

Letter to the Editor

Potential role of extracellular vesicle-mediated antigen presentation in *Helicobacter pylori* hypersensitivity during eradication therapy

To the Editor:

Helicobacter pylori is a gram-negative bacterium that colonizes the human stomach. *H. pylori* is causally related to gastritis/gastric cancer, and eradication of *H. pylori* reduces the incidence of gastric cancer.¹

Generalized skin rash is caused by a variety of infections and adverse drug reactions. It is often difficult to identify the cause of rashes, particularly when patients are administered antibiotics or antiviral agents. The most common clinical features of a cutaneous adverse drug reaction (cADR) are generalized and progressively confluent macular and papular lesions.²

In this study our results support a specific anti-*H. pylori* immune reaction in patients with skin reactions after completing *H. pylori* eradication therapy using antibiotics and a proton pump inhibitor. Additionally, we found that extracellular vesicles (EVs) derived from cultured dendritic cells exposed to *H. pylori* contain *H. pylori* components and induce an immune response *in vitro*. These findings suggest that *H. pylori* mediates the development of extragastric diseases through an anti-*H. pylori* immune reaction.

The characteristics of patients' skin eruptions after *H. pylori* treatment (7 days) are presented in Table E1 in this article's Online Repository at www.jacionline.org. Thirteen patients had at least 3 negative responses to the drug lymphocyte transformation test (LTT) for *H. pylori* eradication therapy drugs, which uses *in vitro* methods to determine whether a patient has a T-cell response to a certain drug. There were no differences between patients with negative drug LTT responses and patients with positive drug LTT responses (eg, drug eruption) on physical examination or histopathologic findings of skin lesions (Fig 1, A, and see Fig E1 in this article's Online Repository at www.jacionline.org). However, differences in clinical course were noted. cADRs generally develop during medication use and resolve within several days of discontinuation. By contrast, patients with negative drug LTT responses had skin manifestations 1 to 4 days after completing *H. pylori* eradication therapy (Fig 1, B). Based on these findings, we assumed that there might be another pathogenesis other than drug eruption that could be caused by *H. pylori*.

To determine whether patients with negative drug LTT responses had an *H. pylori*-specific reaction, we measured cytokine levels in supernatants of *H. pylori*-exposed PBMCs. Concentrations of IL-2, IL-4, IL-6, IFN- γ , and TNF- α were significantly greater in samples from patients with negative drug LTT responses with skin eruptions ($n = 4$; patients 1, 8, 11, and 13) than in samples from patients with negative drug LTT responses without skin eruptions ($n = 3$) and healthy control subjects ($n = 3$; Fig 1, C). The control bacterium (ie, *Escherichia coli*) did not induce cytokine expression in any groups. Although an endotoxin contained in *H. pylori* can mediate nonspecific cell stimulation, *H. pylori* has considerably lower endotoxic and immune activities.³ Indeed, cytokine upregulation was not detected in samples from *H. pylori* noncarriers. Taken together,

these results suggest that a *H. pylori*-specific immune reactions were elicited in patients with negative drug LTT responses.

The *H. pylori*-LTT assay also revealed PBMC proliferation in 1 of 8 patients with negative drug LTT responses (stimulation index [SI] > 2.0) when PBMCs were exposed to *H. pylori* (Fig 1, D). In PBMCs from a patient without skin eruptions, *H. pylori* did not induce PBMC proliferation.

Furthermore, an IFN- γ ELISpot assay revealed that *H. pylori* increased the number of IFN- γ -producing T cells in PBMCs from 1 of 7 patients with negative drug LTT responses, whereas drug exposure did not (Fig 1, E). These data indicate that the *H. pylori*-LTT assay and the ELISpot assay identify a minor population of patients with negative drug LTT responses with *H. pylori*-specific immune responses.

We then investigated *H. pylori*-specific CD4⁺ T cells based on CD154 expression, which is expressed transiently after ligation of the T-cell receptor, thus providing direct access to an antigen-specific population after *ex vivo* stimulation.⁴ In most patients ($n = 7$) *H. pylori*-specific CD4⁺ T cells were identified after *H. pylori* exposure (Fig 2, A, and see Table E2 in this article's Online Repository at www.jacionline.org). Frequencies of CD154⁺CD4⁺ T cells in patients with negative drug LTT responses significantly differed after exposure to *H. pylori*, drugs, and PBS (Fig 2, B). In particular, patients with skin eruptions 3 or more days after completing *H. pylori* treatment (after 10 days) exhibited a significantly increased frequency of CD4⁺CD154⁺ T cells on exposure to *H. pylori*. However, patients with skin eruptions 2 days or less after completing *H. pylori* treatment did not exhibit a significant difference (Fig 2, B). Notably, 1 patient exhibited a high frequency of CD154⁺CD4⁺ T cells on drug exposure but not with *H. pylori*, demonstrating that the patient had a drug-specific immune reaction.

These data indicate that skin eruptions associated with *H. pylori* eradication therapy could be caused by *H. pylori*, especially eruptions that occur 3 or more days after completion of *H. pylori* eradication therapy.

Next, we examined the mechanism of *H. pylori* recognition and elicitation as an antigen. Although some bacteria are found inside host cells, as exemplified by the case of *Staphylococcus aureus*,⁵ it is unclear whether *H. pylori* also exists in host cells. Therefore we consider that *H. pylori* components are required to invade the body to induce an immune reaction against *H. pylori*. Moreover, even if *H. pylori* components invade the body, they would be rapidly degraded by enzymes. EVs, including exosomes and microvesicles, contain proteins and nucleic acids that have roles in a variety of physiologic and pathologic processes.⁶ A recent study reported that serum-derived exosomes from patients infected with *H. pylori* contain *H. pylori*-specific peptides.⁷ In addition, EVs play a role in antigen presentation and modulation of immune responses.⁸ Therefore we hypothesized that if *H. pylori* proteins are contained in these EVs, they might elicit an anti-*H. pylori* immune reaction. Although we used historically popular exosome purification protocol and exosome markers, we could not exclude the possibility that *H. pylori* proteins are also secreted through other EVs. Hence we used the term EVs in this report. EVs purified by means of ultracentrifugation were analyzed by using liquid chromatography coupled with tandem mass spectrometry

