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Enhanced Expression of Interferon Regulatory Factor-1 in the Mucosa of Children with Celiac Disease

VIRGINIA M. SALVATI, THOMAS T. MACDONALD, GIOVANNA DEL VECCHIO BLANCO, GIUSEPPE MAZZARELLA, IVAN MONTELEONE, PIERO VAVASSORI, SALVATORE AURICCHIO, FRANCESCO PALLONE, RICCARDO TRONCONE, AND GIOVANNI MONTELEONE

Department of Pediatrics and European Laboratory for the Investigation of Food-Induced Diseases, University Federico II, Naples, Italy [V.M.S., S.A., R.T.], Division of Infection, Inflammation and Repair, University of Southampton, School of Medicine, Southampton General Hospital, Southampton, U.K. [T.T.M.], Dipartimento di Medicina Interna, Università Tor Vergata, 00133 Rome, Italy [G.D.V.B., I.M. P.M., F.P., G.Mo.], Istituto di Scienze dell'Alimentazione, CNR, Avellino, Italia [G.Ma]

ABSTRACT

Celiac disease (CD) is an enteropathy characterized by a Th1-type immune response to the dietary gluten. The transcriptional mechanisms or factors that control Th1 cell development in this condition remain to be elucidated. The aim of this study was to analyze in CD the expression of interferon (IFN) regulatory factor (IRF)-1, a transcription factor that regulates the differentiation and function of Th1 cells. Duodenal biopsies were taken from children with untreated CD and control children, and analyzed for IRF-1 by Southern blotting of reverse-transcriptase PCR products and Western blotting. IRF-1 DNA-binding activity was assessed by electrophoretic shift mobility assay. The effect of gliadin stimulation on IRF-1 induction was investigated in an *ex vivo* organ culture of treated CD biopsies. Enhanced IRF-1 was seen in untreated CD in comparison with controls. This was evident at both the RNA and protein level. Furthermore, untreated CD samples exhibited stronger nuclear accumulation and DNA-binding activity of IRF-1 than controls. In contrast, IRF-2, a transcriptional repressor that binds the same DNA element and competes with IRF-1, was expressed at the same level in nuclear proteins extracted from both untreated CD and control patients. In explant cultures of treated CD biopsies, gliadin enhanced both IRF-1 RNA and protein. This effect was prevented by a neutralizing IFN-γ antibody. Furthermore, stimulation of normal duodenal biopsies with IFN-γ enhanced IRF-1. These data indicate that IRF-1 is a hallmark of the gliadin-mediated inflammation in CD and suggest that IFN-γ/IRF-1 signaling pathway can play a key role in maintaining and expanding the local Th1 inflammatory response in this disease. **(*Pediatr Res* 54: 312–318, 2003)**

Abbreviations

CD, celiac disease
IRF-1, interferon regulatory factor-1
STAT1, signal transducer and activator of transcription 1
Th1, T-helper cell type 1
IFN, interferon
IEL, intraepithelial lymphocytes
EC, epithelial cells
LPMC, lamina propria mononuclear cell
ECL, enhanced chemiluminescence

CD is a gluten-mediated enteropathy of the proximal small intestine characterized by villous atrophy, crypt cell hyperplasia, and increased number of IEL (1). Accumulating evidence indicates that CD4+ T-cell-mediated hypersensitivity plays a major role in tissue injury in CD. Lamina propria CD4+ T cells are phenotypically activated and produce large amounts of Th1 cytokines in response to gluten stimulation (2, 3). Functional studies have demonstrated that activation of lamina propria Th1 cells can modulate extracellular matrix deposition and epithelial proliferation (4, 5). Enhanced synthesis of Th2 cytokines, such as IL-4, has also been reported in some studies (6).

Polarization of Th cells along the Th1 or Th2 pathway is influenced by a number of factors, such as the nature and concentration of the antigen, the type of antigen-presenting cells, and the microenvironment at the time of antigen expo-

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Correspondence: Giovanni Monteleone, Cattedra di Gastroenterologia, Dipartimento di Medicina Interna, Università Tor Vergata, Via Montpellier, 1, 00133 Rome, Italy; e-mail: Gi.Monteleone@Med.uniroma2.it
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sure (7). In addition, evidence has accumulated to show that distinct cytokine signaling and specific transcription factors are crucial in controlling commitment to a Th1 or Th2 phenotype (8).

