Whipple Disease: Intestinal Infiltrating Cells Exhibit a Transcriptional Pattern of M2/Alternatively Activated Macrophages

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Whipple disease (WD) is a rare systemic disease caused by *Tropheryma whipplei* and is characterized by the presence of foamy macrophages with periodic acid-Schiff–positive inclusions in tissues such as lamina propria. For the first time, we report the gene-expression profile of macrophages in intestinal lesions from a patient with WD. Microarray and real-time polymerase chain reaction revealed that genes encoding CCL18, cathepsins, scavenger receptor, interleukin-10, and lipid metabolites were up-regulated in intestinal lesions. This transcriptional pattern corresponds to that of M2/alternatively activated macrophages. Our results suggest that the T helper 2 response in the intestinal environment may account for the pathophysiological properties of WD.

Whipple disease (WD) is a rare systemic disease that includes arthropathy, weight loss, and gastrointestinal symptoms, leading to a picture of malabsorption syndrome [1]. Otherwise, the disease is characterized by lymphadenopathy, skin hyperpigmentation, and cardiac and neurological manifestations that can be observed in the absence of intestinal manifestations [1]. The causative agent of WD is a novel bacterium named *Tropheryma whipplei*, and the complete genome sequences of 2 strains of *T. whipplei* have been reported [2, 3]. The pathophysiological properties of WD remain largely unknown, but a role for host immune factors has been suggested. First, lamina propria contains a large number of macrophages with periodic acid-Schiff (PAS)–positive inclusions [1]. Second, macrophages have a microbicidal defect in WD and are unable to produce sufficient amounts of interleukin (IL)–12 [4]. Finally, T cells probably undergo Th2 polarization [1]. Since the pathophysiological mechanism of WD has been inferred from a few studies performed with circulating monocytes and/or macrophages, we investigated the transcriptional profile in duodenal lesions rich in tissular macrophages from 1 patient with typical WD. We found that the transcriptional pattern of duodenal macrophages included up-regulation of CCL18, lipid metabolites, and cathepsins. Hence, they exhibit the features of M2/alternatively activated macrophages, which reflects a Th2 orientation of local immune response.

Materials and methods. Biopsy samples of duodenal lesions were obtained from one patient with WD and one control subject with *Helicobacter pylori*–free peptic duodenitis, after informed consent had been obtained. These samples were used for histopathological examination, microarray, and real-time polymerase chain reaction (RT-PCR) experiments. The former samples were fixed in formalin and embedded in paraffin, and the latter samples were immediately conserved in RNA protect (Qiagen). Monocytes from the patient with WD were isolated from blood by use of Ficoll gradient and adherence and were differentiated in macrophages by 7-day culture, as described elsewhere [5].

Duodenal lesion biopsy samples were cut to a thickness of 3 μm and were stained with hematoxylin-eosin–saffron. Serial sections were also obtained for diastase-digested PAS staining and immunohistochemical investigation. The detection of *T. whipplei* organisms was performed by immunohistochemical examination, as described elsewhere [6]. To evaluate the presence of macrophages in lamina propria, paraffin sections were stained with the macrophage marker CD68 (Dako) at a dilution of 1:50. Bacteria and CD68 were revealed by use of the Immunostain-Plus kit (Zymed; Clinisciences), in accordance with the manufacturer's protocol.

Monocyte-derived macrophages were stimulated with *T. whipplei* (bacterium:cell ratio, 50:1) for 4 h, washed, and lysed with Trizol (Invitrogen). Total RNA was purified in accordance with the manufacturer's protocol. Frozen intestinal biopsy samples were homogenized by use of Mixer Mill MM300 (Qiagen), and total RNA was extracted by use of RNeasy columns (Qiagen). Contaminating DNA was removed with 2 U of Turbo DNase (Ambion). RNA quantity and quality were assessed by use of the

