Autoimmune Thrombocytopenic Purpura and Helicobacter pylori Infection

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Background: The mechanisms triggering the production of platelet autoantibodies in autoimmune thrombocytopenic purpura (AITP) are poorly understood. Recently, marked improvements in platelet counts have been reported in patients with AITP and concurrent Helicobacter pylori infection after eradication of H pylori by a standard antibiotic regimen. We looked for an association between H pylori infection and AITP in adults.

Methods: Fifty-one adults of white French origin, negative for human immunodeficiency virus (mean±SD age, 40±19.8 years), with AITP and a platelet count of less than 50×10^3/μL at onset were included. Thirty-five consecutive nonthrombocytopenic patients (mean±SD age, 43±22 years) of the same origin and with unknown H pylori status served as control subjects. Antibodies against H pylori were detected by means of an agglutination method in both patients and control subjects. Sex ratio, mean age, hemorrhagic manifestations, response to corticosteroid therapy, and final outcome were compared in H pylori–negative and H pylori–positive patients with AITP. To test for a possible molecular mimicry mechanism, we also used an immunoblot assay to look for specific H pylori antibodies in platelet eluates from 3 H pylori–positive patients with AITP.

Results: Seroprevalence of H pylori in patients with AITP (15 [29%]) was not significantly different from that in control subjects (10 [29%]). The H pylori–positive and H pylori–negative patients with AITP did not differ in main characteristics at AITP onset, response rate to corticosteroids, and final outcome. None of the 3 patients investigated had H pylori antibodies in platelet eluates.

Conclusion: Although the role of H pylori in a subgroup of patients with AITP cannot be excluded, we found no evidence of an association between H pylori infection and AITP.

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AUTOIMMUNE thrombocytopenic purpura (AITP) is an acquired bleeding disorder in which autoantibodies bind to platelet surface, leading to platelet destruction. The mechanisms triggering the production of platelet autoantibodies are poorly understood. In childhood, AITP is usually an acute self-limited problem; in contrast, AITP in adults is most often chronic, and up to 25% of cases of chronic AITP are refractory to standard therapy. Recently, it was suggested that Helicobacter pylori may contribute to AITP pathogenesis, as partial or even complete remission of thrombocytopenia has been reported in a few patients after eradication of H pylori. However, although there is evidence implicating H pylori in some autoimmune disorders, the link between H pylori infection and AITP remains speculative.

The aim of our study was to compare the prevalence of H pylori infection in a group of patients with AITP with that in a group of nonthrombocytopenic control subjects. We also compared the main characteristics and outcome of AITP between H pylori–positive and H pylori–negative patients and looked for cross-reactivity between platelet and H pylori antibodies.

RESULTS

PATIENT CHARACTERISTICS

Fifty-one patients, including 13 men (25%) and 38 women (75%), with AITP were included. The mean ages of patients (40±19.8 years) and control subjects (43±22 years) were not significantly different. The mean platelet count in patients at AITP onset was 21×10^3/μL (range, 1-50×10^3/μL). The
Patients and Methods

Patients

Fifty-one unselected adults (older than 18 years) of white French origin with AITP and whose H pylori infection status was not known were included in the study. All patients had a definite diagnosis of AITP according to usual criteria and presented with a platelet count of less than 50 x 10^9/L at AITP onset. Patients infected with the human immunodeficiency virus were not included.

Initial treatment of AITP is standardized in our center. Briefly, all patients received corticosteroids at a mean dosage of 1 mg/kg per day for 3 weeks as a first-line therapy. Patients with life-threatening hemorrhages also initially received intravenous immune globulin at a dosage of 1 to 2 g/kg per day. Treatment was considered effective if the platelet count rose to 50 x 10^9/L and increased at least 2-fold. Splenectomy was considered only in patients with a chronic outcome, ie, when the platelet count remained below 50 x 10^9/L after at least 6 months of follow-up. Acute AITP was defined by a treatment-free remission of thrombocytopenia (platelet count >150 x 10^9/L) within 6 months of AITP onset.

The severity of the hemorrhagic syndrome at AITP onset was assessed by means of a clinical severity scale taking the following items into account: cutaneous purpura (localized, score of 1; extensive and/or progressive, 3; associated with large ecchymoses, 4), hemorrhagic oral bullae and/or hemorrhagic oral bullae and/or hemorrhagic oral bullae and/or hemorrhagic oral bullae and/or macroscopic hematuria (score, 5), gastrointestinal tract hemorrhage (score, 5), major menorrhagia and/or metrorrhagia (score, 5), and bleeding on the fundus oculi in the absence of other causes (score, 5). The age at onset was considered as an independent risk factor of bleeding (age >65 years, score of 1; age >80 years, 3).

