IL-1 regulates the IL-23 response to wheat gliadin, the etiologic agent of Celiac Disease

Kristina M. Harris[1], Alessio Fasano[2] & Dean L. Mann[1]

[1]*Pathology Department and* [2]*Mucosal Biology Research Center, University of Maryland School of Medicine, Baltimore, MD, USA*

[1]10 S. Pine Street, MSTF Bldg., Room 8-56 and [2]20 Penn Street, HSF II Bldg., Room S351, Baltimore, MD 21201

Celiac disease (CD) is an autoimmune disease prevalent in ~1% of the general population[1]. CD is unique because both the major genetic (Human Leukocyte Antigen-DQ2/DQ8 alleles) and etiologic factors (dietary glutens) for susceptibility are known[2,3]. While these alleles are responsible for the inappropriate T cell response that characterizes CD, they are not sufficient since most HLA-DQ2$^+$/DQ8$^+$ individuals exposed to glutens never develop disease. The reasons for this have not been explained; however our novel findings strongly advocate a role for interleukin-23 (IL-23) in the immunopathogenesis of CD. We demonstrate that wheat gliadin stimulates monocytes to produce significantly higher amounts of inflammatory cytokines IL-1β, IL-23, and tumor necrosis factor-α (TNFα) in CD patients compared to HLA-DQ2$^+$ healthy individuals. Furthermore, we determine that IL-1 signalling is obligatory for production of IL-23, since IL-1β triggers IL-23 secretion in a dose-dependent manner and IL-1 receptor antagonist (IL-1ra) blocks IL-23 responses to gliadin. Our results suggest that gliadin activation of monocytes and the subsequent robust secretion of IL-1β and IL-23 initiate the immune response cascade that is manifest as CD, and reveal for the first time that the IL-1 system regulates production of IL-23. The discovery of IL-23 has

highlighted the critical role of the innate immune response in autoimmunity and other inflammatory conditions[4]. We anticipate that our novel findings will lead to the discovery of therapeutic targets for this disease and other inflammatory diseases mediated by IL-23.

Celiac disease (CD) is a chronic inflammatory disorder of the small intestine triggered by dietary glutens in genetically susceptible individuals[1]. More than a decade of research has defined the T cell response to gluten-derived gliadin peptides in CD, yet the early events that initiate its activation are not well understood[3,5,6]. Clearly, the compromised intestinal epithelial barrier that characterizes CD allows gliadin access to the intestinal submucosa, where it must be acquired and processed by antigen presenting cells (APC) for presentation and activation of gliadin-specific $CD4^+$ T cells. While augmented levels of zonulin and potent inflammatory cytokines IL-1β and TNFα have all been reported to increase intestinal permeability by disrupting the integrity of tight junctions in individuals with CD and other forms of inflammatory bowel disease (IBD), the precise mechanisms involved remain to be determined [7-9].

A dynamic relationship between intestinal epithelial cells (IEC) and dendritic cells (iDC) regulates the processes of immunologic tolerance to harmless food and commensal antigens and adaptive immunity to pathogens[10]. The aberrant response to dietary glutens in CD immediately calls into question the maturation and activation state of iDC in these individuals. Indeed, a subset of activated lamina propria $DQ2^+$ DC derived from circulating blood monocytes was recently implicated in the pathogenesis of CD [11]. Moreover, circulating monocytes from CD patients have been demonstrated to produce substantially more TNFα and IL-8 in response to gliadin than monocytes from healthy individuals [12]. Together, these findings suggest that CD ultimately results from accumulation of normally quiescent circulating monocytes that are activated upon encounter with gliadin in the gut.

Monocytes and their progeny are integral components of the innate immune system. In response to environmental antigens, conserved pattern recognition receptors (PRR) trigger cytokine production directing the immune response to the encountered antigen [13]. New evidence shows that activated monocytes producing IL-1β and IL-23 are the most potent stimulators of the memory subset of pathogenic T helper cells (termed Th17) that secrete tissue destructive cytokines IL-17, IL-21 and IL-22 [14-16].