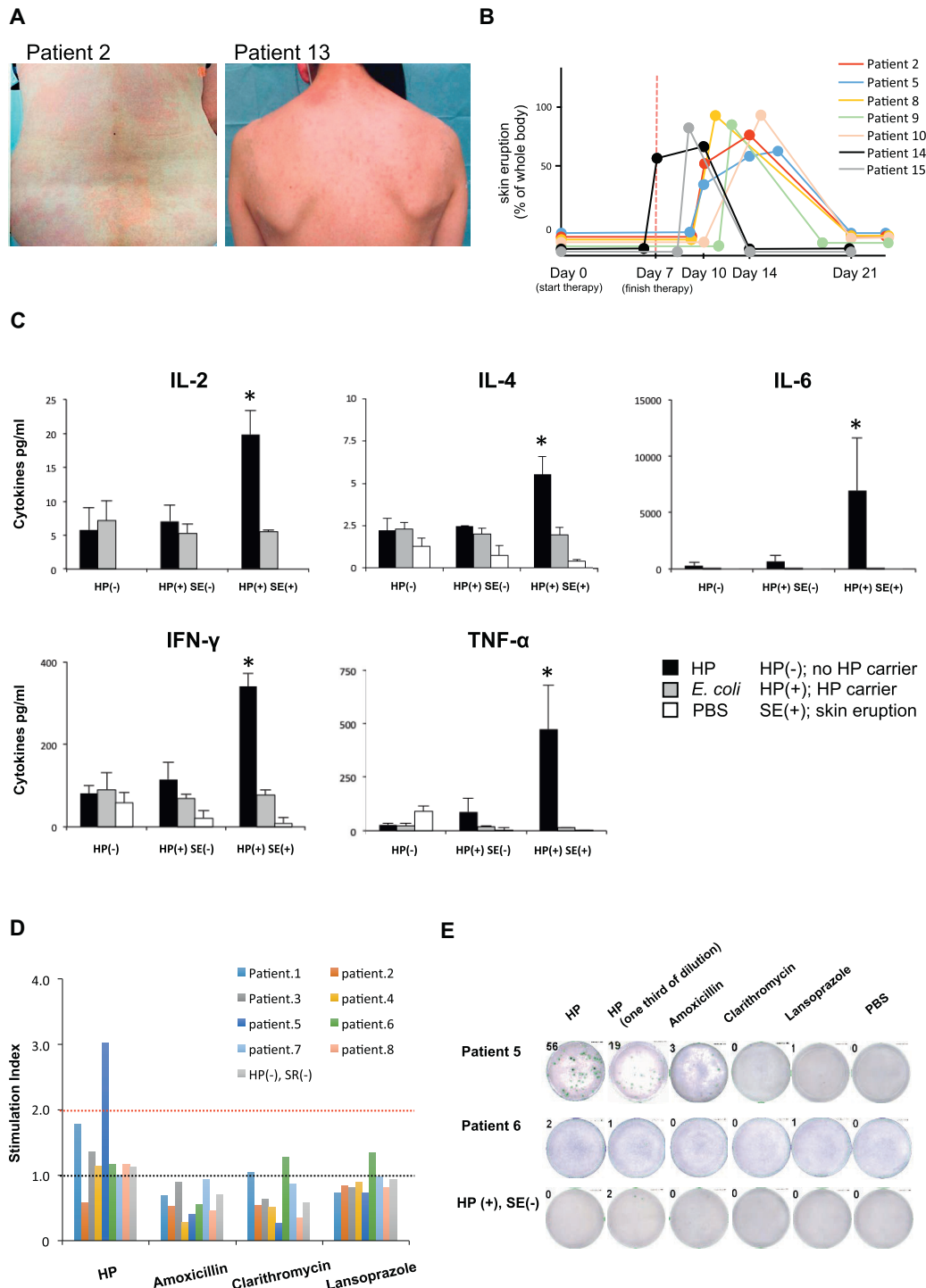


FIG 1. Clinical features and analysis of ELISA, LTT, and ELISpot results in patients with skin reactions after *H pylori* (HP) eradication therapy. **A**, Skin manifestations of patients with skin eruptions after *H pylori* eradication therapy (patients 2 and 13; patients with negative drug LTT responses). A maculopapular rash developed on the chest and back. **B**, Clinical course of patients with skin eruptions after *H pylori* eradication therapy assessed as the mean percentage of skin area affected in each patient. **C**, Cytokine levels in cell-culture supernatants from PBMCs exposed to *H pylori*, *E coli*, or PBS for 24 hours. PBMCs were sampled from patients with and without skin eruption (SE) after *H pylori* eradication therapy and healthy volunteer control subjects. Supernatants were assayed for multiple cytokines by using the Bio-Plex Human Cytokine 27-Plex panel (Bio-Rad Laboratories, Hercules, Calif). Values are means of 3 independent experiments. * $P < .05$. **D**, Proliferation of T cells in the PBS, drug, or *H pylori* groups was measured by using an LTT. Results are presented as an SI. **E**, IFN- γ secretion from PBMCs was measured by using an ELISpot in response to *H pylori*, drugs, or PBS. Data are from patients with skin eruptions after *H pylori* eradication therapy. The number of spots per well is indicated next to the corresponding well image.

