPase (MI range, 28–179), but not to \textit{H. pylori} lysate or porcine albumin. Interestingly, a third group of 13 CD4\textsuperscript{+} clones was found that proliferated almost equally well to both H\textsuperscript{+},K\textsuperscript{−}-ATPase and \textit{H. pylori} lysate, but not to porcine albumin (Fig. 1).

T cell blasts from each of the 28 H\textsuperscript{+},K\textsuperscript{−}-ATPase–reactive T cell clones were screened for proliferation in response to 205 overlapping peptides for the \alpha chain and 56 peptides for the \beta chain of H\textsuperscript{+},K\textsuperscript{−}-ATPase (Table I). In the series of 15 H\textsuperscript{+},K\textsuperscript{−}-ATPase–specific clones that failed to proliferate to \textit{H. pylori} lysate, 6 clones recognized an epitope in the \alpha chain and 9 clones found their epitope in the \beta chain of the proton pump. Interestingly, the \alpha881–895 epitope was recognized by a couple of clones from donor 2 (2.P02 and 2.P14), in spite of their different expression of TCR-\textgamma. Likewise, different TCR-\textgamma regions were expressed by the two clones from donor 3 and the three clones from donor 4 that recognized the same epitopes (\textgamma331–245 and \textgamma76–90, respectively).

In the series of 13 clones that proliferated to both H\textsuperscript{+},K\textsuperscript{−}-ATPase and \textit{H. pylori} lysate, 11 recognized their epitope in the \alpha chain and 2 clones in the \beta chain. Two clones from different patients (2.R37 and 3.A30, bearing different TCR-\textbeta) recognized the same \alpha836–850 epitope, and two other clones in this series (1.C31 and 4.A05) recognized the same \alpha621–635 epitope. No overlap was found between the H\textsuperscript{+},K\textsuperscript{−}-ATPase epitopes recognized by clones reactive only to H\textsuperscript{+},K\textsuperscript{−}-ATPase and the H\textsuperscript{+},K\textsuperscript{−}-ATPase epitopes recognized by clones able to proliferate to both H\textsuperscript{+},K\textsuperscript{−}-ATPase and \textit{H. pylori} lysate (Table I).

Evidence for clonality of the cross-reactive CD4\textsuperscript{+} T cell clones was provided by the unique products of PCR analysis of TCR-\textbeta mRNA expression in clones 1.A12 (\textbeta19) and 2.R37 (\textbeta15; unpublished data) or by the cytofluorimetric patterns of single TCR-\textbeta expression shown by
The clonality of T cell clones reactive to both H\(^+\),K\(^+\)-ATPase and \textit{H. pylori} lyase epitopes was analyzed using a bioinformatic method. For clone 1.A12 reactive to the H\(^+\),K\(^+\)-ATPase epitope and clone 4.C13 reactive to H\(^+\),K\(^+\)-ATPase, no cross-reactive candidate was predicted in the genomes of \textit{H. pylori} J99 and 26695 strains. Screening for cross-reactive H\(^+\),K\(^+\)-ATPase epitopes. Homologies between the 13 H\(^+\),K\(^+\)-ATPase epitopes and peptides present in both genomes of \textit{H. pylori} J99 and 26695 strains were screened by using a bioinformatic method. For clone 1.A12 reactive to the allogeneic H\(^+\),K\(^+\)-ATPase epitope, the six predicted H\(^+\),K\(^+\)-ATPase epitopes and peptides were synthesized, but none of them induced significant proliferation (MI > 5). It is of note that the H\(^+\),K\(^+\)-ATPase epitopes recognized by the other 10 gastric clones were 100% identical in the pig and human molecules. For each of these 10 gastric clones, a cross-reactive peptide was found in the series (\(n = 73\)) of candidate \textit{H. pylori} peptides we had synthesized (Table II). The two clones from different patients (2.R37 and 3.A30), that shared recognition of the 11-25 sequence of a lipopolysaccharide biosynthesis protein of \textit{H. pylori}, the rela-
was consistently higher (1.4–2-fold) than that obtained with the corresponding microbial peptides (Fig. 3). However, at concentrations as low as 1 pM, both self- and cross-reactive microbial peptides were still stimulatory. To know the MHC restriction elements required for recognition of self- or cross-reactive epitopes, T cell clones were stimulated by the appropriate H+,K+-ATPase and H. pylori cross-reactive peptides in the presence of irradiated autologous APCs treated with anti–HLA-DR or anti–HLA-DQ monoclonal antibodies. Addition in culture of anti–HLA-DR consistently resulted in virtual abrogation of the proliferative response by T cell clones, whereas anti–HLA-DQ was unable to affect peptide-induced proliferation (Table III).