The factors responsible for the Th1 response in CD are not known. We have recently demonstrated that IFN-α, a cytokine produced by virally infected cells that can promote IFN-γ synthesis and Th1 cell differentiation in humans, is produced in excess in CD (9). We have also provided evidence that activation of lamina propria T cells in the fetal gut model with anti-CD3 and IFN-α stimulates Th1 cytokine production and crypt cell hyperplasia, indicating a role for IFN-α in the pathogenesis of CD (10). Furthermore, in CD but not in normal duodenal mucosa, there is production of active IL-18, which is able to enhance Th1-cell polarization (11). Finally, it has recently been proposed that IL-15, a cytokine that is able to expand Th1-cell differentiation under particular conditions, may be involved in the CD immune response (12, 13). However, the transcriptional mechanisms that underlie the distinct Th1-type cytokine repertoire in CD remain unknown.

IRF-1 is a member of a family of transcription factors that share structural similarity in their DNA-binding domain and binding specificity (14). IRF-1 RNA is expressed in a variety of cell types, and its expression is dramatically up-regulated after viral infection or IFN stimulation (14, 15). IRF-1 was originally identified as a protein binding to the virus-inducible enhancer of the human IFN-α/β genes (15). Initial studies using a cDNA overexpression system revealed that high-level expression of IRF-1 was sufficient to induce IFN-α/β in the absence of virus infection, indicating that IRF-1 functions as a transcriptional activator of IFN-α/β genes (16). More recently, studies in knockout mice have shown that lack of IRF-1 results in an impaired release of IL-18, defective synthesis of IL-15 and IFN-γ, and enhanced production of Th2 cytokines (17–19). Taken together, these observations reveal a critical role for IRF-1 in the Th1-cell differentiation programs.

METHODS

Patients and controls. Three or more biopsy specimens from the distal duodenum of 18 children with untreated CD (median age, 11 y; range, 6–16 y) were obtained during upper gastrointestinal endoscopy. One specimen was used for routine histologic examination, whereas the remaining were immediately frozen in liquid nitrogen and stored until tested. The histopathologic diagnosis was based on typical mucosal lesions with crypt cell hyperplasia, villous atrophy, and increased number of IEL. All CD patients were positive for anti-endomysial (EMA) and anti-gliadin (AGA) antibodies. Duodenal biopsies were also collected from seven children with treated CD (median age, 13 y: range, 10–15 y), undergoing upper endoscopy for recurrent abdominal pain. All treated CD patients had normal histology and were AGA and EMA negative. Age-matched control patients ($n = 14$; median age, 14 y; range, 13–16 y) were under investigation for gastrointestinal symptoms, but had normal histology and were EMA and AGA negative. This study received ethical approval from the local committee (University Federico II, Naples, Italy). Informed consent was obtained from parents of all children enrolled.

Organ culture and cell isolation. The mucosal specimens were cultured as described elsewhere (20). Briefly, biopsies were placed on iron grids with the mucosal face upward in the central well of an organ culture dish in culture medium containing RPMI 1640 (Sigma-Aldrich SRL, Milan, Italy) supplemented with 10% HL-1 (BioWhittaker, Wokingham, U.K.), penicillin (100 U/mL) and streptomycin (100 μg/mL) (Invitrogen Italia SRL, San Giuliano Milanese, Italy). Cultures were performed with or without the addition of 1 mg/mL peptic-tryptic digest of gliadin (PT) (Sigma-Aldrich SRL) for 8 and 24 h in the presence or absence of a neutralizing IFN-γ or isotype control antibody (5 μg/mL final concentration; R&D Systems, Minneapolis, MN, U.S.A.). Furthermore, normal duodenal biopsies were stimulated with recombinant human IFN-γ (30 ng/mL; Peprotech EC Ltd., London, U.K.) for 24 h. The dishes were placed in a tight container with 95% O_2/5% CO_2 at 37°C, at 1 bar. At the end of the culture, biopsies were snap-frozen and stored at −80°C until used. EC and LPMC were isolated from duodenal biopsies taken from three active CD patients and three normal controls by the DTT-EDTA-collagenase procedure (21) and used for protein extraction. An aliquot of EC was also used for excluding the presence of LPMC contaminating the EC samples as described elsewhere (22).

Protein extraction and Western blot analysis. Western blot analysis was performed on whole mucosal duodenal samples from eight active CD children and eight control children. For cytosolic and nuclear extracts, the method described by Schreiber *et al.* (23) was used with minor modifications. Briefly, snap-frozen biopsies were mechanically homogenized in liquid nitrogen, and cytosolic extracts collected in buffer A containing 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, and 0.2 mM EGTA. Nuclear extracts were prepared by solubilizing the remaining nuclei in buffer C containing 20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, and 10% glycerol. Both buffers were supplemented with 1 mM DTT, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 1 mM phenylmethanesulphonyl fluoride (all reagents were from Sigma-Aldrich SRL).

