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INTERFERON γ-SIGNATURE TRANSCRIPT PROFILING AND IL-23 UPREGULATION IN RESPONSE TO *HELICOBACTER PYLORI* INFECTION

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***Helicobacter pylori* infection is the major cause of gastroduodenal pathologies including gastric cancer. The long persistence of bacteria and the type of immune and inflammatory response determine the clinical issue. In this study, the global gene expression profile after 6 and 12 months of *H. pylori* infection was investigated in the mouse stomach, using the Affymetrix GeneChip Mouse Expression Array A430. Genes related to the inflammatory and immune responses were focused. Levels of selected transcripts were confirmed by reverse transcription polymerase chain reaction. Twenty- five and nineteen percent of the differentially expressed genes observed at 6 and 12 months post-infection respectively, were related to immune response. They are characterized by an interferon (IFN)γ-dependent expression associated to a T helper 1 (Th1) polarised response. In-depth analysis revealed that an up-regulation of IL-23p19, took place in the stomach of *H. pylori* infected-mice. Strong IL-23p19 levels were also confirmed in gastric biopsies from *H. pylori*-infected patients with chronic gastritis, as compared to healthy subjects. Our microarray analysis revealed also, a high decrease of H^+K^+-ATPase transcripts in the presence of the *H. pylori* infection. Association of gastric Th1 immune response with hypochlorhydria through the down-regulation of H^+K^+-ATPase contributes to the genesis of lesions upon the *H. pylori* infection. Our data highlight that the up-regulation of IL-23 and of many IFNγ signature transcripts occur early on during the host response to *H. pylori*, and suggest that this type of immune response may promote the severity of the induced gastric lesions.**

Half of the world population is chronically infected by *Helicobacter pylori*, a bacterium that specifically colonizes the human stomach. The *H. pylori* infection is the major cause of gastroduodenal pathologies such as chronic gastritis, peptic ulcer diseases, gastric adenocarcinoma and lymphoma (1). The most important co-factor in the induction of *Helicobacter*-related disease is the host immune response. Studies in humans and in animal models have shown that a pro-inflammatory T helper (Th)1 response, with high levels of interferon (IFN)γ, interleukin (IL)-12, IL-17 and IL-18, is associated

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with severe lesions but does not clear the infection (2-3). Various bacterial factors have been reported to promote the mucosal Th1 polarization, one of which is the *H. pylori* neutrophil-activating (HP-NAP), previously described to promote neutrophil adhesion to endothelial cells, which leads to production of high levels of IFNγ and tumor necrosis factor (TNF)α with increased expression of class II major histocompatibility molecules (MHC II) on the dendritic cell surface (4-5).

Transcriptome studies on human and animal host response have demonstrated that *H. pylori* targets specific host factors involved mainly in cytoskeletal changes, activation of different signal transduction pathways, change in cell morphology, cytokine induction, alteration of cell proliferation pattern and apoptosis (6-12). In the present study, the global host gene expression profiling in response to chronically *H. pylori* SS1 infected and non-infected C57BL/6 mice was investigated after six and twelve months, the same conditions previously demonstrated to induce an active gastritis with moderate metaplasia associated with the induction of a gastric mutagenic effect (13), known to be among the earliest events in the carcinogenic process. We focused our investigations on the differentially expressed genes related to the inflammatory/immune responses and on gastric expression of IL-23. We provided *in vivo* data supporting a characteristic IFNγ-dependent signature transcript associated with the chronic gastritis induced by the *H. pylori* infection, and consequent IFN-related regulation of gene expression that might drive the induction of severe gastric lesions.

MATERIALS AND METHODS

Animal infections

Six-week-old specific-pathogen-free C57BL/6 male mice (Charles Rivers, France) were infected orogastrically with $1x10^8$ cfu/100 µl of the *H. pylori* strain SS1 (n=6) or received peptone broth (n=6) (13). Non-infected (n=3) and *H. pylori*-infected mice (n=3) were sacrificed after 6 and 12 months. The stomachs were isolated and used for quantification of *H. pylori* colonization, histological analysis as described previously (13), and RNA isolation. All experiments were performed in accordance with institutional guidelines as determined by the Central Animal Facility Committee of the Institut Pasteur, in conformity with the French Ministry of Agriculture guidelines for animal care.

Microarray hybridisation and data mining

Analysis of gene expression was conducted using GeneChip Mouse Expression Array 430A (Affymetrix, Santa Clara, CA). Total RNA were isolated from the stomach samples by TRIzol extraction (Invitrogen Life Technologies), purified on RNeasy minicolumns (Qiagen, Courtaboeuf, France) and quality checked with an Agilent Bioanalyzer (Agilent Technologies; Palo Alto, CA). 20 µg of total RNA was amplified and biotin labeled (Enzo Diagnostic, New York, NY), and then hybridised to the array according to recommended protocols (Affymetrix, Santa Clara, CA). Four arrays, for each analysed condition were used, two arrays to hybridize cRNA from two mice independently and two arrays to hybridize cRNA from the third mouse in duplicate. Hybridization and scanning of the chips were performed according to the recommended protocols of Affymetrix. Gene microarray data (ArrayExpress accession: E-MEXP-1135) from this study have been deposited in the Miamexpress data bank (http://www.ebi.ac.uk/miamexpress/).