**Cytokine Production Induced by H+,K+-ATPase and H. pylori Cross-reactive Peptides.** Mapping of the H+,K+-ATPase peptide specificity and the cross-reactive recognition of H. pylori peptides based on proliferative response was accomplished with the assessment of IFN-γ, IL-4, and IL-5 production induced by the relevant peptides. Upon appropriate stimulation, all gastric clones produced IFN-γ, but neither IL-4 nor IL-5, thus showing a Th1 profile (Fig. 4). In contrast, either H+,K+-ATPase peptides or H. pylori cross-reactive peptides that failed to induce proliferation also failed to induce cytokine production. From these data, we concluded that the appropriate H. pylori cross-reactive peptides are as powerful as the specific H+,K+-ATPase peptides in inducing a number of gastric T cells to proliferate and to express their Th1 functional profile. All gastric clones reactive to both H+,K+-ATPase and H. pylori lysate expressed effector functions typical of Th1 cells, such as perforin-mediated cytotoxicity (range of specific 51Cr release, 36–67%) and Fas–Fas ligand–mediated proapoptotic activity (range of specific 51Cr release, 29–51%), which was substantially inhibited (range 37–69%) by an anti–Fas antagonistic antibody. Likewise, gastric clones reactive only to H+,K+-ATPase were able to express both perforin-mediated cytotoxicity (range of specific 51Cr release, 24–72%) and proapoptotic activity in the same target cells (range of specific 51Cr release, 22–59%).

**Discussion**

The presence in H. pylori–infected AIG patients of gastric T cells reactive to H. pylori antigens is in agreement with
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Recognition of Their Epitope in the
zyme, whereas most of the latter (11 out of 13) found their
epitope suitable for cross-reaction with T cell epitopes of
gastric H. pylori–antigen. For each of 10 cross-reactive gastric clones, bioinformatics provided us with a cross-reactive H. pylori epitope able to induce significant T cell clone proliferation. Clones R37 from patient 2 and clone A30 from patient 3, which shared recognition of the α836–850 H⁺,K⁺-ATPase epitope, also shared cross-reactivity with the 11–25 peptide of a lipopolysaccharide biosynthesis protein of H. pylori. In contrast, for clone C31 from patient 1 and clone A05 from patient 4, both reactive to α621–635 H⁺,K⁺-ATPase, the bioinformatic method had predicted two different series of 11 and 8 cross-reactive candidates, respectively. Indeed, clone 1.C31 reacted quite well only to the 264–278 peptide of histidine kinase, whereas clone 4.A05 showed cross-recognition of the 35–49 epitope of porphobilinogen deaminase of H. pylori. In summary, our search led to the identification of nine H. pylori proteins, each harboring a T cell epitope suitable for cross-reaction with T cell epitopes of gastric H⁺,K⁺-ATPase α chain.

T cell recognition of cross-reactive H. pylori epitopes resulted in both proliferation and expression of functional properties by cross-reactive T cell clones. In all clones, the Th1 cytokine profile expressed upon stimulation with

Table II. Cross-reactive H⁺,K⁺-ATPase and H. pylori Peptides Recognized by Gastric T Cell Clones