For the detection of IRF-1, cytosolic proteins (150–250 μg/sample) were separated on a 10% SDS-PAGE gel. IRF-1 was detected using a polyclonal rabbit anti-human IRF-1 antibody (1:300 final dilution) (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) followed by a horseradish peroxidase–conjugated goat anti-rabbit IgG MAb (DAKO, Carpinteria, CA, U.S.A.) (final dilution 1:2500). The reaction was detected with ECL Plus kit (Amersham Pharmacia Biotech U.K. Ltd., Little Chalfont, Buckinghamshire, U.K.). To confirm equal loading and transfer of proteins, ponceau S (Sigma-Aldrich SRL) staining was performed. Computer-assisted scanning densitometry was used to analyze the intensity of the immunereactive bands in the autographs.

To analyze the content of IRF-1 in the nuclear extracts, 10 μg of nuclear protein/sample were separated on a 10% SDS/PAGE gel and analyzed using the polyclonal human IRF-1 antibody as indicated above. After detection of IRF-1, blots

were stripped by and subsequently incubated with a mouse anti-human histone-1 MAb (1:300 final dilution, Santa Cruz Biotechnology) followed by a goat anti-mouse antibody conjugated to horseradish peroxidase (1:1500 final dilution, DAKO). In addition, each sample was analyzed for the content of IRF-2. For this purpose, 10 μg of nuclear protein/sample were separated on a 10% SDS/PAGE gel and analyzed using the rabbit anti-human IRF-2 polyclonal antibody (1:300 final dilution, Santa Cruz Biotechnology) followed by a goat anti-mouse antibody conjugated to horseradish peroxidase (1:1500 final dilution, DAKO).

RNA extraction, cDNA preparation, and Southern blotting of RT-PCR products. RNA was extracted from biopsy specimens from eight CD patients and eight normal controls using 1 mL of a monophasic solution of phenol and guanidine isothiocyanate (TRizol, Invitrogen Italia SRL) and chloroform, followed by isopropanol (Sigma-Aldrich SRL) precipitation. The integrity of RNA was checked by electrophoresis on a 1.5% agarose gel. A constant amount of RNA (150 ng/sample) was retrotranscribed into complementary cDNA, and 1 μL of cDNA/sample was then amplified using the following conditions: denaturation 1 min at 94°C, annealing 1 min at 55°C for β-actin and 54°C for IRF-1, and extension 1 min and 15 s at 72°C as previously reported (24)

RNA expression for IRF-1 and β-actin was then assessed semi-quantitatively by Southern blotting. In preliminary experiments, we established the optimal number of cycles to obtain a PCR product within the linear phase of the amplification. For this purpose, an equivalent amount of cDNA for sample was amplified using specific primers for β-actin (1 μL of cDNA for 19, 21, 23, and 25 cycles) and for IRF-1 (1 μL of cDNA for 28, 30, 31, and 33 cycles). For Southern blot experiments, cDNA samples were amplified with β-actin primers for 20 cycles and with IRF-1 for 28 cycles. The IRF-1 primers were as follows: FWD: 5'-GTGGAAGTTGTGCCGGACA-3' and REV: 5'-CTTGCCTAGAGGAATAAGAGG-3'; β-actin primers have been published previously (24). Parallel experiments were carried out using RNA as substrate for PCR assay to exclude the amplification of genomic DNA contaminating the RNA samples. PCR product specificity was confirmed by restriction analysis. The cDNA probes used in the Southern blotting were DNA fragments encoding the full-length PCR product. Reverse-transcriptase (RT)-PCR products were run on a 1% agarose gel, and Southern blotting was carried out according to a commercially available chemiluminescence detection kit (Amersham Pharmacia Biotech). Intensity of the bands was measured by densitometry.

Electrophoretic mobility shift assay. Electrophoretic mobility shift assay (EMSA) was used to detect specific binding of IRF-1 to an oligonucleotide from the type IV CIITA promoter (5'-TGCAGAAAGAAAGTGAAAGGGAAAAAGAAC-3'). Nuclear protein–DNA binding studies were carried out for 20 min at room temperature in a 20 μL reaction volume containing 10 mM Tris, 50 mM KCl, 1 mM DTT, 2.5% glycerol, 5 mM $MgCl_2$, 1 μg Poly (dI-dC) (all the reagents were from Sigma-Aldrich SRL), 50 fmol biotin-labeled probe, and 10 μg nuclear proteins. The DNA probe was prepared by annealing the two consensus oligonucleotides, which were labeled at the 3' end with biotin using a commercially available kit (Pierce Chemical, Rockford, IL, U.S.A.). The binding specificity was confirmed by incubating the nuclear protein samples with unlabelled IRF-1/CIITA probe or unlabelled nonspecific oligonucleotide (AP-1, 5'-CGCTTGATGACTCAGCCGGAA-3') in 30-fold molar excess. In antibody blocking assays, a rabbit anti-human IRF-1 (Santa Cruz Biotechnology) or control isotype IgG antibody (DAKO) (both used in the amount of 1.5 μg/mL) were incubated with the nuclear proteins. A 7% nondenaturing polyacrylamide gel was used for electrophoretic separation. After blotting to a membrane, labeled oligonucleotides were detected with a chemiluminescence EMSA kit (Pierce Chemical).