Data analysis was performed using MicroArray Suite software MAS-5.0 algorithms (Affymetrix) and Splus ArrayAnalyser software (Insightful). Pre-processing by robust multi-chip analysis (RMA) (14), was applied to normalize summary values for each probe set (transcript). An LPE (local pooled error) test was carried out for each gene (15), followed by Benjamini-Hochberg correction for multiple testing (16). The probe sets corresponding to differentially expressed genes according to the infection status were selected according to a change call that were two times higher or lower than those of the controls with P values less than 0.05. ArrayMiner software™ 2 (Optimal Design) was then used for clustering according to gene expression profiles.

Detection of transcripts by reverse transcriptase polymerase chain reaction. (RT-PCR) and real-time PCR analysis

RT-PCR analyses were performed to confirm differential expression of selected genes of interest according to infection status, with 100 pg of total RNA in each case, using the Superscript™ One-Step RT-PCR kit (Life Technologies, Cergy Pontoise, France). Primer sequences for the selected genes and the endogenous control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene are listed in Table I.

Real-time PCR analysis for IL-23p19 gene expression were performed with RNA extracted from mouse stomach, which was reverse transcribed and amplified with p19 primers and probe generated according to the

published sequence of p19 in the Applied Biosystems (Foster City, CA) Primer Express software program (17). The primer sequences used are as follows: forward primer, 5'-CAGCAGCTCTCTCGGAAT-3' reverse primer, 5'-ACAACCATCTTCACACTGGATACG-3'; and probe, 5'-CATGCTAGCCTGGAACGCACATGC-3'. Amplifications of p19 and 18S cDNA for the endogenous control, were carried out with Taqman (Applied Biosystems) reagents and the ABI Prism 7700 sequence detection system (Applied Biosystems).

Total RNA was isolated from gastric antrum biopsies from five *H. pylori*-infected patients with a chronic gastritis and five healthy subjects who gave informed consent prior to their inclusion in the study. None of the patients had taken antibiotics or gastric proton pump inhibitors within the two months before the study. Total RNA was prepared with RNAbee™ solution (Duotech, Friendswood, Texas) according to the manufacturer's instructions. RNA was reverse transcribed and then amplified with the following primers: 5'-AGCAACAGGGTGGTGGAC-3', 5'-GTGTGGTGGGGGACTGAG-3' for GAPDH; 5'-TCCACCAGGGTCTGATTTTT-3', 5'-TTGAAGCGGAGAAGGAGACG-3' for IL-23p19. After amplification, data analysis was performed with the 'Second Derivative Method' algorithm. For each sample, the amount of IL-23p19 mRNA was expressed as an *n*-fold of the normalised amount of mRNA from untreated cells (1 AU = mRNA IL-23p19 concentration (fmol/µl) / mRNA GAPDH (fmol/µl), as described previously (4). This part of the study on human samples was approved by the appropriate ethics committee.

RESULTS

H. pylori gastric colonization and associated histological lesions

H. pylori SS1 strain efficiently colonized the stomach of infected mice during the entire experiment, with a mean level of $2x10^5$ and $8x10^6$ cfu/g of tissue at 6 and 12 months post-infection respectively. No bacteria were observed in non-infected tissues. Analysis of histological lesions in the infected gastric mucosa indicated an active gastritis correlated with the presence of mixed inflammatory infiltrates, including polymorphonuclear cells and lymphocytes in the antrum and fundus, as compared to non-infected mucosa (data not shown). Presence of low grade metaplasia was observed mostly at 12 months post-infection, as reported previously (13). In addition, we observed loss of parietal cells, irregular features, cytoplasmic swelling with cell membrane irregularities and karyolysis in infected-mice (Fig.

Table I. *Primers list for RT-PCR analysis.*

Gene name	Primer	Product size (bp)
Glyceraldehyde-3-phosphate Dehydrogenase (*Gapdh*)	F: 5' GATGACATCAAGAAGGTGGTGA 3' R: 5' TGCTGTAGCCGTATTCATTGTC 3'	199
Indoleamine-pyrrole 2,3 dioxygenase (*Ido*)	F: 5' GGGGGTCAGTGGAGTAGACA 3' R: 5' TGGGCAGCTTTTCAACTTCT 3'	199
Chitinase (*Chia*)	F: 5' AGGGCTACACTGGGGAGAAT 3' R: 5' TAGGGGCACCAATTCCATTA 3'	198
Upstream transcription factor 2 (*Usf2*)	F: 5'GACACACCCCTATTCTCCGAAA 3' R: 5' TCCAGGTTGTGCTGCTGTAG3'	361
H+/K+ ATPase transporting, alpha polypeptide (*Atp4a*)	F: 5' GTCTGGAGGGAACAGCTCAG 3' R: 5' TACCACAATGGCCATGAAGA 3'	242
Pancreatic phospholipase A2 (group IB) (*Pla2glb*)	F: 5' GGAGTGATCCCCTGAAGGAT 3' R: 5' GTTGTACGGGACCTTGGAGA 3'	293