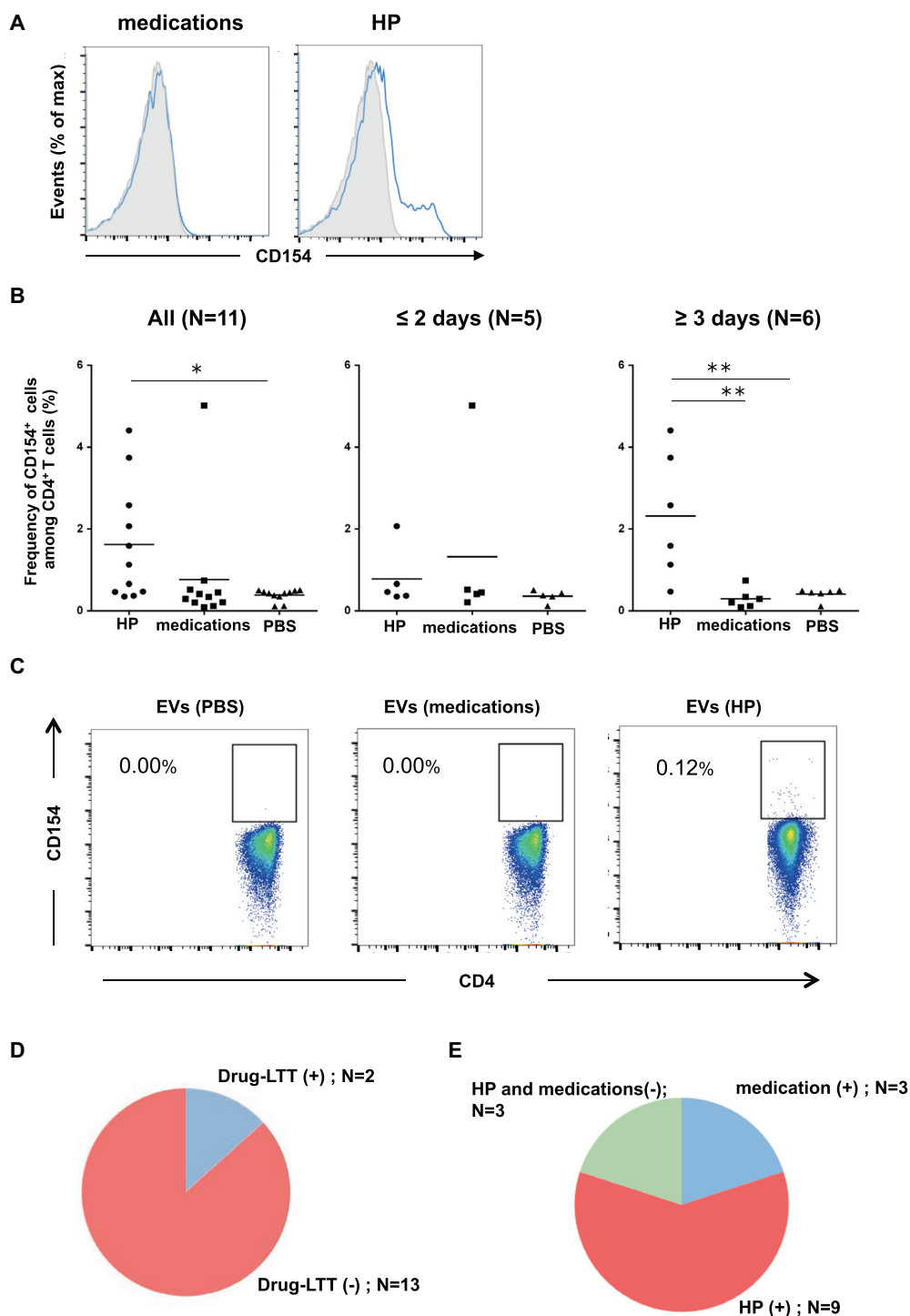


FIG 2. Expression of CD154 on CD4⁺ T cells after incubation of patients' PBMCs stimulated with *H. pylori* (HP) or *H. pylori* peptides containing EVs. **A**, Flow cytometric analysis of CD154 expression by CD4⁺ T cells from a patient with skin eruptions after *H. pylori* treatment after 24 hours of culture with drugs or *H. pylori* (shaded area; isotype control). **B**, CD154 expression in CD4⁺ T cells from patients with and without skin eruptions after *H. pylori* treatment after 24 hours of culture with PBS, drugs, or *H. pylori* (n = 11). ≥ 3 days, Skin eruptions develop 3 or more days after completing *H. pylori* eradication treatment; ≤ 2 days, skin eruptions develop 2 days or less after completing *H. pylori* eradication treatment. * $P < .05$ and ** $P < .01$. **C**, Flow cytometric analysis of CD154 expression in CD4⁺ T cells from a patient with skin eruptions after *H. pylori* eradication (patient 1) after 24 hours of culture with EVs from MDDCs cultured with PBS, eradication drugs, or *H. pylori*. **D**, Number of patients with negative drug LTT responses or patients with positive drug LTT responses with skin eruptions after *H. pylori* treatment. **E**, Numbers of patients with a positive result for any immunologic test for *H. pylori* and those with a positive result for any immunologic test for drugs.

(LC-MS/MS). Some of the *H pylori*-specific proteins were present in 4 of 4 patients with skin eruptions after *H pylori* eradication therapy, whereas no *H pylori* peptides were detected in patients without skin eruptions and *H pylori*-uninfected donors (see Table E3 in this article's Online Repository at www.jacionline.org). In addition, detected *H pylori* proteins varied among patients (see Table E4 in this article's Online Repository at www.jacionline.org).

To reduce the possibility that *H pylori*-specific peptides could escape from a leaky gut and be copelleted in the EV fraction, the fractions were purified based on CD9 and CD81 (exosome marker) sorting. *H pylori*-specific proteins were also observed in CD9- and CD81-purified EVs (see Table E5 in this article's Online Repository at www.jacionline.org).

Next, we investigated whether EVs with proteins identical to *H pylori* components were functional mediators of the *H pylori*-specific immune reaction. It is likely that *H pylori* components contained in serum EVs could induce an immune response. In fact, we used EVs from patients' sera; however, the EVs did not successfully induce an immune reaction, as determined by using flow cytometric analysis of CD154⁺ *H pylori*-specific T cells. Given that the process of purification reduced the amount of EVs, we assumed that the concentration of EVs from patients' sera was very low. The amount of *H pylori*-specific peptides identified in EVs purified from patients' serum samples was very low (see Table E3).