Statistical analysis. Differences between groups were compared using the nonparametric Mann-Whitney *U* test.

RESULTS

In vivo *expression of IRF-1 in CD.* IRF-1 was analyzed by Western blotting using proteins extracted from duodenal mucosal samples of eight untreated CD patients and eight controls. Immunoreactivity for IRF-1 was seen in all CD patients and six of eight control samples. There was a clear increase in the intensity of the IRF-1 bands in CD compared with controls (Fig. 1*A*). The median IRF-1 protein was 68 densitometry arbitrary units (range, 56–89) in CD and 28 (range, 5–43) in controls (Fig. 1*B*) ($p = 0.002$). IRF-1 was more pronounced in both EC and LPMC samples taken from patients with active CD in comparison to normal controls (Fig. 1*A*, inset).

To analyze whether IRF-1 is regulated at the transcriptional level, mucosal samples from untreated CD patients and con-

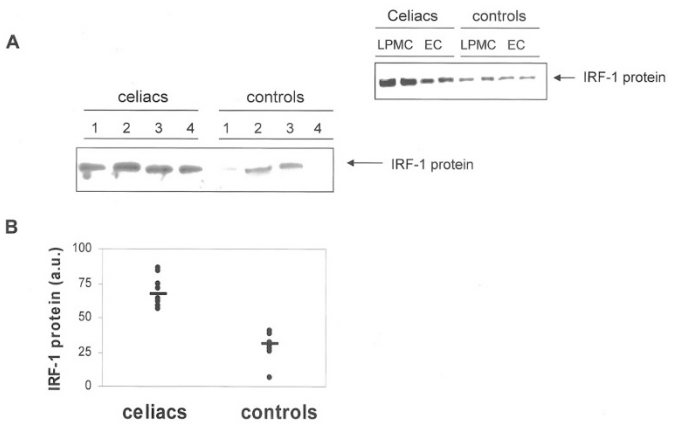

Figure 1. Enhanced IRF-1 protein in untreated CD. (*A*) Representative expression of IRF-1 protein in mucosal samples taken from four patients with untreated CD and four normal controls. The example is representative of three separate experiments analyzing in total mucosal samples from eight patients with CD and eight normal controls by Western blotting. (*Inset*) Western blot analysis of IRF-1 in LPMC and EC isolated from duodenal biopsies from patients with untreated CD and normal controls. The example is representative of two separate experiments analyzing in total mucosal samples from three patients with CD and three controls. (*B*) Quantitative analysis of IRF-1 protein in mucosal samples from eight patients with CD and eight normal controls, as measured by densitometry scanning of Western blots. Values are expressed in arbitrary units (a.u.). Each point represents the value (a.u.) of IRF-1 protein in mucosal samples taken from a single subject. *Horizontal bars* indicate the median.

trols were also assessed for the content of IRF-1 RNA by Southern blotting of RT-PCR products. All samples from CD and controls contained transcripts for IRF-1 (Fig. 2). However, analysis of the ratio of IRF-1 and β-actin transcript bands showed a more pronounced expression of IRF-1 in CD compared with controls (Fig. 2*B*), thus confirming the Western blot data.

Although the arbitrary units may not directly reflect the biologic quantities of IRF-1 RNA and protein within the intestinal mucosa, the fact that in each CD sample the intensity of IRF-1 band was increased compared with that seen in controls suggests that this transcription is increased in CD.

Increased IRF-1 DNA-binding activity in CD. After its induction, IRF-1 rapidly translocates to the nucleus, where it binds to specific DNA sequences (14). Consistent with this, we found that immunoreactivity for IRF-1 was greater in nuclear extracts of duodenal biopsies from untreated CD patients than in controls (Fig. 3). Another IRF family member, IRF-2, binds the same DNA sites and competes with IRF-1 (25). Indeed, the level of IRF-2 within the nucleus is inversely related to the IRF-1-binding DNA activity (25). Therefore, we then analyzed the expression of IRF-2 in nuclear protein taken from untreated CD patients and controls by Western blotting. As shown in Figure 3, IRF-2 was expressed at the same level in CD and controls, although, in both groups, expression was low.