IL-1 was one of the first cytokines to be described and has since proved to be an important mediator of multiple immunologic processes throughout the body, including inflammatory conditions in the gut[17]. The IL-1 family consists of proinflammatory cytokines IL-1α and IL-1β and anti-inflammatory IL-1ra, which prevents IL-1 signalling by binding the active IL-1 receptor (IL-1RI)[18]. An imbalance between IL-1β and IL-1ra, resulting from amplified levels of IL-1β has been associated with inflammation in CD[19]. Interestingly, elimination of dietary glutens significantly increases levels of IL-1ra in these individuals without substantially altering IL-1β, suggesting that individuals with CD inherently produce more IL-1β and IL-1ra, and that dietary glutens may induce inflammation by shifting the balance toward IL-1β in individuals with CD.

IL-23 is a relatively new inflammatory cytokine composed of the IL-12/23p40 subunit and the IL-23p19 protein that is preferentially secreted in specific tissues by APC [20,21]. It perpetuates chronic inflammation by stimulating both adaptive and innate cells to produce additional proinflammatory mediators [4]. CD has been considered a typical Th1 disease, however emergence of the IL23-Th17 paradigm has prompted reanalysis of cell-mediated tissue damage previously attributed to the IL12-Th1 axis, and emphasized the decisive role of the innate arm in adaptive immunity. Although novel studies have detected augmented levels of IL-23 in rheumatoid arthritis, psoriasis, Crohn's disease, ulcerative colitis and multiple sclerosis, and other cytokines associated

with Th17-mediated inflammation (IL-1β, IL-6, IL-15 and TNFα) have been implicated in the pathogenesis of CD, an association with IL-23 has not yet been reported [19,22-29].

Given the strong genetic requirement associated with CD, we investigated gliadin's capacity to activate the IL-23 pathway in HLA-DQ2$^+$ individuals with and without CD. We predicted that gliadin would induce increased levels of IL-23 and related inflammatory cytokines in HLA-DQ2$^+$ individuals with CD compared to healthy individuals. To test this hypothesis, we exposed PBMC from CD patients and HLA-DQ2$^+$ healthy individuals to a pepsin-trypsin digest of gliadin (PTG) and analyzed culture supernatants for IL-1β, IL-1ra, IL-6, IL-12p70, IL-23 and TNFα. We discovered that PTG stimulated production of IL-23, IL-1β, IL-6 and TNFα and reduced secretion of IL-1ra in all donors tested, however levels of IL-1β, IL-23, IL-6 and TNFα were significantly higher, and IL-1ra substantially reduced, in CD patients (Figure 1a). Importantly, PTG did not induce IL-12p70 in any of the donors tested (negative data not shown). These results confirm that gliadin stimulates robust production of IL-1β and TNFα in individuals with CD[12,19] and demonstrate gliadin's ability to disrupt the balance between IL-1β and IL-1ra by simultaneously inducing high levels of IL-1β and decreased levels of IL-1ra. Moreover, our novel findings strongly advocate a role for IL-23 mediated inflammation in the pathogenesis of CD.

In order to demonstrate that production of these potent mediators depended on gliadin exposure, dose response curves were generated with PTG or β-glucan, an agent known to activate the IL-23 pathway. Both stimuli induced dose-dependent production of IL-1β and IL-23, although PTG proved to be far more effective as evidenced by detectable levels of IL-23 achieved with 100μg/ml versus 500μg/ml of β-glucan (Figure 1b). These stimulatory effects of PTG were not due to endotoxin contamination, since the presence of LPS in this preparation of PTG was ruled out in earlier studies[29].

Several immunodominant epitopes of α-gliadin that preferentially bind HLA-DQ2 and DQ8 molecules as well as an innate peptide p31-43 have been implicated in the pathogenesis of CD[30]. To determine if any of these epitopes were involved in activation of the innate immune response, we incubated PBMC with synthetic overlapping peptides spanning the entire sequence of α-gliadin. None of the overlapping peptides tested individually or in combination stimulated secretion of IL-1β or IL-23, indicating that other subtypes of gliadin (γ- or ω-gliadin) or additional properties of gliadin are required for induction of these cytokines (negative data not shown). Since gliadin is a glycoprotein and β-glucan recapitulates the inflammatory cytokine response generated by PTG, posttranslational modifications are likely necessary for pattern recognition and activation of APC.