Meanwhile, a previous report demonstrated that *H pylori* is phagocytized by dendritic cells in Peyer patches in mice.⁹ Therefore we investigated whether EVs are released from dendritic cells. EVs from *H pylori*-exposed monocyte-derived dendritic cells (MDDCs; see Fig E2 in this article's Online Repository at www.jacionline.org) displayed specific markers (see Fig E3 in this article's Online Repository at www.jacionline.org). Using LC-MS/MS, we identified *H pylori*-derived peptide in EVs from *H pylori*-exposed MDDCs (see Table E6 in this article's Online Repository at www.jacionline.org). One *H pylori* component (Gene HP_0426) appears in both serum EVs and EVs derived from cultured dendritic cells exposed to *H pylori*.

We next assessed whether EVs from *H pylori*-exposed MDDCs could activate *H pylori*-specific CD4⁺ T cells. EVs from *H pylori*-exposed MDDCs exhibited significant induction in 2 of 5 patients (Fig 2, C; see Table E7 in this article's Online Repository at www.jacionline.org), indicating that EVs with proteins identical to *H pylori* components are released from *H pylori*-exposed MDDCs and might be functional mediators of *H pylori*-specific immune reactions. However, it should be noted that our data do not exclude the possibility that *H pylori* protein can also mediate T-cell activation directly.

Among 15 patients with skin eruptions, only 2 patients had positive results on drug LTTs (Fig 2, D). We identified 9 patients with *H pylori*-specific immune responses (Fig 2, E). In addition, 1 patient had drug-specific hypersensitivity based on a CD154 expression assay. These data indicated that patients with skin eruptions associated with *H pylori* eradication therapy exhibit drug or *H pylori* hypersensitivity.

cADRs are more frequently induced by antibiotics compared with other drug classes. We demonstrated that combined testing identified *H pylori*-specific hypersensitivity. In particular, CD154 expression enables differentiation between antibiotic- and *H pylori*-mediated skin eruptions. These results suggest that identification of bacterial

hypersensitivity will release patients from unnecessary avoidance of suspected drugs. Furthermore, identification of *H pylori* hypersensitivity is of great benefit when considering medication strategies for patients with skin eruptions caused by *H pylori* eradication therapy. As a result, it would be possible to reattempt or resume *H pylori* eradication therapy when skin eruptions develop because drug eruptions could be ruled out.

Our findings that microbe components are present in serum EVs and have the potential to elicit a specific immune reaction suggest a relationship between the immune system and gut microbes. We hypothesize that a considerable amount of degraded *H pylori* is absorbed by the small intestine during *H pylori* eradication therapy. The increased quantity of *H pylori* components in serum EVs might subsequently induce a CD4⁺ cell-mediated immune reaction.

Finally, *H pylori* components in EVs can induce skin manifestations during antibiotic treatment. A test to identify *H pylori* sensitivity would be highly useful to distinguish such sensitivity from antibiotic hypersensitivity.

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METHODS

Patients, PBMCs, and sera

Fifteen patients with skin eruptions associated with *H pylori* eradication therapy (Table E1) and 3 subjects who did not experience skin eruptions after *H pylori* eradication therapy as control subjects were enrolled in this study. The institutional review board of Hokkaido University Graduate School of Medicine approved this study, and written informed consent for all diagnostic procedures and analyses was obtained from all patients.

PBMCs were collected at least 2 weeks to 3 months after skin eruptions disappeared. Sera were collected while skin eruptions were present.

Bacterial strains and culture conditions

H pylori (ATCC43504; American Type Culture Collection, Rockville, Md) was maintained at -80°C in brain-heart infusion broth. Bacteria were cultured on Columbia agar plates with 10% sheep blood (Becton Dickinson, Cockeysville, Md). All cultures were incubated microaerobically at 37°C in jars flushed with 5% O_2 , 10% CO_2 , and 85% N_2 , unless otherwise stated. Antibiotic-treated *H pylori* or *E coli* (ATCC25992) was used in the experiments as bacterial controls.

In vitro activation assays

For cell stimulation studies, PBMCs from patients and control subjects were cultured with *H pylori* (1.0×10^7 /colony-forming unit [CFU]), drugs (4 $\mu\text{g}/\text{mL}$ amoxicillin, 4 $\mu\text{g}/\text{mL}$ clarithromycin, and 4 $\mu\text{g}/\text{mL}$ lansoprazole), or PBS for 24 hours. The cultured PBMCs and supernatants were used for flow cytometry, ELISAs, ELISpot assays, or LTTs.

Measurement of cytokines/chemokines in culture supernatants

PBMCs were obtained from patients with eruptions after *H pylori* eradication therapy, patients without skin eruptions after *H pylori* eradication therapy, and subjects with no *H pylori* infection. Cell-culture supernatants from PBMCs exposed to *E coli* (1.0×10^7 /CFU), *H pylori* (1.0×10^7 /CFU), or PBS for 24 hours were centrifuged at 2500 rpm for 5 minutes at 4°C . Supernatants were assayed for multiple cytokines by using the Bio-Plex Human Cytokine 27-Plex panel (Bio-Rad Laboratories, Hercules, Calif). Supernatants were reanalyzed for IL-2, IL-4, IL-6, IFN- γ , and TNF- α by using ELISA kits (R&D Systems, Minneapolis, Minn).

Tritiated thymidine incorporation assay for the LTT

An LTT using tritiated thymidine (PerkinElmer, Boston, Mass) was performed, as previously described.^{E1} The results are expressed as an SI, which is the ratio of the highest count per minute of the samples cultured with *H pylori*, drugs, and PBS. An SI value of greater than 2.0 was interpreted as positive.