Table II. *List of the genes most differentially expressed after 6 and 12 months of H. pylori infection as compared to the non-infected control mice at the same time point.*

Locus link	Gene	Description	6 Months Fold expression	6 Months *P* value	12 Months Fold expression	12 Months *P* value
81600	Chia	Chitinase	-3	0.00E+00	-30	$5x10^{-236}$
18778	Pla2g1b	Pancreatic PhospholipaseA2	-4	0.00E+00	-19	$5x10^{-236}$
109820	Pgc	Progastricsin	-2	$1.4x10^{-8}$	-18	$2x10^{-200}$
109791	Clps	Pancreatic colipase	-2	$1.3x10^{-10}$	-11	$9x10^{-126}$
11944	Atp4a	H+/K+ ATPase α subunit			-10	$3x10^{-82}$
11945	Atp4b	H+/K+ ATPase β subunit			-10	$4x10^{-82}$
14077	Fabp3	Fatty acid binding protein 3			-6	$1.3x10^{-62}$
18947	Plrp2	Pancreatic lipase-related protein2	-2	$2.1x10^{-5}$	-6	$6.2x10^{-7}$
11806	Apoa1	Apolipoprotein A1			-5.5	$6x10^{-56}$
22282	Usf2	Upstream transcription factor2	-1.5	$2x10^{-2}$	-5	$2x10^{-33}$
14603	Gif	Gastric intrinsic factor	-3	$1.7x10^{-11}$	-5	$3x10^{-93}$
11459	Acta1	Actin α 1 skeletal muscle			8	0.00E+00
21925	Tnnc2	Troponin C2, Fast			8	0.00E+00
69060	Pnlip	Pancreatic lipase			5	$6.2x10^{-7}$

1B) but not in those non-infected (Fig. 1A).

Global pattern of gene expression in H. pylori infected mice

We analysed the global patterns of gene expression in *H. pylori*-infected and non-infected mice after 6 and 12 months, according to the changes in transcripts evaluated as the fold change shown as logarithmic scale, between the average signal of infected samples and the baseline signal of non-infected control at each time point. There were no differences in gene expression profiles of non-infected samples between the 6 and 12 month time points, supporting the reliability of the changes observed between gene expression profiles for infected *vs* non-infected mice at each time point. The expression levels of 92 (40 up- and 52 down-regulated) genes and 171 (81 up- and 90 down-regulated) genes after 6 and 12 months of infection respectively were different from time-matched non-infected controls. These genes were mainly related to immune response, signal transduction pathways, cytoskeleton components, membrane proteins and transport, mitochondrial components, digestion process, general metabolism, transcription regulation, apoptosis and cell proliferation (Fig. 2). Overall, gene expression levels were between two to four times higher or lower in infected-samples than in non-infected controls. Fourteen genes were down- or up- regulated more than five fold at 6 and/or 12 months after infection (Table II). Chia and Pla2g1b, coding for an acidic chitinase and the pancreatic phospholipase A2 group IB respectively, were two of the genes most affected by *H. pylori* infection (Table II). The transcript levels of the α and β subunits of the H^+K^+-ATPase (Atp4a and Atp4b) primarily responsible for gastric acidification were ten times lower in infected mice than in controls, 12 months after infection (Table II). Moreover, we found down-regulated expression of Atp4a, Atp4b and of the gastric intrinsic factor (Gif) after chronic *H. pylori* SS1-strain infection (Table II).

Confirmation of microarray data by RT-PCR

Microarray analysis showed that several probe sets for the same transcript detected similarly modulated expression due to infection, supporting the validity of the data obtained. We analysed, by

Table III. *Differentially expressed genes related to immune response in H. pylori infected mouse stomachs as compared to non-infected.*