| T cell clones (epitope) | Amino acid sequence recognized | H⁺,K⁺-ATPase bacterial peptide | MI ± SD | H. pylori protein including the cross-reactive peptide (position) |
|------------------------|-------------------------------|--------------------------------|--------|---------------------------------------------------------------|
| 1.C31 (α621–635)       | IRVIMVTGCHFPITAK               | 79 ± 9                         | Histidine kinase (264–278) |
|                        | VRVDVRLDLHCWMLI               | 86 ± 5                         |        |
| 1.A04 (α781–795)       | NLRKPSHYLTVK                 | 194 ± 16                       | Dimethyl adenosine transferase (99–113) |
|                        | ISNLPPYEATRLVNL              | 108 ± 12                       |        |
| 2.P24 (α46–60)         | KKEEMINDHQESVAE              | 23 ± 3                         | Penicillin-binding protein 2 (104–118) |
|                        | LNNYQKEMLVYNHL               | 27 ± 2                         |        |
| 2.R37 (α836–850)       | KAEIDMLHLPFLRPQK             | 50 ± 7                         | LPS biosynthesis protein (11–25) |
|                        | NMRVFQHTSLSPKTC              | 19 ± 2                         |        |
| 3.A30 (α836–850)       | KAEIDMLHLPFLRPQK             | 49 ± 6                         | LPS biosynthesis protein (11–25) |
|                        | NMRVFQHTSLSPKTC              | 16 ± 1                         |        |
| 4.A15 (α181–195)       | VVRGKREDQINADQL              | 39 ± 2                         | Acetate kinase (93–107) |
|                        | VVQIDKREHPVVLVD               | 20 ± 1                         |        |
| 4.C32 (α241–255)       | CTHESSELTNNIAFP              | 87 ± 9                         | VirB4 homologue (78–92) |
|                        | VIQIGEMTPAIAFL               | 75 ± 8                         |        |
| 4.C27 (α256–270)       | STMCEGACTGOLVNN              | 137 ± 17                       | Phosphoglucomutase (70–84) |
|                        | ALDSLEKVVVARLYVK              | 34 ± 2                         |        |
| 4.C26 (α516–530)       | VMKESGRQVRSLGQCS             | 104 ± 9                        | GidA (571–585) |
|                        | VFPQGHPGLEAVEK                | 47 ± 4                         |        |
| 4.A05 (α621–635)       | IRVIMVTGCHFPITAK             | 99 ± 12                        | Porphobilinogen deaminase (35–49) |
|                        | IRIVKSTDQKILDAP              | 51 ± 6                         |        |

For each gastric T cell clone reactive to both H⁺,K⁺-ATPase and H. pylori, a single H. pylori cross-reactive peptide was identified. Identical amino acid residues in the recognized H⁺,K⁺-ATPase peptide and the cross-reactive H. pylori peptide are in boldface. H. pylori proteins containing a cross-reactive epitope recognized by one of the T cell clones in this study are as follows: histidine kinase [HP0392]; dimethyl adenosine transferase [HP1431]; penicillin-binding protein 2 [HP1563]; LPS (lipopolysaccharide) biosynthesis protein [HP0805]; acetate kinase [HP0903]; phosphoglucomutase [HP0075]; VirB4 homologue [HP0017]; GidA, glucose-inhibited division protein A [HP0213]; and porphobilinogen deaminase [HP0237]. Codes in brackets indicate the annotated gene number of H. pylori 26695.
Table III. Effect of Addition in Culture of Anti-DR or Anti-DQ Monoclonal Antibodies on the Proliferative Response to Self and Cross-reactive Peptides by Gastric T Cell Clones Reactive to both H+,K+-ATPase and H. pylori Lysate