IFN- γ ELISpot assay

PBMCs were incubated with the drug or *H pylori* in the same manner. After incubation for 24 hours, cells were collected and analyzed in an IFN- γ ELISpot assay (human IFN- γ ELISpot^{PRO} kits; Mabtech AB, Nacka Strand, Sweden) to determine the frequency of epitope-specific IFN- γ -secreting T cells. Data are presented as spot-forming units per 2×10^5 PBMCs. The positive control was a polyclonal activator anti-CD3 antibody. Spot counting was performed with a CTL-ImmunoSpot S5 UV Analyzer (Cellular Technology, Cleveland, Ohio) and a CTL ImmunoSpot Professional Software version 5.0 reader (Cellular Technology).

Flow cytometry

PBMCs were cultured with the drug or *H pylori*. After cultivation for 24 hours, cells were collected and stained with the following antibodies and

reagents: fluorescein isothiocyanate-conjugated CD4 (BD Biosciences, San Jose, Calif), phycoerythrin (PE)-Cy7-conjugated CD3 (BD Biosciences), PE-conjugated CD154 (BD Biosciences), fluorescein isothiocyanate-conjugated CD81 (BioLegend, San Diego, Calif), PE-conjugated CD9 (BioLegend), Pacific Blue-conjugated CD63 (BioLegend), PE-Cy7-conjugated HLA-ABC (BioLegend), peridinin-chlorophyll-protein complex (PerCP)-Cy5.5-conjugated HLA-DR (BioLegend), and Sytox Blue (Thermo Fisher Scientific, Bartlett, Ill). All stained cells were analyzed with the FACSCanto II or Aria III cytofluorometer (BD Biosciences). Subsequent analysis was performed with FlowJo software (TreeStar, Ashland, Ore).

MDDC generation

PBMCs were plated in 10-cm dishes and maintained at 37°C in a 5% CO_2 atmosphere. After 90 minutes, nonadherent cells were removed, and adherent cells were cultured for 7 days at 37°C in RPMI with 10% exosome-depleted FBS (System Bioscience, Palo Alto, Calif), 4 mmol/L L-glutamine, penicillin/streptomycin, and 100 ng/mL recombinant human GM-CSF (R&D Systems) and 50 ng/mL recombinant human IL-4 (R&D Systems) for 7 days.

Isolation of EVs from serum and cell cultures

EV isolation was performed by using differential centrifugation.^{E2} Serum (1 mL) or cell-culture supernatants from MDDCs (2×10^4 cells) with *H pylori* (1.0×10^7 /CFU), drugs (4 $\mu\text{g}/\text{mL}$ amoxicillin, 4 $\mu\text{g}/\text{mL}$ clarithromycin, and 4 $\mu\text{g}/\text{mL}$ lansoprazole), or medium (RPMI with 10% exosome-depleted FBS (System Bioscience), 4 mmol/L L-glutamine or penicillin/streptomycin) alone for 24 hours at 37°C was diluted with PBS before centrifugation at 300g for 10 minutes to remove larger debris. Then the supernatant was filtered through a 0.22- μm spin filter (Agilent Technologies, Santa Clara, Calif) and centrifuged at 100,000g for 70 minutes. The collected pellet was washed in ice-cold PBS and then centrifuged at 100,000g for 70 minutes. The collected pellet was resuspended in a small volume of PBS for further analysis. Exosome markers (CD9, CD81, HLA-DR, and HLA-ABC; BD Biosciences) were analyzed by using flow cytometry, and morphologic characteristics were observed by using transmission electron microscopy.

EV isolation was also performed by using exosome-specific marker sorting (CD9 and CD81). Sera of patients with skin eruptions associated with *H pylori* eradication therapy were mixed and sorted with the FACSARIA (BD Biosciences) and the exosomal markers CD9 and CD81 (BD Biosciences). A sorted population was centrifuged at 300g for 10 minutes to remove larger debris. Then the supernatant was filtered through a 0.22- μm spin filter (Agilent Technologies, Santa Clara, Calif) and centrifuged at 100,000g for 70 minutes. The collected pellet was washed in ice-cold PBS and then centrifuged at 100,000g for 70 minutes. LC-MS/MS analysis was performed to examine the proteomic profile of these EVs.

LC-MS/MS and proteomic data analysis

Proteolytic digestion was performed with a phase transfer surfactant protocol.^{E3} Extracted exosomal proteins were digested at 37°C overnight with 1% (wt/wt) trypsin. Digested peptides were analyzed with a Q-Exactive Mass Spectrometer (Thermo Scientific, Bremen, Germany) with an UltiMate 3000 nano-flow HPLC system (Dionex, Sunnyvale, Calif) and an HTC-PAL autosampler (CTC Analytics, Zwingen, Switzerland). Raw data files were processed with MaxQuant software (version 1.5.1.2). Peak lists were searched against the UniProt HP protein database by using the Andromeda search engine.^{E4} The precursor mass tolerance was set to 7 ppm, and fragment ion mass tolerance was set to 0.01 Da. Peptides and proteins were accepted with a false discovery rate of less than 1%, which was estimated on the basis of the number of accepted hits from the reverse database.

Scanning electron microscopy

Purified EV solution was dried on a cover glass for 30 minutes at 40°C and then coated with platinum-palladium by using a Hitachi E-1030 ion coater

(Hitachi, Tokyo, Japan). Samples were observed with a Hitachi S-4500 scanning electron microscope (Hitachi).

PBMCs cultured with EVs from *H pylori*-exposed MDDCs

Exosomes were purified by means of differential centrifugation of the supernatants (10 μ L) of autologous MDDC cultures (2×10^4 cells/100 μ L) with *H pylori* (1.0×10^7 /CFU), eradication drugs (4 μ g/mL amoxicillin, 4 μ g/mL clarithromycin, and 4 μ g/mL lansoprazole), or PBS. PBMCs from patients with eruptions after *H pylori* eradication therapy were cultured with EVs. After cultivation for 24 hours, cells were collected and analyzed for CD154 expression, as described above.