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2100 Bioanalyzer and the RNA 6000 Nano LabChip kit (Agilent Technologies). Labeled cRNA probes were prepared by use of the Low RNA Input Linear Amplification kit (Agilent), in accordance with the manufacturer's protocol, hybridized with Oligo Human 1A (V2) microarray (Agilent) probes for 17 h at 60°C, and washed, before scanning by use of a ScanArray Express HT scanner (Packard BioScience). Spot intensities were determined by use of Spotfinder from the TM4 Suite (available at: http://www.tigr.org). Intensities with a median value 50% higher than that of the background were corrected by Lowess normalization. We analyzed only genes whose expression was altered at least 2.5-fold in one of the conditions tested. To identify genes with related function, manual searches of the GenBank, Online Menedelian Inheritance in Man, and LocusLink databases were combined with the ontology search tool DAVID [7]. Additionally, by use of TMEV from the TM4 Suite, common modulated genes were organized into 8 clusters by use of the *k*-means partitional algorithm with Euclidean and average linkage distance metrics between the data and clusters, respectively.

RNA from macrophages and duodenal tissue (DT) was reverse transcribed (Maloney murine leukemia–reverse transcriptase; Invitrogen) by use of an oligo(dT) primer. PCR was performed as described above, with the following primers: *CCL18,* 5′-TACCTCCTGGCAGATTCCAC-3′ (forward) and 5′-TTAGAAG-AGGTGGCCTCCAG-3′ (reverse); *CTSZ,* 5′-CGAGTGTTCCTGAGAGTTGAAAG-3′ (forward) and 5′-CCCCCACCCTTGATTCTAAA-3′ (reverse); *MMP9,* 5′-CACCACCACAACATCACCTATT-3′ (forward) and 5′-C-AGGGACCACAACTCGTCAT-3′ (reverse); *CTSK,* 5′-GAAAACTGGGGAAAC-AAAGGA-TA-3′ (forward) and 5′-GGAGAGAAGCAAAGTAGGAAGGA-3′ (reverse); *LTB,* 5′-AGAG-GAGGAGCCAGAAACAGA-3′ (forward) and 5′-GGTAGCCGACGAGACAGTAGAG-3′ (reverse); *ACP5,* 5′-GTGGCTTTGCCTATGTGGAG-3′ (forward) and 5′-GCTGTGTTTCCCCTTCCTG-3′ (reverse); *PLA2G7,* 5′-CTGAAACAAGAGGAGGAGACACA-3′ (forward) and 5′-ATCCAGGGCAA-TACCACAT-C-3′ (reverse); *CTSB,* 5′-AGGGTCTGAAGGACTGGATTG-3′ (forward) and 5′-CCTGGG-TCTCTGTCTTGC-TC-3′ (reverse); *MSR1,* 5′-CTTTCACAATCAACAGGAGGAC-3′ (forward) and 5′-ATTCTTCGTTTC-CCACTTCAGG-3′ (reverse); *IL16,* 5′-CTCCTGCCAAGCTGAACCCAA-GAC-3′ (forward) and 5′-AAG-GGGCATCTCCAACATCATCAT-3′ (reverse); *IL12,* 5′-TCAGCAACA-TGCTCCAGAAGG-C-3′ (forward) and 5′-TGCATTCATGGTCTTGAACTCCACC-3′ (reverse); *IL4,* 5′-TGGAAGAGAGGT-GCTGATTGG-3′ (forward) and 5′-CAGGAAGAACAGAGGGGGAAG-3′ (reverse); *IFNγ,* 5′-GTTTTGGGTTCTCTTGGCTGTTA-3′ (forward) and 5′-ACACTCTTTTGGATGCT-CTGGTC-3′ (reverse); *IL10,* 5′-GCTCCAAGAGAAAGGCATCTACA-3′ (forward) and 5′-GGGGGTT-G-AGGTATCAGAGGTAA-3′ (reverse); *TNF,* 5′-GAGGGAGAGAAGCAACTACAGACC-3′ (forward) and 5′-AGGAGAA-GAGGCTGAGGAACAAG-3′ (reverse); and *ACTB,* 5′-GGAAATCGTGCG-TGACATTA-3′ (forward) and 5′-AGGAAGGAAGGCTGGAAGAG-3′ (reverse). The fold change (FC) in expression of the target gene relative to β-actin was determined as follows: $FC = 2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct_{target} - Ct_{actin})_{sample} - (Ct_{target} - Ct_{actin})_{reference}$ [8]. Cycle threshold (Ct) values were defined as the cycle numbers at which the fluorescence signals were detected.

