INFECTION AND IMMUNITY, Nov. 2003, p. 6664–6667 Vol. 71, No. 11
0019-9567/03/$08.00+0 DOI: 10.1128/IAI.71.11.6664–6667.2003

Helicobacter pylori cag Pathogenicity Island Is Associated with Reduced Expression of Interleukin-4 (IL-4) mRNA and Modulation of the IL-4δ2 mRNA Isoform in Human Gastric Mucosa

Barbara Orsini,[1]* Barbara Ottanelli,[1] Amedeo Amedei,[2] Elisabetta Surrenti,[1] Marco Capanni,[1] Gianfranco Del Prete,[2] Andrea Amorosi,[3] Stefano Milani,[1] Mario Milco D'Elios,[2] and Calogero Surrenti[1]

Gastroenterology Unit, Department of Clinical Pathophysiology,[1] Department of Internal Medicine,[2] and Department of Pathology,[3] University of Florence, Florence, Italy

Received 21 April 2003/Returned for modification 9 June 2003/Accepted 23 July 2003

Interleukin-4 (IL-4) and IL-4δ2 mRNA gastric expression was evaluated in healthy subjects and patients who did not have ulcers but were infected with *Helicobacter pylori* with or without the *cag* pathogenicity island (*cag* PAI). IL-4 mRNA was physiologically expressed by gastric epithelium and negatively influenced by *H. pylori*. Also, nonepithelial cells in the lamina propria of *H. pylori*-infected patients expressed IL-4 mRNA, whereas IL-4δ2 mRNA was found only in *cag* PAI-negative patients. Thus, gastric IL-4 takes part in the local immune response to *H. pylori*.

Interleukin-4 (IL-4), a pleiotropic cytokine mainly produced by activated T lymphocytes (13), mast cells, and basophils, regulates a wide spectrum of functions in B cells, T cells, macrophages, and other cell types (21). IL-4 promotes T-cell proliferation and differentiation into IL-4-producing Th2 or Th0 T helper cells, while suppressing the development of gamma interferon (IFN-γ)-producing Th1 cells (22). In nonulcer chronic gastritis induced by *Helicobacter pylori*, infection usually raises a local T-cell response with a predominant Th0 profile and production of both IFN-γ and IL-4 (5, 8, 16, 18, 19, 24), whereas it results in a polarized Th1 response in patients with peptic ulcers (9), suggesting that the type of host T-cell response, together with bacterial virulence factors encoded by genes of the *cag* pathogenicity island (*cag* PAI), may contribute to the clinical outcome of *H. pylori* infection (7, 14, 15, 20).

An alternative splicing of IL-4 mRNA lacking exon 2, the IL-4δ2 mRNA isoform (2, 3), was found in human lymphoid tissue (2, 17), in nasal and endobronchial biopsy specimens (12), and bronchoalveolar lavage cells (3). In this study, we used in situ hybridization (ISH) (11, 20) and nested reverse transcriptase PCR (RT-PCR) (23) to evaluate the expression of IL-4 mRNA and its IL-4δ2 isoform in human gastric mucosa in relation to *H. pylori* infection with *cag* PAI-positive or -negative strains.

Thirty *H. pylori*-infected patients (15 males and 15 females; mean age, 42.2 years) with nonulcer dyspepsia, undergoing endoscopy for upper gastrointestinal tract symptoms gave their informed consent to this study, which was approved by the local ethics committee. Exclusion criteria were the following: peptic ulcer disease; history of gastric surgery; active gastrointestinal bleeding; malignancy; or treatment with steroids or nonsteroidal anti-inflammatory drugs, recent antibiotics, or omeprazole. Ten healthy age-matched volunteers were recruited as controls. *H. pylori* infection was established by performing a rapid urease test, histology, [^{13}C]urea breath test, and PCR for urease (*ureA*) gene. During endoscopy, biopsy specimens were collected from the gastric antrum. Paraffin-embedded sections were stained with modified Giemsa stain (S. F. Gray, J. I. Wyatt, and B. J. Rathbone, Letter, J. Clin. Pathol. **39:**1279, 1986) to detect *H. pylori* and with hematoxylin and eosin stain to evaluate gastritis (10). Gastritis was graded on a scale of 0 to 3 for inflammation. Additional antrum specimens were immediately frozen in liquid nitrogen until the following tests: (i) PCR for *ureA* and *H. pylori* genotype (20) according to the *cag* PAI designation (6) and (ii) ISH and nested RT-PCR for IL-4 and IL-4δ2 mRNA isoforms, as reported elsewhere in detail (11, 20, 23).