PBMCs were cultured with EVs isolated from the same patients' MDDC culture supernatants (10 μ L). After cultivation for 24 hours, cells were collected and stained with the above antibodies and analyzed by using flow cytometry (FACSCanto II).

Statistical analysis

The nonparametric Mann-Whitney test was used for comparisons between groups. All statistical analysis was performed with Prism software (GraphPad Software, San Diego, Calif).

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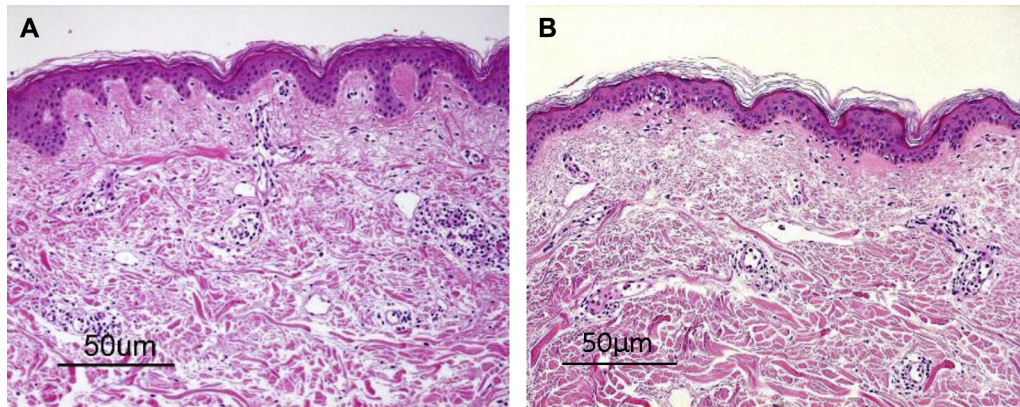


FIG E1. Histopathologic findings for patients with negative drug LTT responses and patients with positive drug LTT responses. Histologic analysis of 5 patients with negative drug LTT responses (**A**) and 15 patients with positive drug LTT responses (**B**) exhibited vacuolar interface dermatitis and superficial perivascular infiltrate of lymphocytes with hematoxylin and eosin staining. Original magnification $\times 100$.

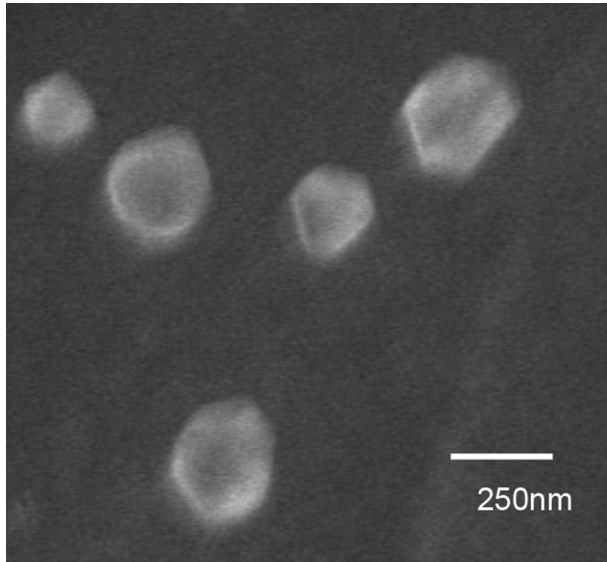


FIG E2. Scanning electron microscope micrograph of whole mounted EVs purified from *H pylori*-exposed MDDC cultures.

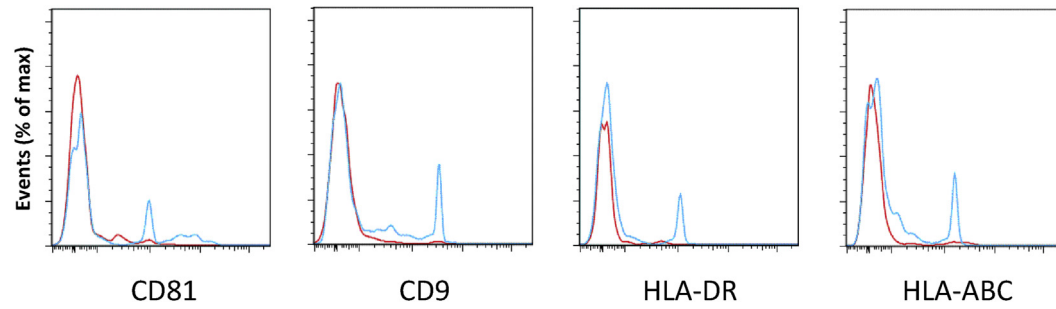


FIG E3. Flow cytometric analysis of MDDC-derived EVs. MDDC-derived EVs were analyzed for CD9, CD81, HLA-ABC, and HLA-DR by using flow cytometry. *Blue histograms*, MDDC-derived EVs; *red histograms*, isotype control.

TABLE E1. Characteristics of patients with skin eruptions after eradication therapy for *H pylori*

	Age (y)	Sex	Interval from start of treatment to onset (d)	Medications	Result of drug LTTs
Patient 1	66	F	11	ACL	All negative (5 times)
Patient 2	72	F	10	ACL	All negative (3 times)
Patient 3	46	M	10	ACR	All negative (3 times)
Patient 4	30	F	10	ACR	All negative (3 times)
Patient 5	62	F	10	ACV	All negative (3 times)
Patient 6	64	M	10	ACR	All negative (3 times)
Patient 7	70	F	9	ACL	All negative (3 times)
Patient 8	74	F	9	ACL	All negative (3 times)
Patient 9	61	F	9	ACR	Amoxicillin (226%)
Patient 10	42	F	8	ACL	All negative (3 times)
Patient 11	74	M	8	ACL	All negative (4 times)
Patient 12	73	F	7	ACR	Rabeprazole (232%)
Patient 13	65	F	4	ACL	All negative (3 times)
Patient 14	60	F	4	ACR	All negative (3 times)
Patient 15	75	F	4	ACL	All negative (3 times)

ACL, Amoxicillin, clarithromycin, and lansoprazole; ACR, amoxicillin, clarithromycin, and rabeprazole; ACV, amoxicillin, clarithromycin, and vonoprazan; F, female; M, male.