Results. A 70-year-old male patient was admitted to the hospital in March 2004 for fever, diarrhea, weight loss, and anemia. The diagnosis of WD was made in 1997 on the basis of the presence of arthralgia, diarrhea, weight loss, anemia, and PAS-positive macrophages on DT. The patient was treated with trimethropim-sulfamethoxazole for 1 year, which resulted in significant clinical improvement. Diarrhea, fever, and anemia were again reported in July 2003. Trimethropim-sulfamethoxazole treatment did not lead to clinical improvement. Histological examination of duodenal lesion biopsy samples revealed numerous foamy macrophages within the lamina propria of flattened villae. Associated with some dilated lymphatic vessels, they represented >80% of the cells and contained numerous intracytoplasmic diastase-resistant PAS-positive granules. *T. whipplei* organisms were detected in macrophage cytoplasm, by immunohistochemical analysis, as coarse granular immunopositive material.

The expression profiles of DT from the patient with WD versus the patient with ulcers and from *T. whipplei*–stimulated versus unstimulated macrophages were compared. Of the 19,569 arrayed sequences, 11,284 and 17,120 were conserved for DT and macrophage analyses, respectively. When analysis was restricted to genes exhibiting a difference of at least 2.5-fold, the expression of 129 sequences was modulated in WD intestinal lesions, but only 60 genes were modulated in response to *T. whipplei* in macrophages. The modulated genes were classified in 14 categories according to their ontology (figure 1*A*). Twenty-five percent and 20% of modulated genes in WD lesions and macrophages were found in the metabolism category, respectively. Genes involved in immune response, nucleic acid and nucleotide binding, and transport were similarly modulated in each sample. Interestingly, genes encoding proteolysis-associated components and extracellular matrix were represented more in DT than in macrophages (7% vs. 1% of the modulated genes, respectively).

A partitional clustering algorithm was used to organize genes into groups with similar expression patterns. Only genes for which transcripts were detected both in DT and macrophages were conserved for analysis: 19 of 60 and 16 of 129 genes were removed, respectively (table 1). The genes specifically up-regulated in DT and silent or repressed in macrophages belonged to clusters 7 and 8 (figure 1*B*). Cluster 8 consists of genes of immune response, proteolysis, and metabolism categories. They