The kinetics of cytokine responses to PTG was determined by exposing PBMC to PTG for 6, 24, 48 and 72h. These studies revealed that IL-1β, IL-6 and TNFα were secreted in as few as 6h following PTG exposure, while IL-23 could not be detected until 24h, suggesting that induction of IL-23 required earlier inflammatory mediators (data not shown). During these initial studies, we also observed a positive correlation between IL-1β and IL-23, which led us to hypothesize that IL-1 is essential for production of IL-23. To directly examine the role of IL-1β in IL-23 responses, we treated PBMC from CD patients with IL-1ra prior to stimulation with PTG or the positive control, β-glucan. IL-1ra completely inhibited induction of IL-23 in response to both PTG and β-glucan, illustrating the fundamental role of IL-1 signalling in IL-23 production (Figure 2a). IL-1ra also markedly reduced levels of IL-1β in PBMC treated with both antigens, suggesting that IL-1β released upon engagement of PTG or β-glucan with their respective pattern recognition receptor (PRR) perpetuates production of IL-1β and facilitates induction of IL-23 (Figure 2a). Additionally, PBMC were treated with physiologic concentrations of exogenous IL-1β in order to ascertain its direct effects on cytokine production. Importantly, IL-1β alone induced IL-23

production at much lower levels than PTG and β-glucan, indicating that additional signalling pathways triggered by these antigens enhance secretion of IL-23 (Figure 2b). These results demonstrate for the first time that the IL-1 system regulates IL-23, and illustrate the powerful anti-inflammatory effects of IL-1ra on induction of IL-23.

While IL-1β is produced by many cell types, IL-23 production is thought to be restricted to activated APC. Recently, TLR activated monocytes were shown to secrete high levels of IL-23 and to be the best inducers of Th17 cells[14], thus we predicted that monocytes were the cellular source of PTG-induced IL-23. To investigate this hypothesis, we exposed purified lymphocytes, monocytes, or monocyte-derived DC (cultured with GM-CSF and IL-4 for 72h) to PTG overnight and analyzed the cell-free culture supernatants for IL-23 and related "Th17" polarizing mediators. Under these conditions, monocytes and not their progeny DC or lymphocytes produced IL-23, IL-1β, IL-6, TNFα and CCL20 in response to PTG, demonstrating a direct interaction between PTG and its anonymous PRR(s) on this population (Figure 3).

As with whole PBMC, IL-1ra significantly inhibited IL-23 responses to PTG and β-glucan in purified monocytes (Figure 4a), and addition of exogenous IL-1β to this subset triggered a dose-dependent IL-23 response (Figure 4b). These results illustrate that gliadin directly stimulates monocytes to secrete IL-23 and related inflammatory mediators and further support a primary role for the IL-1 system in IL-23 mediated inflammation.

In summary, our studies demonstrate that enzymatically digested wheat gliadin stimulates monocytes to produce significantly more IL-23, IL-1β and TNFα in CD patients than HLA-DQ2^{+} healthy individuals, and reveal a fundamental role for the IL-1 system in the IL-23 pathway. We show that IL-1β directly induces monocytes to secrete IL-23, while its natural inhibitor, IL-1ra, substantially inhibits both the IL-1β and IL-23

responses generated by monocytes exposed to gliadin. Moreover, our data indicate that gliadin initiates the inflammatory cascade by disrupting the balance between these two IL-1 members, which could be targeted therapeutically for treatment of this disease and other conditions associated with IL-23 mediated inflammation.

Methods

Cells. Peripheral blood mononuclear cells (PBMC) were isolated from Celiac patients' and healthy donors' whole blood by density gradient centrifugation in Lymphocyte Separation Medium (ICN Biomedicals Inc.). PBMC were viably cryopreserved in RPMI-1640 media (Invitrogen Corp.) containing 20% human AB serum (hAB) (Gemini Bioproducts) and 10% Dimethylsulfoxide (Sigma) using an automated cell freezer (Gordinier Electronics), and stored in the vapor phase of liquid nitrogen until used. Highly purified monocytes (95% purity) were obtained from healthy donors as above followed by countercurrent centrifugal elutriation. The resulting cells were viably cryopreserved in fetal bovine serum (Summit Biotechnology) containing 10% DMSO and 5% glucose (Sigma) for later use. All individuals gave informed consent for peripheral blood drawn for this study. The study protocol was approved by the Institutional Review Board at the University of Maryland School of Medicine.