Next, we wished to determine whether in CD there is increased IRF-1 DNA-binding activity. To accomplish this,

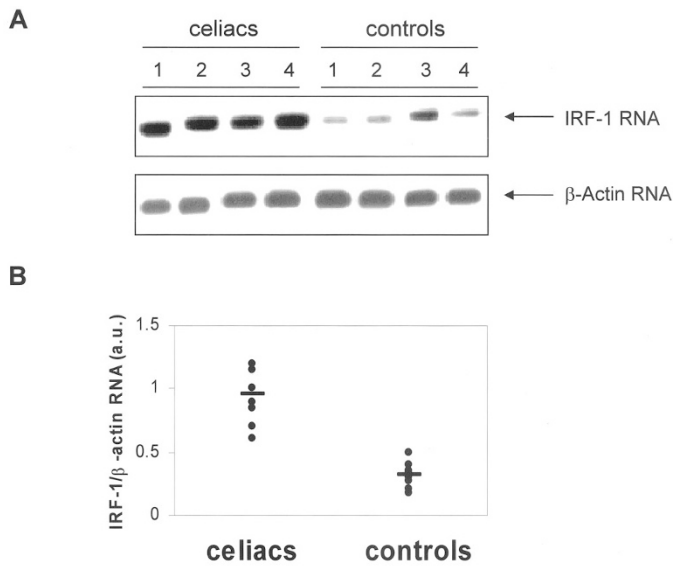

Figure 2. IRF-1 RNA transcripts in untreated CD. (*A*) Southern blot analysis of transcripts for IRF-1 and β-actin in duodenal mucosal tissue homogenates from four patients with untreated CD and four normal controls. cDNA samples were amplified using specific primers for IRF-1 or β-actin respectively. RT-PCR products were separated on agarose gel, blotted, and hybridized with specific probes for IRF-1 or β-actin. The example is representative of three separate experiments analyzing in total mucosal samples from eight patients with CD and eight controls. (*B*) Quantitative analysis of IRF-1/β-actin ratio in mucosal samples from eight patients with CD and eight normal controls, as measured by densitometry scanning of Southern blots. Values are expressed in arbitrary units (a.u.). Each point represents the value (a.u.) of IRF-1/β-actin ratio in mucosal samples taken from a single subject. *Horizontal bars* indicate the median.

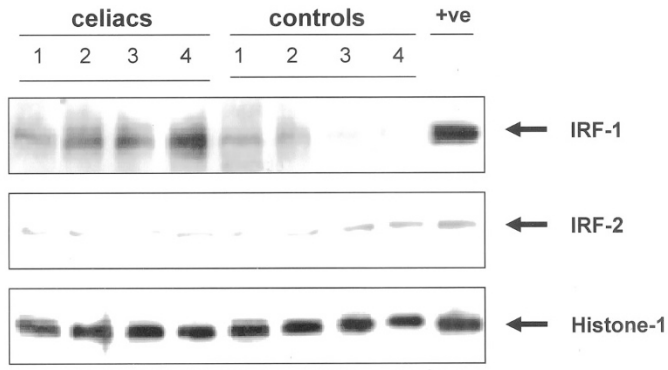

Figure 3. Enhanced nuclear accumulation of IRF-1 in untreated CD. Representative Western blot of IRF-1 (*upper panel*), IRF-2 (*middle panel*), and histone-1 (*lower panel*) in nuclear proteins extracted from four patients with untreated CD and four normal controls. The example is representative of three separate experiments analyzing in total mucosal samples from eight patients with CD and eight normal controls. *+ve,* THP-1 cells stimulated with recombinant human IFN-γ (30 ng/mL) for 4 h.

nuclear proteins were prepared from untreated CD and control biopsies, and binding to an oligonucleotide containing the IRF-1 site from the type IV CIITA promoter was assessed. As shown in Figure 4, nuclear extracts from all untreated CD and control samples formed an IRF-1 protein-DNA complex, but the intensity of this band was more pronounced in CD. The specificity of complex formation was examined by competition experiments using a 30-fold molar excess of unlabelled IRF-1/CIITA probe and supershift analysis.

Enhancement by gliadin of IRF-1 expression in treated CD biopsies. To show that in CD the gliadin-mediated immune response is related to IRF-1, we used an established *ex vivo* organ culture in which biopsies from treated CD patients or normal controls were stimulated with gliadin. In explants from treated CD biopsies, but not controls, stimulation with gliadin resulted in an enhanced induction of IRF-1 at both RNA and protein level (Fig. 5). Similarly, challenge of treated CD biopsies with gliadin for 24 h enhanced IRF-1 protein, and this effect was inhibited by a neutralizing IFN-γ antibody (Fig. 6*A*). In addition, stimulation of normal duodenal biopsies with IFN-γ increased IRF-1 protein, thus suggesting a role for this cytokine in mediating IRF-1 induction at the duodenal level (Fig. 6*B*).