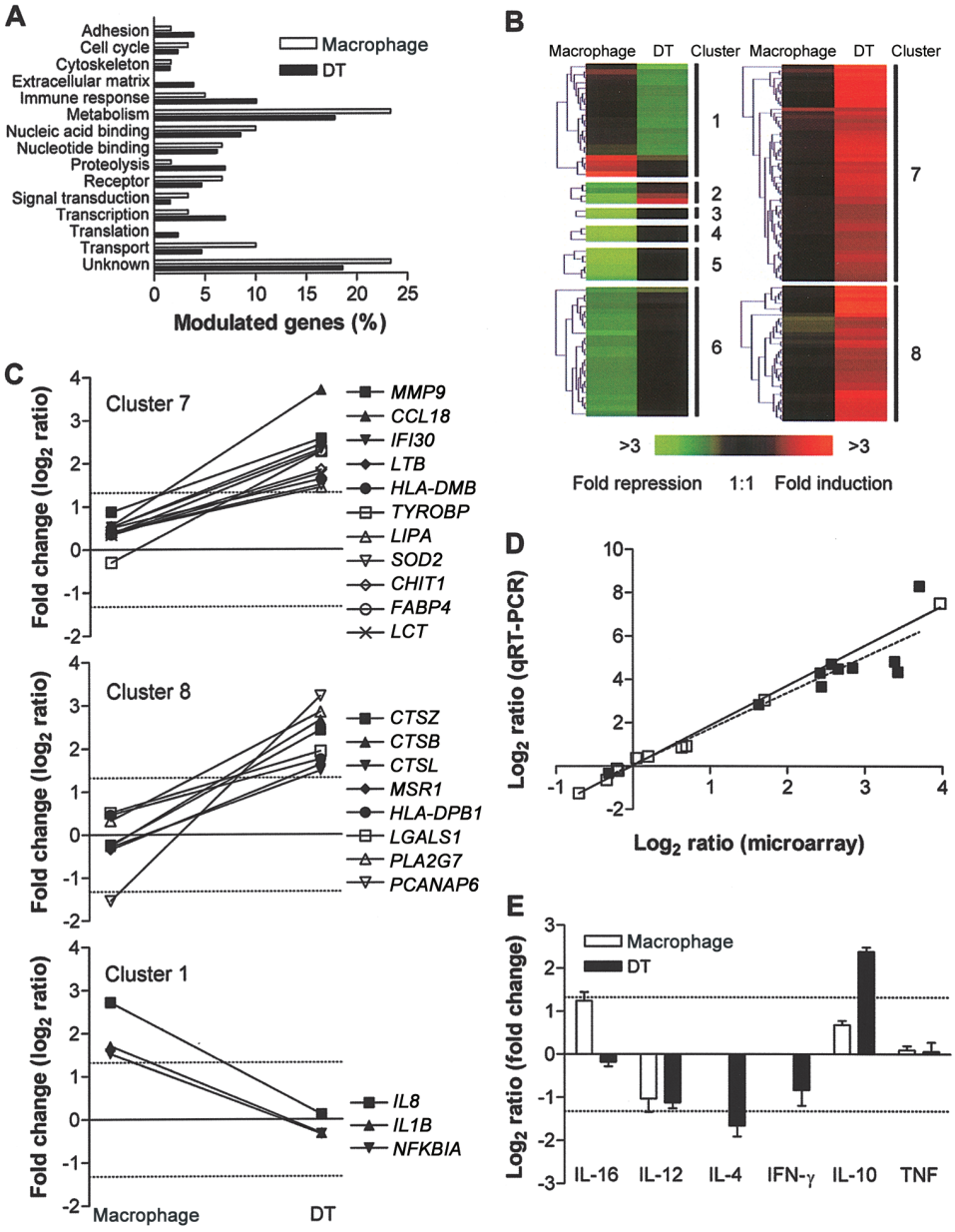

Figure 1. Differential responses of Whipple disease duodenal tissue (DT) and macrophages. *A,* Modulated genes in macrophages and DT, classified in 14 categories according to their ontology and presented as percentages. *B,* Common modulated genes in both macrophages and DT, organized into 8 clusters by the *k*-means partitional algorithm. Data are represented by a color gradient from green (down-regulation) to red (up-regulation). *C,* Some of the genes belonging to clusters 7, 8, and 1, represented as fold change ($\log_2$ ratio). Dotted lines stand for significant cut off at a 2.5-fold change value. *D,* Ten genes selected to validate microarray analysis by quantitative real-time polymerase chain reaction (qRT-PCR) for macrophages *(white squares)* and DT *(black squares).* Results are expressed as the $\log_2$ ratio of the fold change obtained by qRT-PCR and microarray experiments. Dots were then analyzed by linear regression, to determine the slope and the coefficient of determination (r^2) for both macrophages and DT ($y = 1.675 + 0.043$ and $r^2 = 0.976$ for macrophages [*solid line*] and $y = 1.656 + 0.073$ and $r^2 = 0.813$ for DT [*dotted line*]). *E,* RNA from macrophages and DT, extracted and processed for qRT-PCR. Results are expressed as the ratio of expression levels ($n = 3$) after normalization to the β-actin control. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

included cathepsins *(CTSB, CTSZ,* and *CTSL),* macrophage scavenger receptor 1 *(MSR1),* HLA-DPβ1 *(HLA-DPB1),* galectin 1 *(LGALS1),* granulin *(PCNAP6),* and phospholipase A2 *(PLA2G7)* (figure 1*C*). In cluster 7, the expression of genes encoding CCL18, matrix metalloproteinase 9 *(MMP9),* IFI30, lymphotoxin β *(LTB),* HLA-DMB, and TYRO-binding protein *(TYROBP)* was up-regulated. The expression of 4 genes involved in lipid metabolism was highly increased in DT: fatty

Table 1. Uncommonly modulated genes in macrophages and duodenal tissue (DT).