Locus Link	Gene	Name	6 Months Fold expression	Adjusted P value	12 Months Fold expression	Adjusted P value
14969	H2-Eb1	Histocompatibility 2, class II antigen E beta			2.0	9.40E-13
12260	C1qb	Complement component 1 q, subcomponent, beta polypeptide	2.0	3.85E-02	2.0	2.61E-07
14998	H2-DMa	Histocompatibility 2, class II, locus DMa			2.6	0.00E+00
215384	Fcgbp	IgG Fc binding protien			5.5	0.00E+00
14999	H2-Dmb1	Histocompatibility 2, class II, locus Mb1	2.0	1.93E-04	2.1	0.00E+00
12575	Cdkn1a	Cyclin-dependent kinase inibitor 1A (P21)	2.0	3.72E-03	2.0	1.00E-11
110454	Ly6a	Lymphocyte antigen 6 complex, locus A			2.0	0.00E+00
26365	Ceacam1	CEA-related cell adhesion molecule 1	2.0	9.05E-04	2.7	5.34E-08
21822	Tgtp	T-cell specific GTPase	2.0	4.54E-05	2.0	0.00E+00
56045	Samhd1	SAM domain and HD domain, 1	2.2	4.11E-02		
14469	Gbp2	Guanylate nucleotide binding protein 2	2.4	4.43E-09		
15980	Ifngr2	Interferon gamma receptor 2	2.0	2.00E-02		
15930	Ido	Indolcamine-pyrrole 2,3 dioxygenase	2.2	3.81E-06	2.2	0.00E+00
21356	Tapbp	TAP Binding protein/Tapasin	2.0	1.93E-04		
20701	Serpina1b	Serine (or cysteine) proteinase inhibitor, clade A, member 1b			2.2	0.00E+00
16145	Igtp	Interferon gamma induced GTPase	2.0	3.73E-02		
22375	Tts	Tryptophanyl-tRNA synthetase	2.0	1.14E-02	2.0	2.42E-10
16149	Cd74	Ia-associated invariant chain	2.0	6.08E-04	2.6	0.00E+00
14961	H2-Ab1	Histocompatibility 2, class II antigen A beta 1	3.0	4.51E-10	3.0	0.00E+00
14968	H2-Ea	Histocompatibility 2, class II antigen E alpha	2.0	1.5 E-04	2.5	0.00E+00
15000	H2-Dmb2	Histocompatibility 2, class II, locus Mb2	2.0	2.73E-03	2.3	0.00E+00
14960	H2-Aa	Histocompatibility 2, class II antigen A, alpha			2.0	0.00E+00
17988	Ndrg1	N-myc downstream regulated 1	2.0	3.2E-02	2.0	3.12E-02
16081	Igk-V1	Immunoglobulin kappa chain variable 1 (V1)	2.0	4.38E-05	2.0	0.00E+00
17857	Mx1	Myxovirus (influenza virus) resistance 1			-2.3	7.88E-08
16145	Il1ra1	Interleukin 11 receptor, alpha chain 1/GP130			-2.0	6.32E-06
15957	Ifit1	Interferon-induced protein with tetratricopeptide repeat1	-2.0	3.80E-03	-3	6.61E-07
15959	Ifit3	Interferon-induced protein with tetratricopeptide repeat3	-2.0	6.20E-04	-2.3	6.61E-07
53606	Glp2	Interferon-alpha inducible protein	-2.0	1.41E-04	-2.5	2.75E-08

a: Numbers indicate the fold increase or decrease of the mean expression values for each gene, calculated in each conditions between infected and non-infected mouse stomach samples at each time point, as described in the materials and methods section.

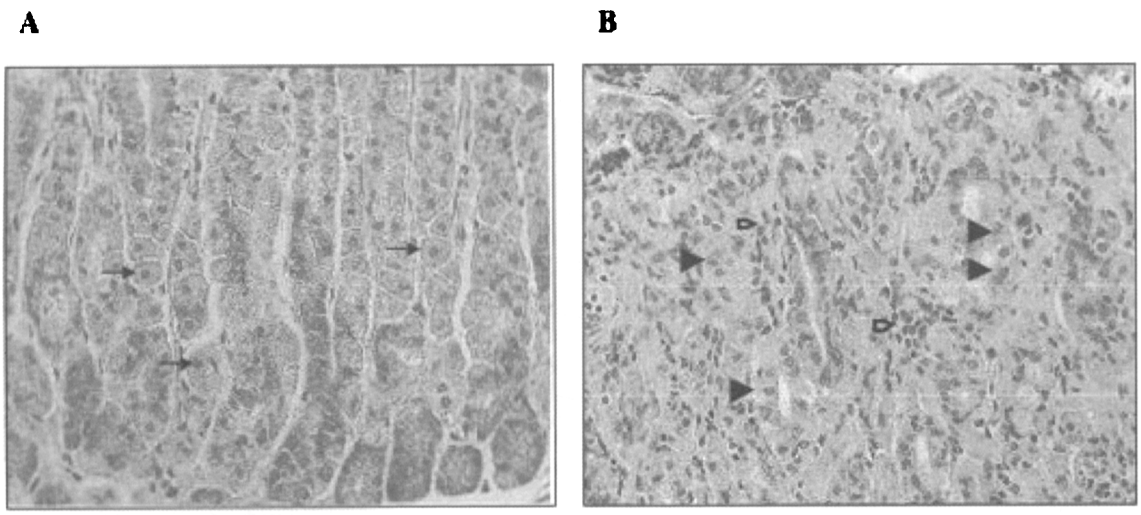

Fig. 1. *Histological lesions in the fundus of gastric mucosa of one representative non-infected mouse (**A**) and H. pylori infected-mouse for 12 months (**B**) revealed by hematoylin/eosin staining. Strong recruitment of lymphocytes and plasma cells were observed in the infected-mucosa as indicated by open symbols (**B**). As compared to the normal architecture of uninfected gastric mucosa and parietal cells as indicated by arrows (**A**), highly altered parietal cells (arrow heads) with atypical morphology as hyperchromatism and karyolysis, associated with atypical chief cells were seen in the infected gastric mucosa (**B**) (original magnification 200x).*