DNA Extraction and HLA Typing. DNA was extracted from a portion of the PBMC using the QIAamp DNA Mini Kit (Qiagen) per the manufacturer's instructions. DNA was analyzed by spectrophotometry to determine quantity and purity and stored at –20°C until used. Alleles of genes encoding HLA were identified using One Lambda Micro SSP™ ABDR Typing Kit, and alleles of genes encoding HLA-DQ were determined by DQA1 and DQB1 SSP UniTray® Kit (Dynal Biotech) following the manufacturer's.

Reagents. Gliadin was prepared by enzymatic digestion as described previously[29]. The presence of contaminating endotoxin in gliadin was determined by *Limulus* amebocyte assay per the manufacturers' instructions. 100mg of β-D-glucan from barley (Sigma) was dissolved in 600ul 95%EtOH followed by 9mL distilled water. The resultant slurry was stirred vigorously at 100°C for 3 minutes, allowed to cool, and stored at 10mg/ml at 4°C until used. 25 overlapping 20mers spanning the sequence of α-gliadin were synthesized and purified >95% at the University of Maryland Biopolymer Lab, and stored at –20°C until used. Recombinant human IL-1β and IL-1ra were purchased from R & D Systems.

PBMC cultures. PBMC from CD patients and HLA-$DQ2^+$ healthy controls was tested as follows. 10^6PBMC/ml were incubated in RPMI-1640 supplemented with 10% heat inactivated hAB, 1% L-glutamine, 1% Pen-Strep and 20mM Hepes Buffer (cRPMI) with and without PTG, β-glucan, 5ng/ml rhIL-1β, or 10μg/ml pooled synthetic 20mers of α-gliadin in 96 well U-bottom plates (Denville Scientific Inc.) at 37 °C in 5% CO_2 for 6, 24, 48, or 72h. Alternatively, 10^6PBMC/ml were incubated with 0.5μg/ml rhIL-1ra at 37 °C in 5% CO_2 for 1h then cultured with and without 100μg/ml PTG or 500μg/ml β-glucan for an additional 20h. Cell-free culture supernatants were harvested for cytokine and chemokine analysis.

Elutriated monocyte cultures. 5 x 10^5monocytes/ml were cultured in cRPMI with and without 100μg/ml PTG, 100μg/ml β-glucan, or 0.5-50ng/ml rhIL-1β in 96 well U-bottom plates at 37 °C in 5% CO_2 for 20h. Alternatively, 5 x 10^5monocytes/ml were incubated with 0.5μg/ml rhIL-1ra at 37 °C in 5% CO_2 for 1h then cultured with and without 100μg/ml PTG or β-glucan for an additional 20h. Cell-free culture supernatants were harvested for cytokine and chemokine analysis.

Cytokine & chemokine analysis. Cell-free culture supernatants were analyzed for IL-1β, IL-1ra, IL-6, IL-12p70, IFNγ, TNFα (Bio-Plex Cytokine Assay kit, Bio-Rad) or IL-

1β, IL-23 (ELISA kit, eBiosciences), IL-1ra and CCL20 (Quantikine ELISA kit, R & D Systems) following the manufacturers' protocols. Appropriate standard curves were included in each assay.

Statistical analyses. Data are presented as mean values + s.d. P values comparing different conditions within the same individuals were calculated using paired two-tailed Student's *t* tests and p values comparing the two study groups were determined by unpaired two-tailed Student's *t* tests (Figure 1a). *P* values < 0.05 were considered statistically significant.

1. Fasano, A. et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med* 163, 286-92 (2003).

2. Louka, A. S. et al. Coeliac disease patients carry conserved HLA-DR3-DQ2 haplotypes revealed by association of TNF alleles. *Immunogenetics* 55, 339-43 (2003).

3. Lundin, K. E., Scott, H., Fausa, O., Thorsby, E. & Sollid, L. M. T cells from the small intestinal mucosa of a DR4, DQ7/DR4, DQ8 celiac disease patient preferentially recognize gliadin when presented by DQ8. *Hum Immunol* 41, 285-91 (1994).