Gene	GenBank accession no.	Description	Macrophages, $\log_2$ ratio	DT, $\log_2$ ratio
ACHE	M55040	Acetylcholinesterase	−2.4	ND
ACP5	BC025414	Acid phosphatase 5	ND	3.4
AMICA	AY138965	Adhesion molecule AMICA	ND	1.8
C11orf23	NM_018312	Chromosome 11 open-reading frame 23	ND	2.0
CP	NM_000096	Ceruloplasmin (ferroxidase)	ND	3.1
CRABP2	NM_001878	Cellular retinoic acid–binding protein 2	−2.9	ND
DKFZp434B0417	NM_013377	Similar to semaF cytoplasmic domain–associated protein 3	1.3	ND
EAF1	NM_033083	ELL associated factor 1	ND	1.9
FLJ22054	NM_018527	Hypothetical protein FLJ22054	−1.4	ND
FLJ40722	AL832734	Hypothetical protein FLJ40722	ND	1.5
FLJ90709	NM_173514	Hypothetical protein FLJ90709	−1.4	ND
GDAP1	NM_018972	Ganglioside-induced differentiation-associated protein 1	ND	1.6
GPNMB	BC032783	Glycoproteinnmb	ND	4.2
IGKV2-40	X59314	Immunoglobulin κ variable 2–40	ND	−1.6
ITGB2	M15395	Integrin β 2	ND	2.2
KIAA0217	D86971	KIAA0217 protein	ND	2.6
KIF3B	AB002357	Kinesin family member 3B	ND	3.3
KITLG	NM_000899	KIT ligand	ND	2
LOC200383	NM_145299	Similar to Dynein heavy chain at 16F	−1.9	ND
LOC90529	NM_178122	Hypothetical protein LOC90529	−2.8	ND
MGC33367	NM_144602	Hypothetical protein MGC32905	−1.8	ND
MGC45866	NM_152259	Hypothetical protein MGC45866	−1.8	ND
PCK1	NM_002591	Phosphoenolpyruvate carboxykinase 1	ND	−1.6
PEPP-2	AL590526	PEPP subfamily gene 2	ND	2.5
PSMD13	AB009398	Proteasome 26S subunit, non-ATPase, 13	ND	2.6
RAB27B	U57093	RAB27B	−1.6	ND
RASGEF2	NM_152643	RasGEF domain family, member 2	−1.4	ND
SLC28A2	U84392	Solute carrier family 28, member 2	ND	−2.1
SMURF2	AY014180	E3 ubiquitin ligase SMURF2	−3.5	ND
TDP1	NM_001008744	Tyrosyl-DNA phosphodiesterase 1	−1.6	ND
TM4SF4	U31449	Transmembrane 4 superfamily member 4	ND	−1.4
TUB	NM_003320	Tubby homolog (mouse)	ND	2.2
ULBP3	AF304379	UL16 binding protein 3	−2.3	ND
ZNF336	AK025447	Zinc-finger protein 336	−1.6	ND
ZNF578	NM_152472	Zinc-finger protein 578	−1.3	ND

NOTE. The genes listed in the table were removed from the clustering analysis since they were not detected in any of the conditions. ND, not detected.