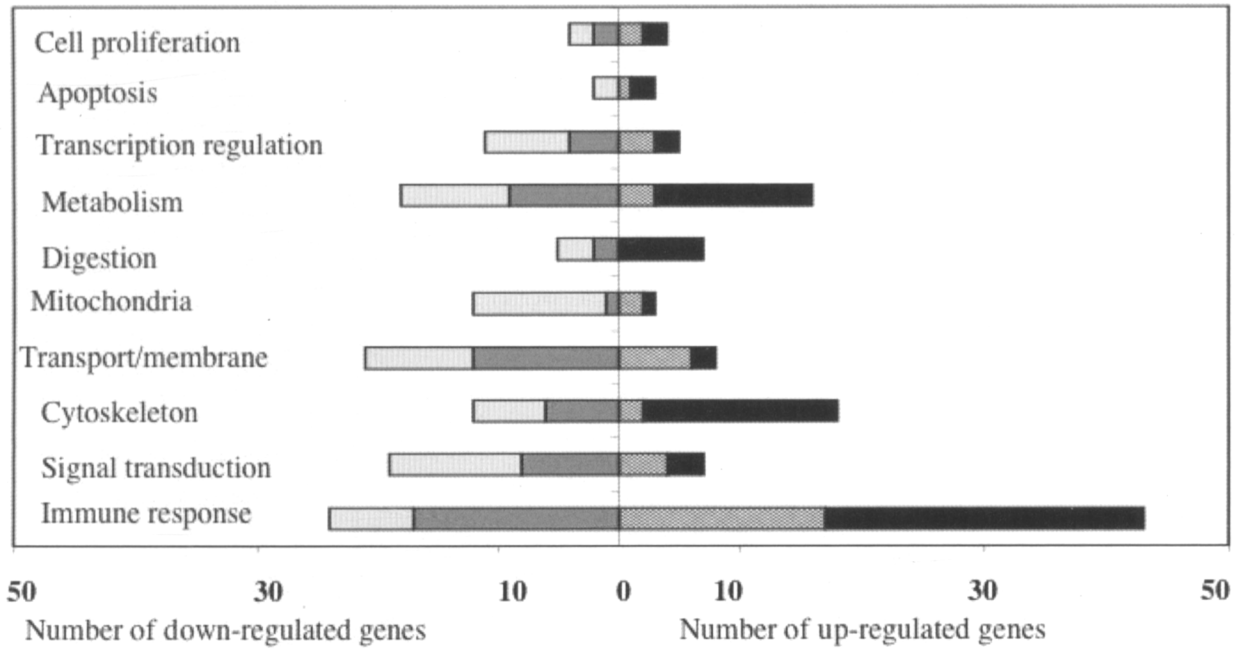

Fig. 2. *Distribution of genes differentially expressed in stomachs of mice after 6 and 12 months of infection according to functional categories. The bars represent the number of genes with an expression up-regulated at 6 months (hatched bars) and 12 months (black bars) or down-regulated after 6 months (open bars) and 12 months (grey bars) of infection as compared to non-infected control.*