Control Group

Thirty-five unselected patients older than 18 years, seen consecutively in our department during a 1-month period (March 1 to April 1, 2000) served as control subjects. Since the seroepidemiologic characteristics of H pylori infection differ between countries, only individuals of white French origin were included. None of them had a history of thrombocytopenia. Their H pylori infection status and history were not known.

Detection of H pylori Antibodies

An agglutination method (Pyloriset kit TMEIA-G; Orion Diagnostica, Helsinki, Finland) was used to detect anti-H pylori antibodies of IgG type in both patients and control subjects. In patients, tests were performed on serum samples collected and frozen when AITP was diagnosed. None of the patients had been previously treated with intravenous immune globulin. All serologic tests in the control group were performed consecutively on fresh samples.

Detection of Platelet Autoantibodies

Patients were screened for circulating and surface-bound IgG and IgM antiplatelet antibodies by an indirect and a direct suspension immunofluorescence technique on paraformaldehyde-fixed platelets. In patients in whom platelet antibodies were detected in serum samples by indirect suspension immunofluorescence technique, the presence of antibodies directed against specific platelet membrane targets was investigated by a direct and indirect monoclonal antibody–specific immobilization of platelet antigens assay with the use of a panel of monoclonal antibodies directed against platelet membrane glycoprotein (Gp) IIb-IIIa, Gpla-Ha, and GpIb-IX as previously described by Kiefel with minor modifications.

Platelet Eluates

To determine whether H pylori antibodies could cross-react with platelet-membrane antigens, an immunoblot assay (Mikrogen, Martinsried, Germany) was used to test platelet eluates from 3 H pylori–positive patients with AITP. This immunoblot assay was used to detect IgG and IgA antibodies directed against specific H pylori antigens (CagA, VacA, UreB, HspA, Hsb, FlaA, and UreA). Platelet eluates were obtained by an ether-elution method and were also tested for the presence of platelet antibodies by means of the indirect suspension immunofluorescence technique and the indirect monoclonal antibody–specific immobilization of platelet antigens assay.

Statistical Analysis

Patients were compared with controls and H pylori–positive patients with H pylori–negative patients for AITP characteristics and outcome. Results are expressed as mean±SD. A χ^2 test (or Fisher exact test if necessary) was used for comparison of categorical data, and the nonparametric Mann-Whitney test was used for comparison of quantitative data. A P value of less than .05 was considered significant.

Course of AITP was chronic in 42 patients (82%) and acute in 9 (18%). Splenectomy was required in 14 patients with chronic AITP. All patients were alive after a median follow-up of 10 months (range, 6 months to 10 years) from AITP onset. None of the patients had a history of gastric or duodenal ulcer. After AITP onset, only 2 (4%) of the 51 patients underwent an upper digestive endoscopy, one for dyspepsia related to a peptic esophagitis and the other for occult bleeding associated with a duodenal ulcer; H pylori was present only in the duodenal biopsy specimen of the second patient. At the time of AITP diagnosis, only 1 patient was being treated by a proton pump inhibitor for dyspepsia; none of the patients had received antibiotics during the 4 weeks before AITP onset.

Fifteen (29%) of the 51 patients with AITP and 10 (29%) of the 35 control subjects (P=.93) were seropositive for H pylori.

Characteristics and Outcome of AITP

The mean age at AITP onset was slightly higher in H pylori–positive patients (47.8 years; range, 19-92 years) than in H pylori–negative patients (35.3 years; range, 18-75 years) (P=.05).
Neither the main features of AITP nor the response to corticosteroids was significantly different between \textit{H pylori}–positive and \textit{H pylori}–negative patients (Table).

\textbf{H PYLORI IMMUNOBLOT ON PLATELET ELUATES}

\textit{Helicobacter pylori}–specific antibodies were sought by immunoblotting on platelet eluates in 3 \textit{H pylori}–positive patients with AITP who had circulating platelet antibodies detected by indirect suspension immunofluorescence technique and in whom platelet eluates were available. None of them had detectable anti-\textit{H pylori} antibodies in platelet eluates. Conversely, platelet-associated antibodies were detected on platelet eluates in 2 of the 3 patients tested, specifically directed against GpIb-IXa and GpIb-IX, as detected by means of monoclonal antibody–specific immobilization of platelet antigens assay in one patient.