Briefly, serial cryostat sections of antrum specimens and cytospin preparations of clonal T cells producing IL-4 (positive control for the probe) were used for IL-4 mRNA ISH. A 27-bp fragment (spanning exons 1 to 3) of the IL-4 cDNA (25) (American Type Culture Collection, Manassas, Va.) was subcloned to obtain the RNA probe labeled with ^{35}S-uridine-5′-(thio)-triphosphate (New England Nuclear, Boston, Mass.). The signal for IL-4 mRNAs was acquired by a charge-coupled device video camera connected to the microscope and analyzed by a computerized video image system Leica QWin Q550 (Leica Microsystems, Cambridge, United Kingdom).

Total RNA was isolated from frozen specimens and the IL-4-producing T-cell clone by TRIzol reagent (Gibco BRL, Gaithersburg, Md.) according to the manufacturer's instructions. First-strand cDNA was synthesized by oligo(dT)$_{12-18}$ primer (Gibco BRL), and all cDNA samples were amplified for β-actin gene (housekeeping gene) (Gibco BRL). First-round and second-round PCR runs were performed to detect IL-4 and IL-4δ2 mRNA isoforms. IL-4 transcripts were measured by densitometry of ethidium bromide-stained gels with a scanner and Kodak 1D 3.5 Network software (Eastman Kodak Company, New Haven, Conn.).

* Corresponding author. Mailing address: Department of Clinical Pathophysiology, Gastroenterology Unit, CIRHeP, Viale Pieraccini 6, I-50139 Florence, Italy. Phone: 39-055-4271301. Fax: 39-055-4271297. E-mail: b.orsini@dfc.unifi.it.