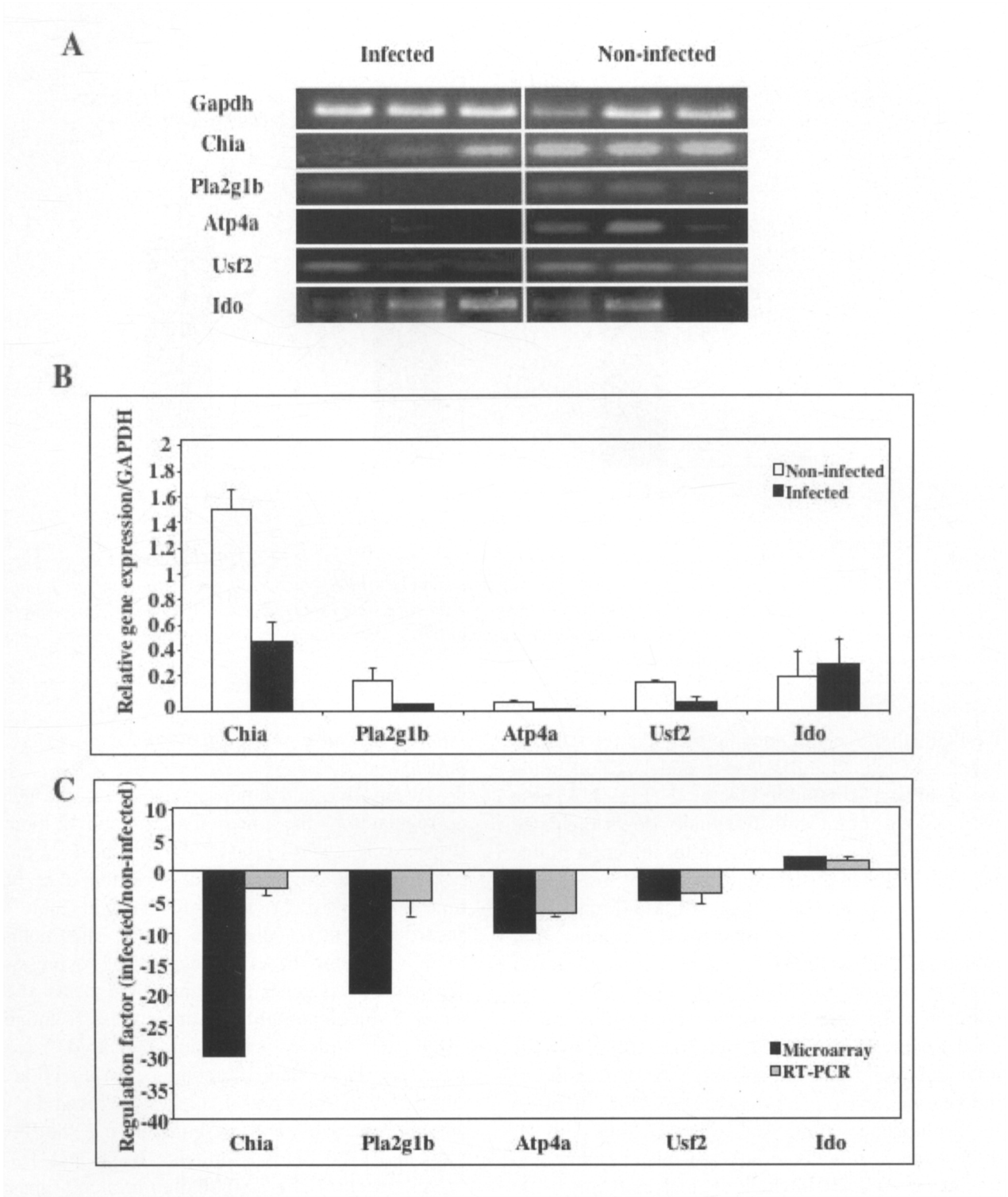

Fig. 3. *Validation of microarray gene expression by RT-PCR analysis. Each band represents one mouse (**A**). Relative quantification of the differential expression of selected genes (**B**), including chitinase (Chia), pancreatic phospholipase A2 group IB pancreatic (Pla2g1b), H^+/K^+ ATPase α subunit (Atp4a), upstream stimulatory factor 2 (Usf2) and the indoleamine-pyrrole 2, 3 dioxygenase after 12 months of infection compared to non-infected samples. Comparison of the mean fold changes observed for gene expression by microarray and RT-PCR analysis (**C**). RT-PCR experiments were performed 3 times.*

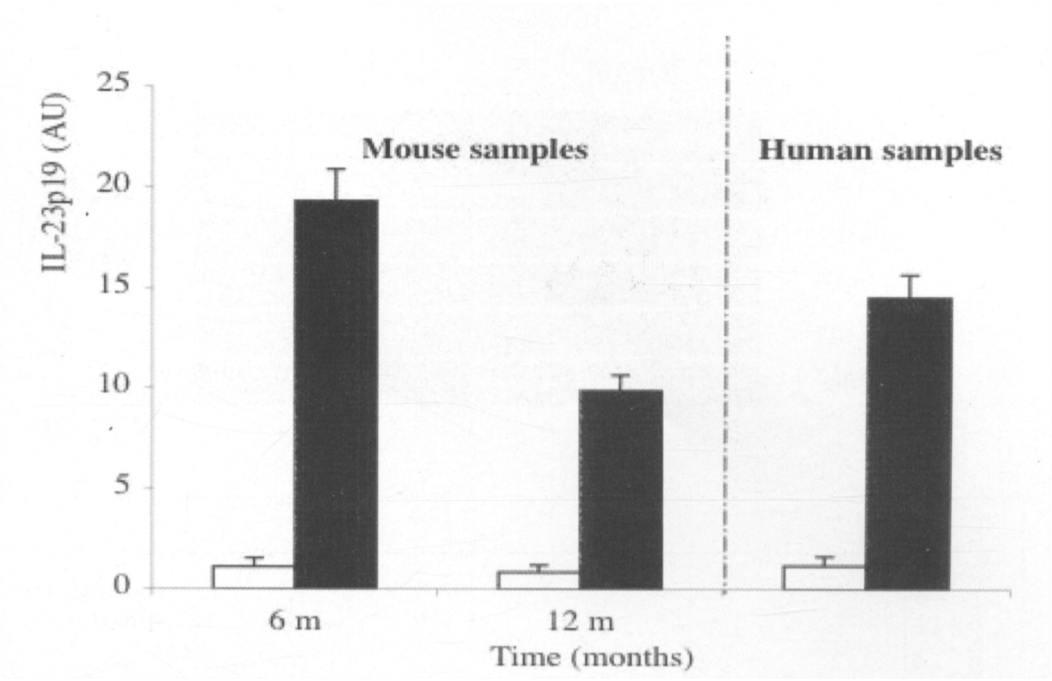

Fig. 4. *IL-23p19 gastric transcript levels of expression in H. pylori infection. IL-23p19/GAPDH average ratio in stomach samples from C57BL/6 mice 6 and 12 months after infection with H. pylori (filled bars), compared to non-infected samples (open bars) at the same time points. At the right part of the graph the levels are reported of IL-13p19 gene expression from gastric biopsies from H. pylori-infected patients with chronic gastritis (filled bars) (n=5) compared to healthy subjects (open bars) (n = 5). Error bars indicate SD.*