4. Hue, S. et al. Interleukin-23 drives innate and T cell-mediated intestinal inflammation. *J Exp Med* 203, 2473-83 (2006).

5. Tollefsen, S. et al. HLA-DQ2 and -DQ8 signatures of gluten T cell epitopes in celiac disease. *J Clin Invest* 116, 2226-36 (2006).

6. van de Wal, Y. et al. Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. *Proc Natl Acad Sci U S A* 95, 10050-4 (1998).

7. Drago, S. et al. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. *Scand J Gastroenterol* 41, 408-19 (2006).

8. Al-Sadi, R. M. & Ma, T. Y. IL-1beta causes an increase in intestinal epithelial tight junction permeability. *J Immunol* 178, 4641-9 (2007).

9. Ma, T. Y. et al. TNF-alpha-induced increase in intestinal epithelial tight junction permeability requires NF-kappa B activation. *Am J Physiol Gastrointest Liver Physiol* 286, G367-76 (2004).

10. Kelsall, B. L. & Leon, F. Involvement of intestinal dendritic cells in oral tolerance, immunity to pathogens, and inflammatory bowel disease. *Immunol Rev* 206, 132-48 (2005).

11. Raki, M. et al. A unique dendritic cell subset accumulates in the celiac lesion and efficiently activates gluten-reactive T cells. *Gastroenterology* 131, 428-38 (2006).

12. Cinova, J. et al. Gliadin peptides activate blood monocytes from patients with celiac disease. *J Clin Immunol* 27, 201-9 (2007).

13. Williams, M. A., Newland, A. C. & Kelsey, S. M. The potential for monocyte-mediated immunotherapy during infection and malignancy. Part I: apoptosis induction and cytotoxic mechanisms. *Leuk Lymphoma* 34, 1-23 (1999).

14. Acosta-Rodriguez, E. V., Napolitani, G., Lanzavecchia, A. & Sallusto, F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* 8, 942-9 (2007).

15. Steinman, L. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. *Nat Med* 13, 139-45 (2007).

16. McKenzie, B. S., Kastelein, R. A. & Cua, D. J. Understanding the IL-23-IL-17 immune pathway. *Trends Immunol* 27, 17-23 (2006).

17. Ligumsky, M., Simon, P. L., Karmeli, F. & Rachmilewitz, D. Role of interleukin 1 in inflammatory bowel disease--enhanced production during active disease. *Gut* 31, 686-9 (1990).

18. Schreuder, H. et al. A new cytokine-receptor binding mode revealed by the crystal structure of the IL-1 receptor with an antagonist. *Nature* 386, 194-200 (1997).

19. Fornari, M. C. et al. Pre- and post-treatment serum levels of cytokines IL-1beta, IL-6, and IL-1 receptor antagonist in celiac disease. Are they related to the associated osteopenia? *Am J Gastroenterol* 93, 413-8 (1998).

20. Oppmann, B. et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 13, 715-25 (2000).

21. Uhlig, H. H. et al. Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. *Immunity* 25, 309-18 (2006).

22. Kim, H. R. et al. The clinical role of IL-23p19 in patients with rheumatoid arthritis. *Scand J Rheumatol* 36, 259-64 (2007).

23. Becker, C. et al. Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells. *J Clin Invest* 112, 693-706 (2003).

24. Piskin, G., Sylva-Steenland, R. M., Bos, J. D. & Teunissen, M. B. In vitro and in situ expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin. *J Immunol* 176, 1908-15 (2006).

25. Cua, D. J. et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 421, 744-8 (2003).

26. Elder, J. T. IL-15 and psoriasis: another genetic link to Th17? *J Invest Dermatol* 127, 2495-7 (2007).

27. Yen, D. et al. IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. *J Clin Invest* 116, 1310-6 (2006).

28. Chowers, Y. et al. Increased proinflammatory cytokine gene expression in the colonic mucosa of coeliac disease patients in the early period after gluten challenge. *Clin Exp Immunol* 107, 141-7 (1997).

29. Thomas, K. E., Sapone, A., Fasano, A. & Vogel, S. N. Gliadin stimulation of murine macrophage inflammatory gene expression and intestinal permeability are MyD88-dependent: role of the innate immune response in Celiac disease. *J Immunol* 176, 2512-21 (2006).