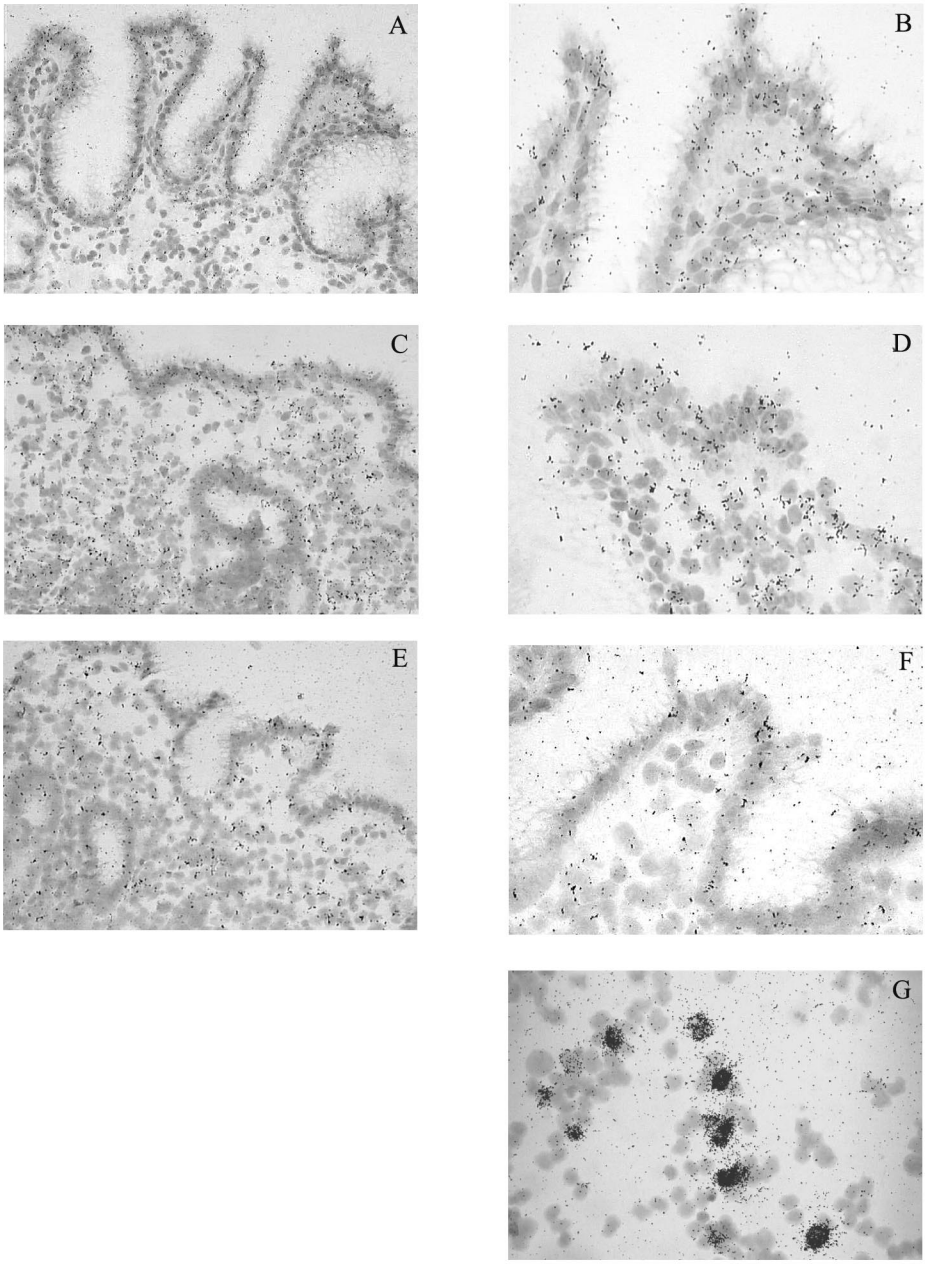

FIG. 1. ISH for IL-4 mRNA in gastric antrum biopsy sections. Bright-field photomicrographs with ^{35}S-labeled antisense IL-4 RNA probe of the surface epithelium and pits from a control subject (A and B), a *cag* PAI-negative patient (C and D), a *cag* PAI-positive patient (E and F) and from a cytospin sample of clonal T cells producing IL-4 (G). Original magnifications, ×20 (left) and ×40 (right).

Chi-square analysis was used to compare mean score values of inflammation of patients with *cag* PAI-positive and *cag* PAI-negative *H. pylori* infections. Student's *t* test was used to compare the ISH and PCR results for patients and controls. The interobserver variability for the computer image analysis was also determined using the paired *t* test (three observations).

All 30 *H. pylori*-infected patients were positive for *ureA*, 18 of them also showing amplification for *cagA*, *cagE*, *cagI*, *cagT*, and *virB11* homologue genes (*cag* PAI-positive patients). No amplification for these genes was obtained in *cag* PAI-negative patients. All *H. pylori*-infected patients had chronic active gastritis, with higher inflammation scores in *cag* PAI-positive patients than in *cag* PAI-negative patients (2.69 ± 0.27 versus 2.28 ± 0.22 [$P < 0.005$]), whereas healthy controls had no abnormalities. In both controls and *H. pylori*-infected patients, ISH consistently showed IL-4 mRNA expression in gastric cells of the surface epithelium and pits (Fig. 1). Though ISH may be prone to low resolution and background artifacts, image analysis suggested that mean IL-4 mRNA expression was higher in gastric epithelial cells of uninfected controls (114.40 ± 12.51 pixel units) than in the epithelium of *cag* PAI-negative patients (105.55 ± 7.72 pixel units) or *cag* PAI-positive patients (100.49 ± 7.8 pixel units). These findings indicate that IL-4 mRNA expression is a physiological function of gastric epithe-