DISCUSSION

In this study, we show for the first time that in the mucosa of patients with untreated CD there is enhanced expression of IRF-1, at both RNA and protein level, as well as increased nuclear accumulation and IRF-1 DNA-binding activity. Furthermore, we provide evidence that stimulation of treated CD biopsies with gliadin is followed by the induction of IRF-1.

The exact mechanisms responsible for the sustained increase of IRF-1 in CD remain to be elucidated. It is well documented that IRF-1 promoter contains STAT1 binding sites and that signaling *via* the IFN receptor promotes transcription of IRF-1 through activation of STAT1 (14). In keeping with this, IFN-induced expression of IRF-1 mRNA is completely abolished in STAT1-deficient cells (26). We have recently shown enhanced

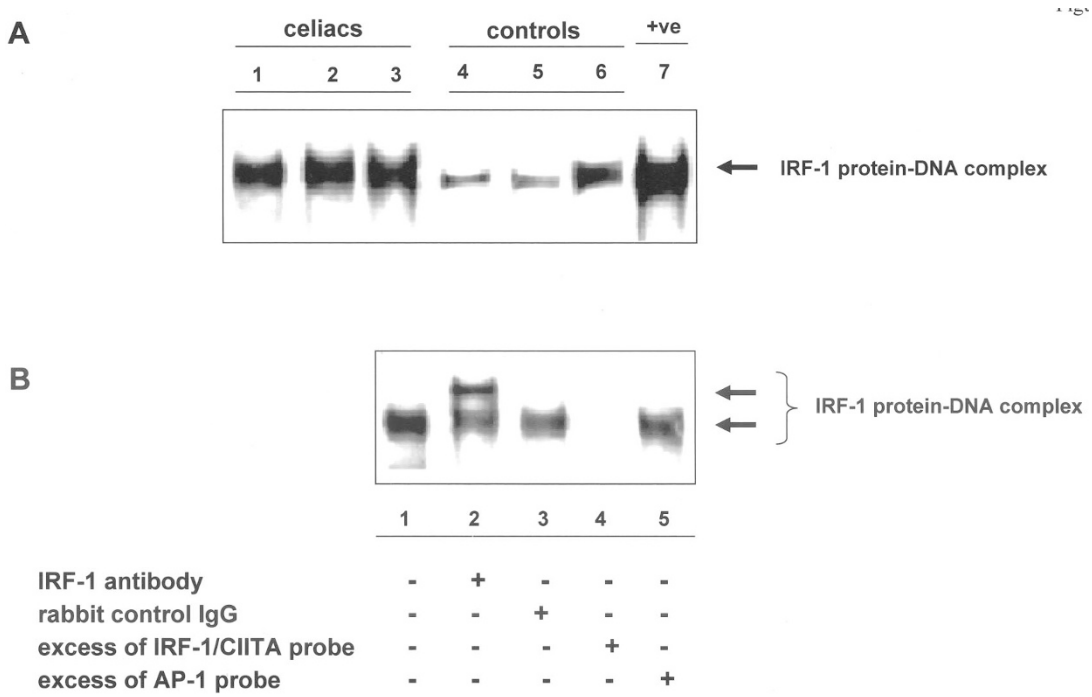

Figure 4. Nuclear extracts from untreated CD exhibit enhanced IRF-1 DNA-binding activity. (*A*) Representative EMSA blot showing enhanced IRF-1 DNA-binding activity in CD. Nuclear proteins extracted from mucosal biopsies from three patients with untreated CD (*lanes 1–3*) and three normal controls (*lanes 4–6*) were analyzed as indicated in materials and methods. IFN-γ–stimulated THP-1 cells were used as a positive control (*+ve*) (*lane 7*). One of two separate experiments analyzing samples from sevem patients with CD and seven controls is shown. (*B*) Representative EMSA blot showing the specificity of the IRF-1 protein-DNA complex. *Lane 1* shows the binding of nuclear proteins extracted from a mucosal biopsy of an untreated CD patient to the IRF-1/CIITA probe. Incubation of nuclear proteins, extracted from the same mucosal biopsy, with a human IRF-1 antibody (*lane 2*) causes a supershift of the complex, whereas with an excess of unlabelled IRF-1/CIITA probe (*lane 4*) causes a complete disappearance of the IRF-1 protein-DNA complex. In contrast, incubation of nuclear proteins with a nonrelevant control (IgG) antibody (*lane 3*) or excess of unlabelled nonspecific oligonucleotide probe AP-1 (*lane 5*) does not affect the IRF-1 protein-DNA complex. One of two representative experiments is shown.

activation of STAT1 and STAT1 DNA-binding activity in untreated CD mucosa, and also that challenge of treated CD biopsies with gliadin results in functionally active STAT1 (unpublished experiments). It is thus highly likely that the IFN/STAT1 signal transduction pathway contributes to enhance IRF-1 expression in CD. Indeed, the addition of a neutralizing IFN-γ antibody to our CD biopsy cultures completely prevented the gliadin-mediated IRF-1 induction. Finally, we provide evidence that IRF-1 is induced in normal duodenal biopsies stimulated *in vitro* with IFN-γ. Because the IRF-1 gene is also regulated by nuclear factor-κB (NF-κB) (26), we cannot exclude the possibility that NF-κB is involved.