TABLE E2. Flow cytometric analysis of CD154 expression in CD4⁺ cells after 24 hours of culture with *H pylori*, medications, or PBS

Interval from start of treatment to onset (d)		Frequency of CD154 ⁺ expression in CD4 ⁺ T cells (%)		
		<i>H pylori</i>	Medications	PBS
Patient 1	11	3.85	0.09	0.43
Patient 2	10	2.58	0.2	0.11
Patient 3	10	1.13	0.34	0.5
Patient 4	10	4.41	0.74	0.46
Patient 5	10	1.59	0.29	0.45
Patient 6	10	0.47	0.12	0.49
Patient 7	9	0.37	0.21	0.51
Patient 8	9	0.66	0.45	0.35
Patient 10	8	0.35	5.02	0.12
Patient 14	4	2.07	0.52	0.43
Patient 15	4	0.46	0.41	0.38

TABLE E3. Specific peptides of *H pylori* in serum EVs

	Time interval from start of treatment to onset (d)	<i>H pylori</i> infection	Skin rash	No. of proteins	
				<i>H pylori</i>	Human
Control subject 1	—	—	—	0	734
Control subject 2	—	—	—	0	581
Control subject 3	—	—	—	0	350
Control subject 4	—	—	—	0	616
Control subject 5	—	—	—	0	526
Control subjects 6	—	+	—	0	911
Patient 3	Day 10	+	+	20	493
Patient 5	Day 10	+	+	19	463
Patient 7	Day 9	+	+	18	466
Patient 11	Day 8	+	+	19	438

+, Positive; —, negative.

TABLE E4. Proteins identified in *H pylori*-infected human serum EVs purified by means of ultracentrifugation and density gradient centrifugation

ID	Gene	Protein	Sequence	Score*	Patients
O07025	<i>flhI</i>	Flagellum-specific ATP synthase	LGIFAGSGVGK	20.79	3, 5, 7, 11
O24884	<i>HP_0043</i>	Mannose-6-phosphate isomerase	MVELNAK	49.59	3, 5, 7, 11
			SVGFLLESLSK	47.30	3, 5, 7, 11
O24925	<i>HP_0099</i>	Methyl-accepting chemotaxis protein (TlpA)	MLETSSNFAKDLANDSANLK	22.63	3
			SKGLSIGNK	68.66	3
O24936	<i>HP_0114</i>	Uncharacterized protein	MDIYQK	42.12	3, 5, 7, 11
O25369	<i>bamA</i>	Outer membrane protein assembly factor BamA	EASQESQK	39.66	3, 5, 7, 11
			LDQLEYDSMR	47.19	3, 5, 7, 11
O25395	<i>HP_0686</i>	Iron(III) dicitrate transport protein (FecA)	AYSSMLNQAK	51.45	3, 5, 7, 11
O25412	<i>HP_0708</i>	Uncharacterized protein	INTLEDQK	45.08	11
			KINTLEDQK	76.22	11
O25446	<i>HP_0750</i>	Uncharacterized protein	QVASSYQNINTTSYNGPK	34.93	3, 5, 7, 11
O25466	<i>HP_0775</i>	Penta-phosphate guanosine -3-pyrophosphohydrolase (SpoT)	LTEEIENK	66.35	3, 5, 7, 11
			QVLLNDCCYPK	20.78	3, 5, 7, 11
			SGDVVKIIK	49.42	3, 5, 7, 11
			VTPKGGIEILR	38.00	3, 5, 7, 11
O25720	<i>HP_1088</i>	Transketolase	LSALMKLK	41.65	5
			VLKGAYVK	27.12	5
O25730	<i>pgl</i>	6-Phosphogluconolactonase	GYQLFEFENLK	45.66	3, 5, 7, 11
O25781	<i>Pgi</i>	Glucose-6-phosphate isomerase	EKIEAMFK	47.08	3, 5, 7, 11
			RAVLHTALR	8.34	3, 5, 7, 11
O25885	<i>HP_1327</i>	Uncharacterized protein	ESLVFK	77.91	3, 5, 7, 11
O25923	<i>HP_1371</i>	Type III restriction enzyme R protein	EDLLEELK	48.56	3, 5, 7, 11
			GGTQIICFIDPKGSK	21.89	3, 5, 7, 11
O26038	<i>HP_1508</i>	Ferredoxin-like protein	KGLQLECIKNCLECDACTITMAK	3.24	3
			LKTIAYMGVIAIVIALLAITSFK	0.71	3
			RSPENECVNLHCVCPTHIDIR	0.16	3
O34810	<i>HP_0426</i>	Uncharacterized protein	SVETIK	70.91	3, 5, 7, 11
P48370	<i>gyrA</i>	DNA gyrase subunit A	MQDNSVNETK	50.03	3, 5, 7, 11
P56013	<i>rpsF</i>	30S ribosomal protein S6	EVEAWHALVDRANKK	35.04	3, 5, 7, 11
			PSHAKEK	111.04	3, 5, 7, 11
P56088	<i>guaB</i>	Inosine-5-monophosphate dehydrogenase	GLITIK	88.73	3, 5, 7, 11
P56157	<i>dnaE</i>	DNA polymerase III subunit alpha	LGITLK	76.55	3, 5, 7, 11
P94846	<i>uvrB</i>	UvrABC system protein B	VLLYAK	78.00	3, 5, 7, 11
P96786	<i>flhD</i>	Flagellar hook-associated protein 2	LDEDTR	69.43	3, 5, 7, 11

*Andromeda score for the associated MS/MS spectra.