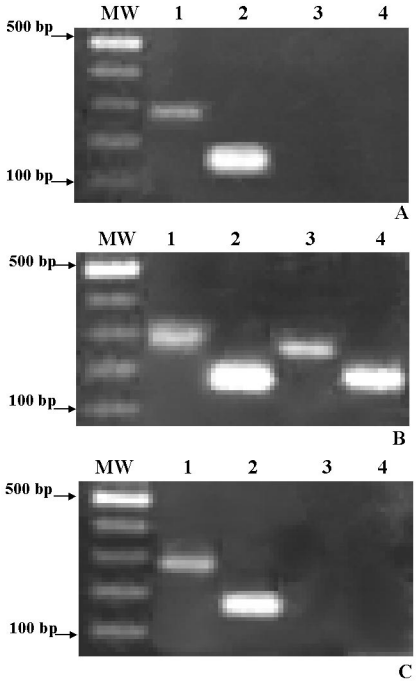

FIG. 2. Nested RT-PCR for IL-4 and IL-4δ2 in a *cag* PAI-positive patient (A), *cag* PAI-negative patient (B), and an uninfected control subject (C). Pictures were obtained from the original Polaroid films for 1.5% agarose gel electrophoresis and ethidium bromide staining. Lane MW, 100-bp DNA ladder; lane 1, first-round PCR (R1) for IL-4; lane 2, second-round PCR (R2) for IL-4; lane 3, R1 for IL-4δ2; lane 4, R2 for IL-4δ2.

lial cells, which is negatively influenced by *H. pylori*, particularly by *cag* PAI-positive strains.

ISH also allowed us to observe that nonepithelial cells in the lamina propria of gastric biopsy specimens from all *H. pylori*-infected patients expressed IL-4 mRNA with a stronger signal in *cag* PAI-negative patients (Fig. 1C and D) than in *cag* PAI-positive patients (184.63 ± 46.38 versus 73.01 ± 10.45 pixel units [$P < 0.005$]) (Fig. 1E and F), whereas only a background level was detected in the few nonepithelial cells of control biopsy specimens (Fig. 1A and B).

The results of nested RT-PCR confirmed that IL-4 mRNA was present in the gastric tissue from either *H. pylori*-infected patients or controls (Fig. 2), though densitometric analysis of the intensity of IL-4 transcripts failed to show a significant difference between patients and controls (data not shown). These results are apparently in conflict with ISH data. However, since RT-PCR does not allow assessment of the relative contributions of gastric epithelial cells to IL-4 transcripts (higher in normal mucosa and lower in *H. pylori*-infected mucosa) versus that of nonepithelial inflammatory cells (virtually absent in controls, but variably high in infected samples), it is possible that the cumulative levels of IL-4 transcripts tend to equalize in the different samples. A major point of this study is the observation that IL-4δ2 mRNA expression was restricted to *cag* PAI-negative patients (Fig. 2B), whereas it was not detected in either *cag* PAI-positive patients (Fig. 2A) or uninfected controls (Fig. 2C). The reason for this difference is unclear. However, one may suspect that IL-4δ2 mRNA is not expressed by gastric epithelial cells and that it represents a peculiar product of some nonepithelial cells recruited in the gastric inflammatory setting by *H. pylori* infection. Although we did not characterize the nonepithelial IL-4 and IL-4δ2 mRNAs producing cells in the lamina propria, these cells were likely to be $CD4^+$ T cells, as suggested by several studies (5, 8, 9, 19, 24). Thus, a possible explanation for the lack of IL-4δ2 mRNA expression in the inflamed mucosa of *cag* PAI-positive patients and its consistent detection in *cag* PAI-negative patients is that T cells that infiltrate the gastric mucosa in the two groups of infected patients express different cytokine profiles, predominantly Th1 in the former and preferentially Th0 in the latter (8, 9).