\textbf{COMMENT}

\textit{Helicobacter pylori} is a ubiquitous gram-positive bacterium involved in the pathogenesis of gastric and duodenal ulcers. Recently, the involvement of \textit{H pylori} has also been suggested in various autoimmune diseases, including megaloblastic anemia\textsuperscript{17,18} and extraintestinal diseases such as Sjögren syndrome and immune thyroiditis.\textsuperscript{7,9} Previous in vitro studies suggested that \textit{H pylori} has the potential to initiate autoreactivity through molecular mimicry, since monoclonal antibodies directed against \textit{H pylori} may also react with ductal cells of the salivary glands and renal tubular cells.\textsuperscript{8} Within the past 2 years, a role for \textit{H pylori} in the pathogenesis of AITP has been suggested because significant and, in some cases, very substantial improvements in platelet count have been reported after \textit{H pylori} eradication with a standard antibiotic regimen\textsuperscript{3-7} from patients with AITP and concurrent \textit{H pylori} infection. However, 3 of the relevant reports are anecdotal case reports\textsuperscript{2,4,5} and 2 are uncontrolled brief reports.\textsuperscript{3,6} Therefore, the implication of \textit{H pylori} in pathogenesis of AITP is not backed by strong evidence.

We compared the prevalence of antibodies directed toward \textit{H pylori} in patients with AITP and in ethically matched controls. Although a positive culture obtained from gastric biopsy samples is considered the gold standard to confirm active \textit{H pylori} infection or carriage,\textsuperscript{10} we used a blood antibody detection method for the following reasons: (1) Endoscopic methods of \textit{H pylori} isolation were not feasible in our patients with a platelet count less than 50 \(\times 10^9\)/µL at AITP onset. (2) Non-endoscopic methods of detection, such as urea breath tests or stool antigen testing, did not allow retrospective determination of the patients’ \textit{H pylori} carriage or previous infection status. (3) The reliability (sensitivity of 95.8\% and specificity of 95.5\%) of the enzyme immunoassay kit has been validated.\textsuperscript{20}

The observed prevalence of anti-\textit{H pylori} antibodies was similar in patients with AITP and control subjects and consistent with the expected value of 25\% to 35\%, observed in the French adult population.\textsuperscript{21}

We also compared \textit{H pylori}–positive and \textit{H pylori}–negative patients with AITP and found that neither clinical and biological characteristics of AITP nor the response to corticosteroids and final outcome were influenced by the serologic \textit{H pylori} status.

Finally, a molecular mimicry mechanism has been demonstrated in human immunodeficiency virus–related immune thrombocytopenia,\textsuperscript{22} and has been suggested by Emilia et al\textsuperscript{4} as the possible mechanism for \textit{H pylori}–associated AITP. We therefore performed an \textit{H pylori} immunoblot assay on platelet eluates obtained in 3 of our \textit{H pylori}–positive patients, and there was no evidence of cross-reactivity between platelet and \textit{H pylori} antibodies.

In conclusion, although the implication of \textit{H pylori} infection in the pathogenesis of AITP in a particular subgroup of patients cannot be expressly ruled out by our case-control study, we found no evidence of an association between these 2 conditions. Moreover, since spontaneous remissions can occur in chronic AITP,\textsuperscript{23} controlled trials comparing the effect of an \textit{H pylori} eradication therapy with that of a placebo are warranted to determine whether the subgroup of patients with AITP and active \textit{H pylori} infection may benefit from eradication of \textit{H pylori}.

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\begin{table}
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\begin{tabular}{|c|c|c|}
\hline
& \textit{H pylori}–Positive & \textit{H pylori}–Negative \\
& Patients & Patients & \\
\hline
\textbf{Age at onset, y} & & \\
Mean & 47.8 & 35.5 \\
Range & 19-92 & 18-75 \\
\hline
\textbf{Sex, No. (\%)} female & & \\
11 (73) & 27 (75) \\
\hline
\textbf{Mean hemorrhagic score at diagnosis} & & \\
3.7 & 3.15 \\
\hline
\textbf{Platelet count at diagnosis, \(\times 10^9\)/µL} & & \\
Mean & 13 & 23 \\
Range & 1-50 & 5-48 \\
\hline
\textbf{Positive for antiplatelet antibodies, No. (\%)} & & \\
10 (67) & 26 (72) \\
\hline
\textbf{Acute outcome, No. (\%)} & & \\
3 (20) & 7 (19) \\
\hline
\textbf{Partial or complete response to corticosteroids, No. (\%)} & & \\
12 (80) & 27 (75) \\
\hline
\end{tabular}
\caption{Comparison of Patients With Autoimmune Thrombocytopenic Purpura Positive and Negative for \textit{Helicobacter pylori}}
\end{table}

2. Garcia Perez S, Valverde de la Osa J, Gimenez Samper M, Alonso Garcia I. Reso-