A major histologic feature of untreated CD is the marked infiltration of the small intestinal mucosa with both IEL and lamina propria T cells, which produce high levels of IFN-γ (1–3, 27). How IFN-γ–secreting Th1 cells are generated in CD remains unknown. A possibility is that naive T cells migrate from the blood into the CD intestinal mucosa where they then differentiate along the Th1 pathway in response to locally produced Th1-inducing factors. It is also possible that Th1 cells primed in the Peyer's patches subsequently seed the lamina propria of CD patients, where locally produced factors may contribute to maintain and expand the gluten-specific Th1 response (28–30). Indeed, we have recently shown that in CD mucosa there is enhanced production of IFN-α and IL-18, two Th1-promoting cytokines (9, 11). The role of IRF-1 in CD remains to be clarified, because the number of mucosal cells we could purify from single small biopsies was not sufficient to carry out functional experiments. However, functional analysis of the role of IRF-1 in other systems has clearly shown that this transcription factor is critical in the differentiation of IFN-γ–secreting cells (14, 15, 25, 31). It is therefore conceivable that IRF-1 plays an important role in the development of the Th1 immune response in CD, as documented in other Th1-mediated immune diseases (32–35). The molecular mechanism that underlies the ability of IRF-1 to promote Th1 cell polarization is not fully understood. It is unlikely that IRF-1 directly affects transcription of the IFN-γ gene, because no binding sites for IRF-1 have been reported in the promoter of the IFN-γ gene. The more plausible explanation is that IRF-1 facilitates indirectly the induction of Th1 cells by positively regulating the synthesis of Th1-inducing cytokines, including IFN-α, IL-15, and IL-18 (16, 17). It would also be important to emphasize that these cytokines can, through the induction of IFN-γ, eventually enhance transcription of IRF-1 and generate a positive feedback loop able to maintain and expand the Th1 response in CD.

In addition to its role in the regulation of Th1 cell differentiation, IRF-1 can contribute to amplify and maintain chronic inflammation and favor autoimmunity (31–34). This largely relies on the ability of IRF-1 to modulate several immune-regulatory genes (*e.g.* inducible nitric oxide synthase, nitric oxide, class II transactivator), as well as to facilitate the recruitment of inflammatory cells within the inflamed tissue