Previous studies reported that recombinant IL-4δ2 antagonizes the effects of IL-4 on T cells, B cells, and macrophages by competing for receptor binding (1, 3), suggesting that the balance between IL-4 and IL-4δ2 may be important in the regulation of IL-4 effects (3, 4). Although the specific role of IL-4δ2 in gastric tissue is still unclear, we hypothesize that its presence in patients with *cag* PAI-negative, but not in *cag* PAI-positive, *H. pylori* infection may influence the host response to *H. pylori* and contribute to quenching of inflammation.

This study was supported in part by grants from Ministero dell' Università, Ministero della Ricerca Scientifica e Tecnologica (MURST), and Associazione Italiana per la Ricerca sul Cancro (AIRC).

REFERENCES

1. **Arinobu, Y., S. P. Atamas, T. Otsuka, H. Niiro, K. Yamaoka, H. Mitsuyasu, Y. Niho, B. White, and K. Izuhara.** 1999. Antagonistic effects of an alternative splice variant of human IL-4, IL-4δ2, on IL-4 activities in human monocytes and B cells. Cell. Immunol. **191:**161–167.
2. **Atamas, S. P.** 1997. Alternative splice variants of cytokines: making a list. Life Sci. **61:**1105–1112.
3. **Atamas, S. P., J. Choi, V. V. Yurovsky, and B. White.** 1996. An alternative splice variant of human IL-4, IL-4δ2, inhibits IL-4-stimulated T cell proliferation. J. Immunol. **156:**435–441.
4. **Atamas, S. P., V. V. Yurovsky, R. Wise, F. M. Wigley, C. J. G. Robinson, P. Henry, W. J. Alms, and B. White.** 1999. Production of type 2 cytokines by $CD8^+$ lung cells is associated with greater decline in pulmonary function in patients with systemic sclerosis. Arthritis Rheum. **42:**1168–1178.
5. **Bamford, K. B., X. Fan, S. E. Crowe, J. F. Leary, W. K. Gourley, G. K. Luthra, E. G. Brooks, D. Y. Graham, V. E. Reyes, and P. B. Ernst.** 1998. Lymphocytes in the human gastric mucosa during *Helicobacter pylori* have a T helper cell 1 phenotype. Gastroenterology **114:**482–492.
6. **Censini, S., C. Lange, Z. Xiang, J. E. Crabtree, P. Ghiara, M. Borodovsky, R. Rappuoli, and A. Covacci.** 1996. *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. USA **93:**14648–14653.
7. **Covacci, A., J. L. Telford, G. Del Giudice, J. Parsonnet, and R. Rappuoli.** 1999. *Helicobacter pylori*, virulence, and genetic geography. Science **284:** 1328–1333.
8. **D'Elios, M. M., M. Manghetti, F. Almerigogna, A. Amedei, F. Costa, D. Burroni, C. T. Baldari, S. Romagnani, J. L. Telford, and G. Del Prete.** 1997. Different cytokine profile and antigen-specificity repertoire in *Helicobacter pylori*-specific T cell clones from the antrum of chronic gastritis patients with or without peptic ulcer. Eur. J. Immunol. **27:**1751–1755.
9. **D'Elios, M. M., M. Manghetti, M. De Carli, F. Costa, C. T. Baldari, D. Burroni, J. L. Telford, S. Romagnani, and G. Del Prete.** 1997. T helper 1 effector cells specific for *Helicobacter pylori* in the gastric antrum of patients with peptic ulcer disease. J. Immunol. **158:**962–967.
10. **Dixon, M. F., R. M. Genta, J. H. Yardley, and P. Correa.** 1996. Classification and grading of gastritis. The updated Sydney System. International Workshop on the histopathology of gastritis, Houston 1994. Am. J. Surg. Pathol. **20:**1161–1181.
11. **Fiorucci, S., L. Lanfrancone, L. Santucci, A. Calabro, B. Orsini, B. Federici, and A. Morelli.** 1996. Epidermal growth factor modulates pepsinogen secretion in guinea pig gastric chief cells. Gastroenterology **111:**945–958.
12. **Glare, E. M., M. Divjak, J. M. Rolland, and E. H. Walters.** 1999. Asthmatic airway biopsy specimens are more likely to express the IL-4 alternative splice variant IL-4δ2. J. Allergy Clin. Immunol. **104:**978–982.
13. **Howard, M., J. Farrar, M. Hilfiker, B. Johnson, K. Takatsu, T. Hamaoka,**