RT-PCR, the levels of specific mRNAs for relevant genes as Chia, Pla2gIB, Atp4a and Usf2 encoding for upstream stimulatory factor 2 (Fig. 3). These were among the most differentially down-regulated genes especially at 12 months after infection (Table II). RT-PCR analysis confirmed the low levels of expression for Chia, Pla2gIB, Atp4a and Usf2 observed by microarray analysis 12 months after infection, that were 3, 4, 7.4 and 3 times lower than in non-infected samples, respectively. We confirmed the infection-mediated inhibitory effect on the expression of Chia and Pla2g1b six months after infection (data not shown). The expression of indoleamine-pyrrole 2,3 dioxygenase (Ido) involved in tryptophane catabolism, was 2.2 times higher after 6 and 12 months in infected-mice as compared to non-infected. By RT-PCR analysis only a 1.5-fold increase was observed in infected-mice, compared to the controls (Fig. 3A, 3B). For each of the selected genes, comparison of microarray data and RT-PCR results indicated that microarray analysis slightly overestimated the fold changes (Fig. 3C), consistent with a previous report (18).

Immune response genes differentially expressed in infected-mice

Twenty-five and nineteen percent of the genes up-regulated by the infection after 6 and 12 months respectively, were related to the host immune response, with more than a third found at both time points (Fig. 2). They are listed in Table III, according to their expression profile. They pointed to a characteristic IFNγ-dependent expression. These included genes encoding components of the major histocompatibility complex class II antigens (MHC II), with an expression increased 2 to 3-fold either at 6 and 12 months post-infection as compared to the control. Genes involved in the antigen processing and presentation, e.g. the IFNγ-inducible GTP binding proteins IGTP and TGTP (T cell specific), the TAP binding protein (Tapasin) and the guanylate nucleotide binding protein 2 (GBP2) were also observed up-regulated (Table III). Transcript levels for the Ia-associated invariant chain (CD74), the IFNγ-regulated GTPase and IFNγ-receptor 2, were also found two times higher at six months in infected than in non-infected samples.

It is interesting to notice that in the presence of the *H. pylori* infection, concomitantly to the induction of the indoleamine-pyrrole 2,3 dioxygenase (Ido), which is a pro-inflammatory enzyme catalyzing the rate-limiting step in the oxidative catabolism of tryptophan, the transcript level for the tryptophanyl tRNA synthetase (Tts) was 2 times higher in infected than in non-infected controls either after 6 or 12 months. The same increase was observed for the four Tts corresponding probe sets present on the arrays.

Another gene product to be mentioned, is CEA-related cell adhesion molecule 1 (Ceacam 1), a major component of the apical surface glycocalyx of the mature epithelial cells. It plays an important role in adherence of enteric bacteria. A 2 and 2.7-fold induction of Ceacam 1 gene expression were observed at 6 and 12 months post-infection respectively.

Induction of IL23p19 associated with the IFNγ –signature in the immune response to H. pylori infection

The cytokines produced by the host in response to *H. pylori* strongly influence the outcome of the infection. A new member of the IL-12 cytokine family, IL-23, has been identified (19). It consists of two subunits, p40 (shared with IL-12) and p19. As cited above, our microarray data documented an induction of the expression of various MHC class II related genes in *H. pylori*-infected mice (Table III). Considering the important relevance of IL-23 in the host immune response and its ability to drive the Th1 response and to get further insights into the immune mechanisms related to the *H. pylori* infection, we compared, by real time-PCR analysis, the IL-23p19 transcript levels from mice stomach samples after 6 and 12 months of infection with non-infected controls. In the infected-mice, the expression of IL-23p19 was 18 times higher than in those non-infected at 6 months ($p<0.005$). This important difference was maintained up to 12 months (Fig. 4).

In vivo relevance of IL-23 up-regulation during H. pylori infection in humans

We investigated the *in vivo* relevance of the IL-23 up-regulation by the *H. pylori* positive patients. RNA from the antrum of five *H. pylori*-infected patients with chronic gastritis and five healthy non-infected subjects were isolated. IL-23p19 transcript levels were found 13 times higher ($p<0.005$) in the *H. pylori*-infected patients than in the non-infected (Fig. 4).