| T cell clones | Peptide added in culture | Proliferative response* in the presence of: |
|---------------|--------------------------|---------------------------------------------|
|               |                          | Isotype control | Anti-DR | Anti-DQ |
| 1.C31         | H+,K+-ATPase α621-635    | 110 ± 7        | 1.6 ± 0.1 | 115 ± 9 |
|               | Histidine kinase, 264-278| 101 ± 5        | 1.5 ± 0.1 | 100 ± 7 |
| 1.A04         | H+,K+-ATPase α781-795    | 173 ± 10       | 1.1 ± 0.1 | 160 ± 8 |
|               | Dimethyl adenosine transferase, 99-113 | 148 ± 11 | 1.0 ± 0.1 | 151 ± 10 |
| 2.P24         | H+,K+-ATPase α46-60      | 31 ± 2         | 1.2 ± 0.1 | 30 ± 3  |
|               | Penicillin-binding protein 2, 104-118 | 38 ± 2 | 0.9 ± 0.1 | 38 ± 3  |
| 2.R37         | H+,K+-ATPase α836-850    | 61 ± 4         | 1.1 ± 0.1 | 58 ± 5  |
|               | LPS biosynthesis protein, 11-25 | 32 ± 3 | 1.2 ± 0.1 | 35 ± 4  |
| 3.A30         | H+,K+-ATPase α836-850    | 42 ± 4         | 1.0 ± 0.1 | 40 ± 5  |
|               | LPS biosynthesis protein, 11-25 | 22 ± 2 | 1.3 ± 0.1 | 23 ± 1  |
| 4.A15         | H+,K+-ATPase α181-195    | 48 ± 3         | 1.4 ± 0.1 | 49 ± 4  |
|               | Acetate kinase, 93-107   | 29 ± 2         | 1.0 ± 0.1 | 30 ± 2  |
| 4.C32         | H+,K+-ATPase α241-255    | 98 ± 9         | 1.1 ± 0.1 | 96 ± 8  |
|               | Phosphoglucosamine mutase, 70-84 | 89 ± 9 | 1.2 ± 0.1 | 90 ± 10 |
| 4.C27         | H+,K+-ATPase α256-270    | 118 ± 10       | 1.9 ± 0.2 | 106 ± 11|
|               | VirB4 homologue, 78-92   | 59 ± 4         | 0.9 ± 0.1 | 58 ± 5  |
| 4.C26         | H+,K+-ATPase α516-530    | 127 ± 9        | 1.4 ± 0.1 | 126 ± 10|
|               | GidA, 571-585            | 56 ± 6         | 1.3 ± 0.1 | 55 ± 7  |
| 4.A05         | H+,K+-ATPase α621-635    | 97 ± 9         | 1.0 ± 0.1 | 96 ± 8  |
|               | Porphobilinogen deaminase, 35-49 | 60 ± 6 | 1.1 ± 0.1 | 62 ± 5  |

*Values are MI ± SD.
GidA, glucose-inhibited division protein A.

cross-reactive H. pylori peptides paralleled that disclosed by stimulation with either bacterial lysate or entire H+,K+-ATPase or the appropriate H+,K+-ATPase epitopes. These data suggest that cross-reactive H. pylori peptides represent signals powerful enough to activate the functional program of gastric cross-reactive Th1 cells. Upon mitogen stimulation, all cross-reactive gastric clones expressed both perforin-mediated cytolysis and induction of Fas–Fas ligand-mediated apoptosis in target cells. Thus, it is tempting to hypothesize that in the inflammatory setting in which cross-reactive T cell clones are activated, parietal cells may express APC functions, becoming target, at the same time, of the cytotoxic and proapoptotic activity of cross-reactive gastric T cells. The end point of this process would be gastric corpus atrophy and hypochloridria.

One may ask whether the T cells which cross-react with H. pylori antigens are simply a chance finding and what is the probability of any T cell response showing some cross-reactivity. However, such a promiscuity of T cell responses to H. pylori lysate could not be found in a series of 206 CD4+ human T cell clones isolated from atherosclerotic plaques, which included 46 clones that recognized Chlamydia pneumoniae antigens (31), although
one might expect more similarity between the proteomes of both bacteria than between the human proteome and that of *H. pylori*.

T cell recognition of a peptide depends on anchor residues involved in binding to the MHC molecule as well as on TCR contact residues. This suggests that the self-peptide and the bacterial peptide recognized by a single T cell, should be considerably homologous in amino acid sequence. However, degeneracy in both TCR (32) and MHC binding–motives (33) reduces this sequence-specific requirement to only a few crucial residues, and it has been demonstrated that in the animal model of myocarditis cross-recognition of the autoantigen (myosin) and the microbial peptide (Chlamydia cysteine-rich outer membrane protein) depends on only four identical residues in the 16 amino acid epitope sequence (34). Another mechanism of TCR cross-recognition may work. Recently, MHC-based molecular mimicry has been reported to underlie TCR cross-reactivity in multiple sclerosis (35). A T cell clone from a patient recognizes both myelin basic protein amino acids 85–99 and Epstein–Barr virus (EBV) DNA polymerase peptide EBV627–641, but recognition of these peptides is restricted by two different DR2 molecules (i.e., DRB1*1501 and DRB5*0101, respectively). Crystal structure determination revealed structural similarities of both DR–peptide complexes at the surface presented for TCR recognition, thus explaining the mechanism that may underlie the mimicry between EBV and myelin basic protein.

Blocking experiments with anti-DR, and anti-DQ antibodies showed that DR represents the MHC restriction element. However, because all our patients are heterozygous for DR alleles, we cannot exclude that the aforementioned mechanism of TCR cross-reactivity is responsible for the mimicry between *H. pylori* and H⁺,K⁺-ATPase observed here.