TABLE E5. Proteins identified in *H pylori*-infected human serum EVs purified by the exosome marker (CD9 and CD81)

ID	Gene	Protein	Sequence	Score*
O07025	<i>fliI</i>	Flagellum-specific ATP synthase	LGIFAGSGVGK	20.79
O24936	<i>HP_0114</i>	Uncharacterized protein	MDIYQK	42.12
O25395	<i>HP_0686</i>	Iron(III) dicitrate transport protein (FecA)	AYSSMLNQAK	51.45
O25730	<i>pgl</i>	6-phosphogluconolactonase	GYQLFEFENLK	45.66
O25885	<i>HP_1327</i>	Uncharacterized protein	ESLVFK	77.91
O34810	<i>HP_0426</i>	Uncharacterized protein	SVETIK	70.91
P48370	<i>gyrA</i>	DNA gyrase subunit A	MQDNSVNETK	50.03
P56088	<i>guaB</i>	Inosine-5-monophosphate dehydrogenase	GLITIK	88.73
P56157	<i>dnaE</i>	DNA polymerase III subunit alpha	LGITLK	76.55
P94846	<i>uvrB</i>	UvrABC system protein B	VLLYAK	78.00
P96786	<i>fliD</i>	Flagellar hook-associated protein 2	LDEDTR	69.43
O25446	<i>HP_0750</i>	Uncharacterized protein	QVASSYQNINTTSYNGPK	34.93

*Andromeda score for associated MS/MS spectra.

TABLE E6. Proteins identified in *H. pylori*-infected cultured cell EVs by using LC-MS/MS

ID	Gene	Protein	Sequence	Score*
O25506	<i>hup</i>	DNA-binding protein HU	FETAEQK	100.19
			GESVELIGFGK	116.55
O25728	<i>hcpC</i>	Putative beta-lactamase HcpC	YAEGCASLGGIYHDGK	121.32
O25776	<i>fldA</i>	Flavodoxin	AIGNAEVVDVAK	138.24
			FVGLVIDEDNQDDLTDER	174.19
			IGIFFGTDSGNAEIAIEK	150.70
			TIGLVGLGDQDTYSETFAEGIFHIYEK	206.00
			VVGQTPTDGYHFEASK	121.99
O25825	<i>HP_1227</i>	Cytochrome c-553	IVNMMSEAEIEK	88.16
P0A0R3	<i>groS</i>	10 kDa chaperonin	EHEACCHDK	257.88
			LEENKTSSGIIPDNAK	77.70
			TSSGIIPDNAK	99.73
P21762	<i>tsaA</i>	Probable peroxiredoxin	APAVLGNNEVDEHFELSK	54.51
			DFTFVCPTEIIAFDK	73.80
			GGIGQVTFPMVADITK	92.70
P43312	<i>sodB</i>	Superoxide dismutase [Fe]	IEIIQTSNAQTPVTDK	152.37
			SSLFDILTK	105.52
P52093	<i>ftnA</i>	Bacterial non-heme ferritin	AYEHEQHISESINNIVDHAIK	37.21
			FEGLTQIFQK	89.68
			LIVFLNENNVPVQLTSISAPEHK	124.31
P55834	<i>rplL</i>	50S ribosomal protein L7/L12	EGVNKEEAETIKK	86.94
P56003	<i>tuf</i>	Elongation factor Tu	ITVELISPALELGTK	103.67
P56112	<i>HP_0175</i>	Putative peptidyl-prolyl cis-trans isomerase HP_0175	DSPVTTYTYEQAKPTIK	85.99
			FIELANR	105.39
			TALVENEAK	105.98
P56431	<i>trxB</i>	Thioredoxin reductase	QVVCAASDGATAALSVISYLEHH	70.07
P66928	<i>trxA</i>	Thioredoxin	GVALVDFWAPWCGPCK	73.83
P69996	<i>ureB</i>	Urease subunit beta	LGDTDLIAEVEHDYTIYGEELK	55.05
Q48271	<i>copP</i>	COP-associated protein	SVVVEFDAPATQDLIK	101.30
O24943	<i>HP_0129</i>	Uncharacterized protein	NLASLSQEDR	113.03
O25043	<i>HP_0263</i>	Adenine specific DNA methyltransferase (Hpaim)	MKPYFSLEK	63.82
O25076	<i>HP_0305</i>	Uncharacterized protein	GVLDLLNFGLK	123.79
O25218	<i>HP_0472</i>	Outer membrane protein (Omp11)	HGNDLYGLNFK	123.26
			LGFVGFANK	119.41
			VYGFLDWFNSTSGTEHTK	118.70
P77872	<i>katA</i>	Catalase	ADDSDYYPQPGDYR	149.35
			DAIKFPDFIHTQK	121.45
			DLFNAIAR	104.44
			EFEVWNWDYR	119.22
			FHFHTMQGVK	144.47
			GPVLLQSTWFLEK	150.11
			GSGAYGTFTVTK	92.86
			HLTNEEAEEVR	108.56
			HMDGFGSHTFSLINAK	199.33
			LHDTIGESLAHVTHK	188.59
			NPENYFAEVEQAAFSPANVVPVPGIGYSPDR	106.56
			YYTEEGNWDLVGNNTPVFFIR	157.73
O25423	<i>HP_0721</i>	Uncharacterized protein	DHDDKDHDHDEHSDKH	206.70
			GNMEDMDDDFGLR	153.39
			KGNMEDMDDDFGLR	77.37
			MAGVVAPQDIVDYTK	110.35
O34810	<i>HP_0426</i>	Uncharacterized protein	MAKIDVELK	64.65

*Andromeda score for associated MS/MS spectra.

TABLE E7. Flow cytometric analysis of CD154 expression in CD4⁺ cells stimulated with EVs from supernatants from MDDCs cultured with *H pylori*, medications, or PBS

	Frequency of CD154 ⁺ expression in CD4 ⁺ T cells (%)		
	EVs (<i>H pylori</i>)	EVs (medications)	EVs (PBS)
Patient 1	0.12	0.00	0.00
Patient 2	0.04	0.02	0.00
Patient 5	0.02	0.03	0.00
Patient 6	0.00	0.00	0.00
Patient 14	0.13	0.03	0.00