DISCUSSION

Important host co-factors in the outcome of the *H. pylori* infection relate to innate and immune response in both humans and experimental animals. In the present study, we investigated the global host gene expression profile in C57BL/6 mice in response to the *H. pylori* infection over 6 and 12 months, taking advantage of the ability of *H. pylori* SS1 strain to chronically colonize the mouse stomach for long periods. Our data focused on genes related to immune response. Multiple IFNγ-dependent signature transcripts were found up-regulated 6 and 12 months after infection, consistent with a Th1-polarized response. An up-regulation for carcinoembryonic antigen-related cell adhesion protein family, Ceacam 1 transcript level, was observed. Ceacam1 isoforms are activation-induced cell surface molecules on T-cells, specifically involved in Th1-mediated inflammation and associated with the release of IL-6 and IL-12, as demonstrated in a murine model of colitis (20). The concomitant up-regulation of indoleamine 2,3-dioxygenase (Ido) and tryptophanyl tRNA synthetase (Tts) genes expression is interesting to mention as it has never been reported so far in the case of the *H. pylori* infection. Ido and Tts are both IFN γ-inducible and responsible for tryptophan degradation and its use in protein synthesis respectively. Ido and Tts display immunomodulatory regulation of T-cell proliferation in human and in animal models (21). It is tempting to speculate that Tts could play a role in the balance between the anti-inflammatory role of Ido and the inhibitory effect on infection that should probably result in a global advantage for the host by turning down the gastric tissue damage.

Our data also documented increased transcript levels for components of the MHC class II system. MHC class II molecules are up-regulated during *Helicobacter* infection due to IFNγ exposure, favouring presentation of *H. pylori* antigens, and leading to activation of signal transduction cascade and apoptosis (22). They are over-expressed in

gastric carcinogenesis and associated with a host genetic polymorphism with higher risk of intestinal-type gastric adenocarcinoma, mainly associated with the *H. pylori* infection (23).

During the infection, the driving of gastric T cell response towards a Th1 type immune response is characterized by a high production of IFNγ with consequences on the severity of the gastric lesions. Various *H. pylori* factors have been reported to play a role in T cell polarisation, associated with a high production of IL-12 and IL-23 (4-5). Recently, a new link between inflammation and tumorigenesis has been proposed through the action of IL-23, which has been described to play an important role in triggering tissue inflammation and facilitating tumour growth (24). Levels of IL-23p19 mRNA, coding for the specific subunit of IL-23, were significantly higher in a large panel of human tumours than in their adjacent normal tissues (24). Induction of IL-23 leads to the recruitment of inflammatory cells, and is crucial for the pathogenesis of different inflammatory diseases, such as experimental colitis and arthritis (25). Moreover, IL-23 is produced by the host in response to many bacterial infections (26). In the case of the *H. pylori* infection, IL-23 expression has been reported to be induced by the bacterial HP-NAP factor, leading to an IFNγ–mediated immune response, associated with an induced expression of MHC class II components (4-5). We show here that IL-23p19 transcript levels were much higher in samples from infected-mice than in non-infected at each time point. In our mouse model it occurred in the context of an active gastritis with the presence of metaplasia, especially at 12 months of infection (13). The up-regulation of IL-23 expression has been found also to be relevant *in vivo* in humans, since the IL-23p19 expression levels were much higher in gastric biopsies from *H. pylori* infected-patients with chronic gastritis than from non-infected healthy subjects. Our data argue for the involvement of IL-23, IFNγ and its related transcripts in the genesis of the Th1-mediated immune response in *H. pylori* infection. While the IL-23 gastric action remains unknown, it is tempting to speculate that its up-regulation favours the severity of the gastric lesions induced by the *H. pylori* infection, as demonstrated in an *H. hepaticus* mouse model of colitis, where IL-23 drives IFNγ and IL-17 response and promotes severe intestinal inflammation (27).

The association between gastric Th1 immune response and hypochlorhydria through down-regulation of H^+K^+-ATPase has been reported previously in the INS-GAS mouse model in which *Helicobacter* infection exacerbates the development of gastric cancerous lesions (28). In the present study, H^+K^+-ATPase is strongly decreased at 12 months after *H. pylori* infection. Inhibition of H^+K^+-ATPase expression may be attributed to the progressive destruction of parietal cells observed in infected samples. The loss of parietal cells and the associated hypochlorhydria represent two crucial events preceding gastric dysplasia and neoplasia in *H. felis*-infected INS-GAS mice (28). In humans, molecular mimicry between *H. pylori* proteins and H^+K^+-ATPase has been reported as a key event in the genesis of *H. pylori*-relative gastric autoimmunity and consequent T-cell attack to H^+K^+-ATPase target on parietal cell surface (29). Although such a mechanism has not been identified in the mouse model, it cannot be excluded. A marked decrease of the gastric intrinsic factor (Gif) in the *H. pylori* infected mice was also observed in the present study. Atp4a, Atp4b and Gif have been previously shown strongly down-regulated in human gastric cancer (30) as well as in the *H. felis*-infected INS-GAS mouse model, previously described to develop metaplasia and gastric cancerous lesions exacerbated by *Helicobacter* infection (28).

The results obtained on long-term *H. pylori* SS1 infection in the present study consistently argued for the occurrence in our mouse model of the early events known to be involved in the promotion of severe gastric lesions. They provide novel insights in the immune response to *H. pylori* infection, demonstrating the *in vivo* involvement of IL-23 associated with the Th1-related IFNγ-signature transcripts, concomitantly with events preceding the induction of severe gastric lesions at the gastritis stage. The gastric IL-23 expression associated with polarized Th1 response might play an important role in the pathogenesis of *H. pylori* infection, constituting a new field of investigations.

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