30. Maiuri, L. et al. Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease. *Lancet* 362, 30-7 (2003).

ACKNOWLEDGEMENTS

We would like to thank the University of Maryland Center for Celiac Research for providing patient samples, PTG and synthetic a-gliadin peptides. Funding for this project was provided by an Internal Research Grant from the Department of Pathology, University of Maryland School of Medicine.

AUTHORS' CONTRIBUTIONS

K.M.H designed and conducted the experiments, analyzed the data and drafted the manuscript. D.L.M. assisted K.M.H. with experimental design, data analyses and manuscript writing. A.F. provided patient material, gave input on experimental design and data evaluation, and reviewed the manuscript.

Correspondence and requests for materials should be addressed to D.L.M. (dmann001@umaryland.edu).

Figure 1 Gliadin induces robust production of IL-23 and related proinflammatory cytokines in PBMC from CD patients. **(a)** PBMC from CD patients generate significantly higher amounts of IL-23, IL-1β and TNFα in response to PTG stimulation than HLA-DQ2^{+} healthy individuals. PTG substantially reduces secretion of the anti-inflammatory cytokine IL-1ra in CD patients but not in

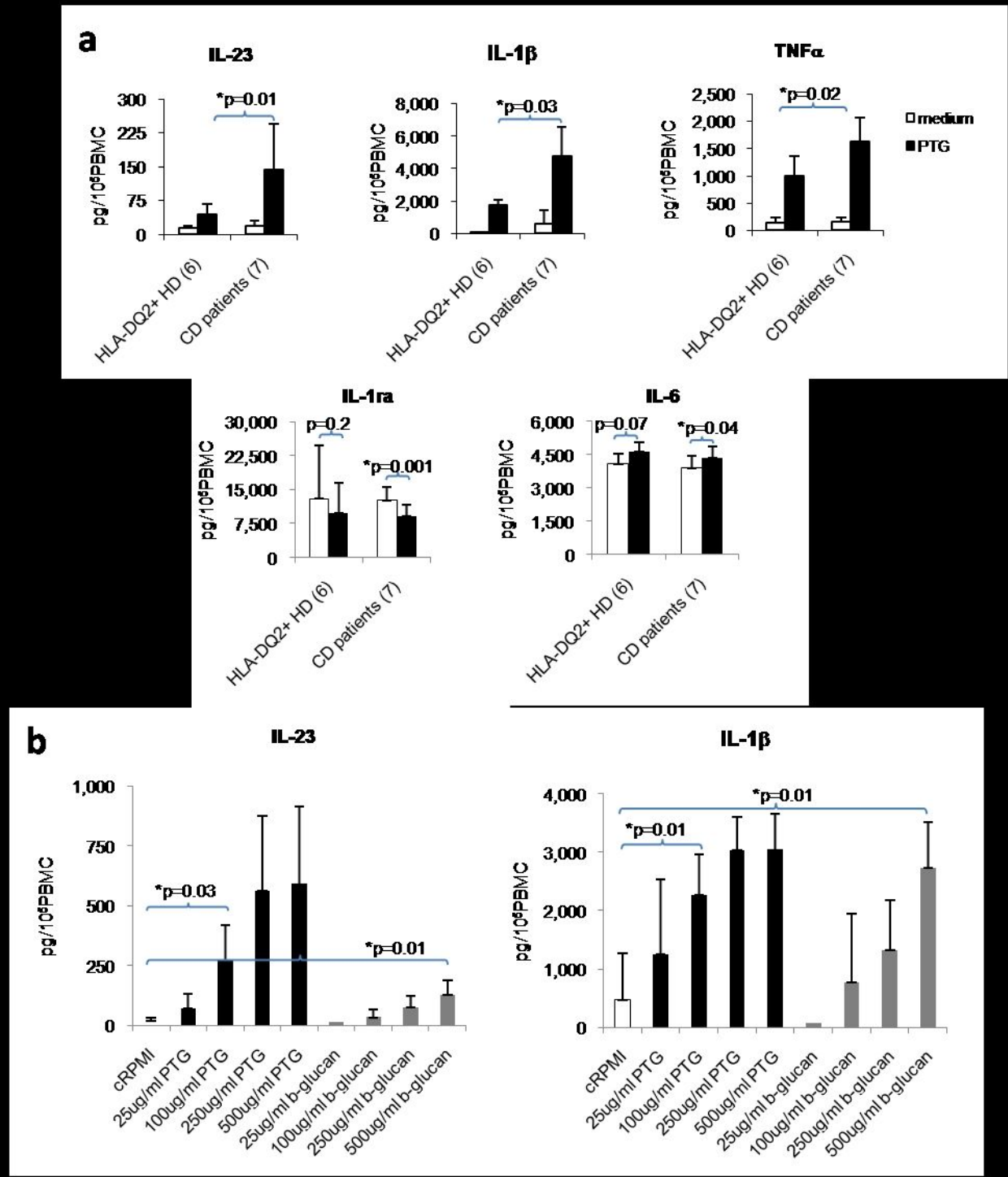
healthy individuals, and only stimulates significant levels of IL-6 in CD patients. PBMC from 7 CD patients and 6 HLA-DQ2^{+} healthy individuals (HD) were incubated with or without PTG (100μg/ml) for 48h, and cell-free culture supernatants analyzed for production of IL-1β, IL-1ra, IL-6, IL-12p70, IL-23 and TNFα. Together, these data illustrate that proinflammatory cytokine responses to PTG are augmented in HLA-DQ2^{+} individuals with CD compared to those without disease. Error bars indicate + s.d. **(b)** PTG stimulation of IL-23 and IL-1β production is dose dependent. PBMC from CD patients were cultured with or without 25, 100, 250 or 500μg/ml PTG for 24h. Increasing doses of β-glucan from barley served as a positive control. Concentrations of IL-23 and IL-1β were quantified by ELISA. Data represents mean values from 3 independent experiments. Error bars indicate + s.d.