and W. E. Paul. 1982. Identification of a T cell-derived B cell growth factor distinct from interleukin 2. J. Exp. Med. **155:**914–923.
14. **Israel, D. A., and R. M. Peek.** 2001. Pathogenesis of *Helicobacter pylori*-induced gastric inflammation. Aliment. Pharmacol. Ther. **15:**1271–1290.
15. **Jenks, P. J., F. Megraud, and A. Labigne.** 1998. Clinical outcome after infection with *Helicobacter pylori* does not appear to be reliably predicted by the presence of any of the genes of the *cag* pathogenicity island. Gut **43:** 752–758.
16. **Karttunen, R., T. Karttunen, H. P. T. Ekre, and T. T. MacDonald.** 1995. Interferon gamma and interleukin 4 secreting cells in the gastric antrum in *Helicobacter pylori* positive and negative gastritis. Gut **36:**341–345.
17. **Klein, S. C., J. G. Golverdingen, D. F. van Wichen, A. G. M. Bouwens, I. Stuij, M. G. J. Tilanus, E. J. E. G. Bast, and R. A. De Weger.** 1996. Expression of two interleukin 4 mRNA isoforms in B lymphoid cells. Cell. Immunol. **167:**259–268.
18. **Lindholm, C., M. Quiding-Jarbrink, H. Lonroth, A. Hamlet, and A. M. Svennerholm.** 1998. Local cytokine response in *Helicobacter pylori*-infected subjects. Infect. Immun. **66:**5964–5971.
19. **Lohoff, M., M. Rollinghoff, and F. Sommer.** 2000. *Helicobacter pylori* gastritis: a Th1 mediated disease? J. Biotechnol. **83:**33–36.
20. **Orsini, B., G. Ciancio, S. Censini, E. Surrenti, G. Pellegrini, S. Milani, H. Herbst, A. Amorosi, and C. Surrenti.** 2000. *Helicobacter pylori cag* pathogenicity island is associated with enhanced interleukin-8 expression in human gastric mucosa. Digest. Liver Dis. **32:**458–467.
21. **Paul, W. E.** 1991. Interleukin-4: a prototypic immunoregulatory lymphokine. Blood **77:**1859–1870.
22. **Romagnani, S.** 1993. Regulatory role of IL-4 and other cytokines in the function and development of human T-cell clones. Res. Immunol. **144:**625–628.
23. **Seah, G. T., and G. A. W. Rook.** 1999. A sensitive, non-radioactive quantitative method for measuring IL-4 and IL-4δ2 mRNA in unstimulated cells from multiple clinical samples, using nested RT-PCR. J. Immunol. Methods **228:**139–149.
24. **Sommer, F., G. Faller, P. Konturek, T. Kirchner, E. G. Hahn, J. Zeus, M. Rollinghoff, and M. Lohoff.** 1998. Antrum- and corpus mucosa-infiltrating $CD4^+$ lymphocytes in *Helicobacter pylori* gastritis display a Th1 phenotype. Infect. Immun. **66:**5543–5546.
25. **Yokota, T., T. Otsuka, T. Mosmann, J. Banchereau, T. DeFrance, D. Blanchard, J. E. De Vries, F. Lee, and K. Arai.** 1986. Isolation and characterization of a human interleukin cDNA clone, homologous to mouse B-cell stimulatory factor 1, that expresses B-cell- and T-cell-stimulating activities. Proc. Natl. Acad. Sci. USA **83:**5894–5898.

Editor: J. D. Clements