acid–binding protein 4 *(FABP4),* chitinase 1 *(CHIT1),* lactase *(LCT),* and lipase A *(LIPA)* (figure 1*C*). Conversely, the genes from cluster 1 represented genes that were up-regulated in *T. whipplei*–stimulated macrophages but were not expressed or were slightly repressed in DT (figure 1*B*). They included the genes encoding IL-8 *(IL8),* IL-1β *(IL1B),* and the NF-κB inhibitor *(NFKBIA)* (figure 1*C*). Finally, only the gene encoding superoxide dismutase 2, *SOD2,* was up-regulated in both macrophages and DT. Ten genes whose expression was markedly modulated in microarray experiments were controlled by quantitative (q) RT-PCR (figure 1*D*). Since some genes of innate immunity were removed from the microarray analysis after normalization, we directly assessed the expression of genes encoding IL-12p35, tumor necrosis factor (TNF), IL-10, IL-16, IL-4, and interferon (IFN)–γ, by qRT-PCR in DT and macrophages stimulated with *T. whipplei* for 4 h (figure 1*E*) or 24 h (data not shown). No significant difference was observed between macrophages stimulated for 4 h and those stimulated for 24 h. We found that TNF expression was not induced in *T. whipplei*–stimulated macrophages and WD lesions; IL-12 expression was repressed in both samples. The expression of IL-16 was up-regulated only in *T. whipplei*–stimulated macrophages, whereas the expression of IL-10 was up-regulated in DT. Expression of both IFN-γ and IL-4 was repressed in DT.

Discussion. In this report, we have investigated the transcriptional profiles of duodenal lesions and *T. whipplei*–stim-

ulated macrophages from one patient with typical WD. This analysis revealed the absence of expression of genes associated with the classic phenotype of activated macrophages. Clustering analysis showed that the gene profile was differently modulated in DT and *T. whipplei*–stimulated macrophages. Indeed, the genes belonging to clusters 7 and 8 were up-regulated in DT, whereas they were silent or repressed in *T. whipplei*–stimulated macrophages. CCL18, a chemokine that is mainly expressed by monocytes and dendritic cells, was the major molecule up-regulated in DT. Increased expression of CCL18 has been reported in pathological conditions with chronic inflammation, such as atherosclerotic plaques, liver inflammation, rheumatoid arthritis, and atopic dermatitis [9]. CCL18 is likely associated with Th2 polarization of the immune response, since increased expression of CCL18 is considered to be a signature of macrophage polarization toward the phenotype of M2/alternatively activated macrophages [10]. Th2 cytokines, such as IL-10 and IL-4, strongly increase CCL18 secretion, whereas Th1 cytokines, such as IFN-γ, inhibit this secretion [11]. We found that expression of the IL-10 gene was increased and that of the IL-12 and IFN-γ genes was repressed in WD duodenal lesions. In addition, the increased expression of MSR1, IL-1 receptor antagonist, CD14, MMP9, and galectin-1 suggested that cells in DT had acquired the phenotype of M2/alternatively activated macrophages. It is noteworthy that galectin-1 inhibits lipopolysaccharide-induced nitric oxide synthase 2 expression and directs the balance toward L-arginase, a marker of M2/alternatively activated macrophages [10]. It is likely that *T. whipplei* favors the differentiation of macrophages toward the phenotype of M2/alternatively activated macrophages in the context of the intestinal immune system, as has been reported in infectious diseases such as helminthiasis [12].

In accordance with these results, macrophages from duodenal lesions include the pattern of M2/alternatively activated macrophages and up-regulated expression of cathepsins L, B, and Z; acid phosphatase; and chitinase. Cathepsin up-regulation may be related to a defective IFN-γ pathway, since IFN-γ decreases expression of cathepsin B in macrophages [13]. Chitinase is secreted by chronically activated and alternatively activated macrophages and is increased in asthmatic Th2 inflammation [14]. The association of M2/alternatively activated macrophages with the up-regulation of acid phosphatase, chitinase, HLA-DBP1, and HLA-DMB has been reported in Gaucher disease, in which the accumulation of glycosphingolipids is related to the genetic deficiency of lysosomal glucocerebrosidase [15]. Nevertheless, the sequence analysis of glucocerebrosidase in the patient with WD did not reveal any mutation similar to those reported in Gaucher disease (data not shown).

In conclusion, WD intestinal lesions are characterized by the presence of macrophages exhibiting phenotypic features of M2/alternatively activated macrophages. This pattern is specific to DT, since macrophages derived in vitro from monocytes did not exhibit this pattern. We suggest that macrophages from blood origin acquire the features of M2/alternatively activated macrophages in the context of the intestinal environment and *T. whipplei* infection.

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