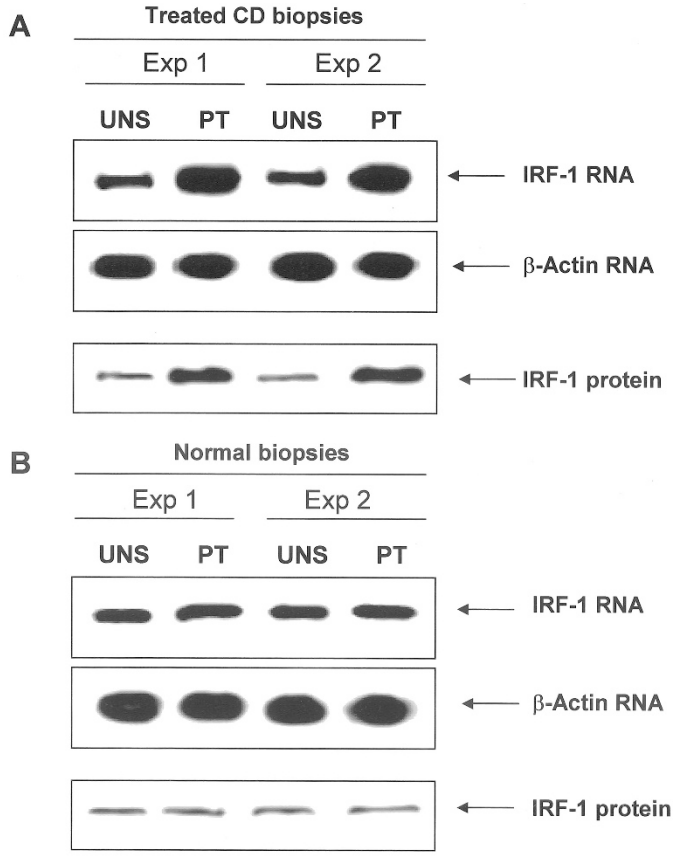

Figure 5. Stimulation of treated CD but not normal control biopsies with gliadin results in enhanced IRF-1 induction. (*A*) Southern blots of IRF-1 (*upper panel*) and β-actin (*middle panel*) RNA transcripts and Western blot of IRF-1 protein (*lower panel*) in mucosal biopsies taken from treated CD patients cultured in the absence (*UNS*) or presence of gliadin (*PT*). Two representative experiments analyzing in total mucosal samples from six patients with treated CD are shown. (*B*) Southern blots of IRF-1 (*upper panel*) and β-actin (*middle panel*) RNA transcripts and Western blots of IRF-1 protein (*lower panel*) in mucosal biopsies taken from normal duodenal biopsies cultured in the absence (*UNS*) or presence of gliadin (*PT*) for 8 or 24 h, respectively. Two representative experiments analyzing in total mucosal samples from three normal controls stimulated with gliadin are shown.

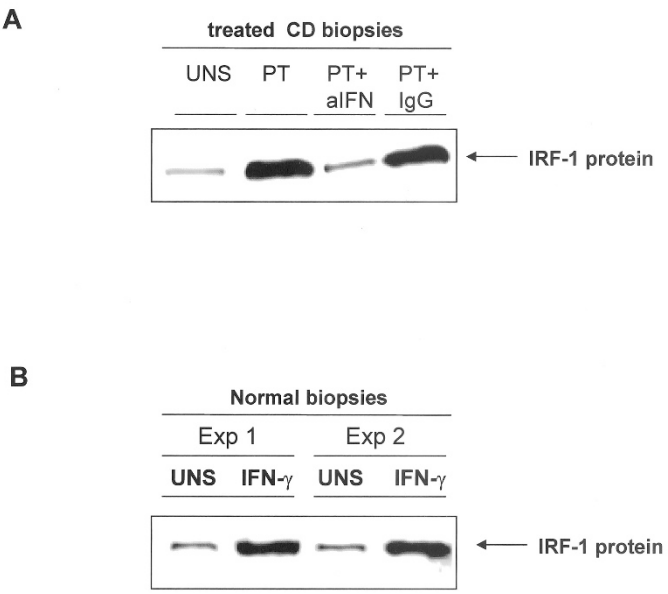

Figure 6. IFN-γ induces IRF-1 in the human duodenal mucosa. (*A*) Stimulation of treated CD biopsies with gliadin enhances IRF-1. This effect is inhibited by a neutralizing IFN-γ (aIFN) but not an isotype control antibody (IgG). One of two representative Western blotting experiments analyzing in total mucosal samples from five patients with treated CD are shown. (*B*) Western blot of IRF-1 in normal duodenal biopsies cultured in the absence (*UNS*) or presence of recombinant human IFN-γ for 24 h. Two representative experiments analyzing in total mucosal samples from five normal controls are shown.

up-regulating the expression of vascular cell adhesion molecule-1 (26, 36, 37). Furthermore, it is well known that IRF-1 can regulate both FasL expression and caspase activity, and promote apoptosis of epithelial cells (38). In this context, it could participate in the complex pattern of events that eventually lead to the villous atrophy. Indeed, recent studies have highlighted the role of FAS-mediated apoptosis in the gliadin-mediated induction of mucosal damage (39). Finally, it should be pointed that IRF-1 *trans*-activating IL-15 could also participate to the development of $CD94^+$ natural killer T cells, which heavily infiltrate the CD mucosa (18, 40, 41).

In contrast to IRF-1, IRF-2 was expressed at the same level in CD patients and controls, clearly indicating that these two transcription factors are differently regulated in CD mucosa. Because IRF-2 binds the same DNA sites and therefore competes with IRF-1 (25), it is possible that the increased IRF-1:IRF-2 expression ratio may contribute to the sustained IRF-1–binding DNA activity seen in CD.

All the mucosal samples taken from controls contain transcripts for IRF-1, confirming previous studies showing that IRF-1 RNA is expressed at low level in a variety of cell types (42). In addition, IRF-1 protein was found in most of the normal duodenal samples. This is, however, not surprising, given that human intestinal lamina propria is infiltrated with IFN-γ–secreting cells and with cells containing active STAT1 and NF-κB, the two inducers of IRF-1 (23, 24, 28, 43).

CONCLUSION

In conclusion, our data indicate that IRF-1 is a hallmark of gliadin-mediated inflammation in CD and suggest that the IFN-γ/IRF-1 signaling pathway may play a role in the maintenance of the Th1 response in CD.

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