None of the bacterial epitopes recognized by the cross-reactive T cell clones in the present paper belong to *H. pylori* immunodominant proteins (i.e., CagA, VacA, and urease), which have been identified previously as targets of the majority of gastric T cells in *H. pylori*-infected patients with chronic antral gastritis (28) and peptic ulcer (26). It remains unknown to what extent the *H. pylori* epitopes recognized by the cross-reactive T cells described here are relevant to bacterial infection.

A clear example of epitope mimicry in humans is Lyme arthritis, in which *Borrelia burgdorferi* disseminates to multiple tissues, including joints. In the synovia of patients with specific MHC class II haplotypes, activation of Th1 cells reactive to the 165–173 peptide of the outer surface protein A of *B. burgdorferi* occurs (29, 30). Such an epitope is similar to the L332–340 peptide of the human leukocyte function-associated antigen 1α, whose expression is up-regulated on synoviocytes by the Th1–derived IFN-γ (36–38). However, some aspects of this model of human molecular mimicry are questioned because it is still unknown what precipitates the disease several months after the borrelial infection, whether the human leukocyte function-associated antigen 1α epitopes are actually being presented in the joints, and what kind of APCs would be involved (5).

With regard to the question of whether it was *H. pylori* infection or *H. pylori*-independent gastric autoimmunity that initiated disease, three hypotheses can be suggested. First, our patients, having inherited MHC haplotypes that predispose to organ-specific autoimmunity (39), already had undiagnosed or subclinical AIG and *H. pylori* infection, by providing a number of epitopes cross-reactive to H⁺,K⁺-ATPase, caused a Th1-mediated inflammation leading to the expansion of both cross-reactive and single (H⁺,K⁺-ATPase)-reactive gastric T cells. The outcome was increased parietal cell destruction and gastric atrophy. Second, *H. pylori* was the initiating factor, and primary activation of gastric Th1 cells reactive to *H. pylori* peptides that cross-react with H⁺,K⁺-ATPase resulted in an inflammatory process in which T cell–derived IFN-γ allowed H⁺,K⁺-ATPase–bearing parietal cells to act as APCs and to become targets of cross-reactive epitope recognition and killing and/or apoptotic suicide. Apoptotic parietal cells would allow cross-priming of T cells specific for private H⁺,K⁺-ATPase epitopes, ultimately leading to full blown AIG by epitope spreading. Third, *H. pylori* infection was an epiphenomenon, playing no role in the natural history of the disease.

Our data fulfill most of the criteria proposed for assessing a case of molecular mimicry (1, 5, 12). In our patients, there was a temporal association between clinical and serological evidence of AIG and *H. pylori* infection, at least at the time of culture of their in vivo–activated gastric T cells. Because *H. pylori* is commonly acquired in young life (19), the infection preceded symptoms of AIG that usually arise later in life. Taking into account the wide diffusion of *H. pylori* (19), one may suspect that some AIG patients, who were found *H. pylori*–negative at the time of their diagnosis of AIG, might have harbored the bacterium previously, and *H. pylori* was lost while mucosal atrophy was ongoing. Strikingly, all our AIG patients lost *H. pylori* during the 4-yr study period, possibly due to hypochloridria that made their gastric environment no more appreciated by the bacterium. The possibility that *H. pylori* gets lost due to increasing gastric atrophy and hypochloridria has already been reported (40).

We have identified a quite broad repertoire of culprit epitopes in both the pathogen and in the self gastric protein associated with AIG. The microbial cross-reactive epitopes were able to elicit vigorous responses in the same gastric T cells that responded at comparable levels to both the corresponding self H⁺,K⁺-ATPase epitopes and the entire self protein. Finally, cross-reactive T cell clones quantitatively represented a significant component of the T cell response at gastric level during the autoimmune disease and the concomitant *H. pylori* infection. This would argue against the possibility that the detection at gastric level of autoreactive, cytotoxic, and proapoptotic Th1 cells that cross-react to *H. pylori* epitopes is simply an epiphenomenon.

Together, our results support the idea that in genetically susceptible individuals, *H. pylori* infection triggers or accel-
erates the development of gastric autoimmunity via molecular mimicry.

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