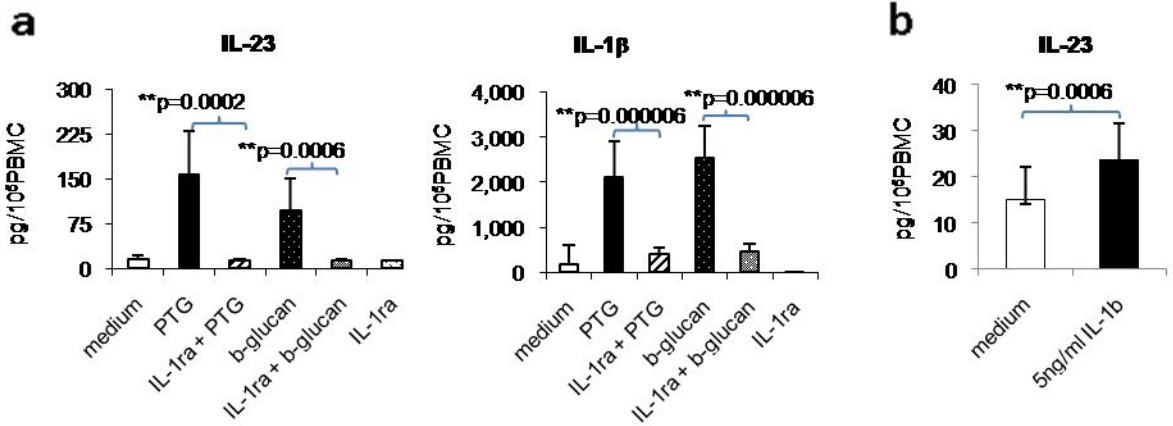
Figure 2 IL-1 cytokines regulate the IL-23 response *in vitro*. **(a)** Addition of IL-1ra significantly inhibits IL-23 and IL-1β responses to PTG and the positive control, β-glucan. PBMC were incubated with or without 0.5μg/ml IL-1ra for 1h prior to stimulation with PTG or β-glucan for 20h. Secretion of IL-23 and IL-1β were determined by ELISA. These data are mean values from 10 independent experiments. Error bars indicate + s.d. **(b)** IL-1β alone stimulates PBMC to produce IL-23, however its capacity to do so is much lower (~10-fold) than that of PTG or β-glucan. PBMC were cultured in the absence or presence of 5ng/ml IL-1β for 20h, and supernatants tested by IL-23 ELISA. These results represent the mean of 10 independent experiments. Error bars indicate + s.d.

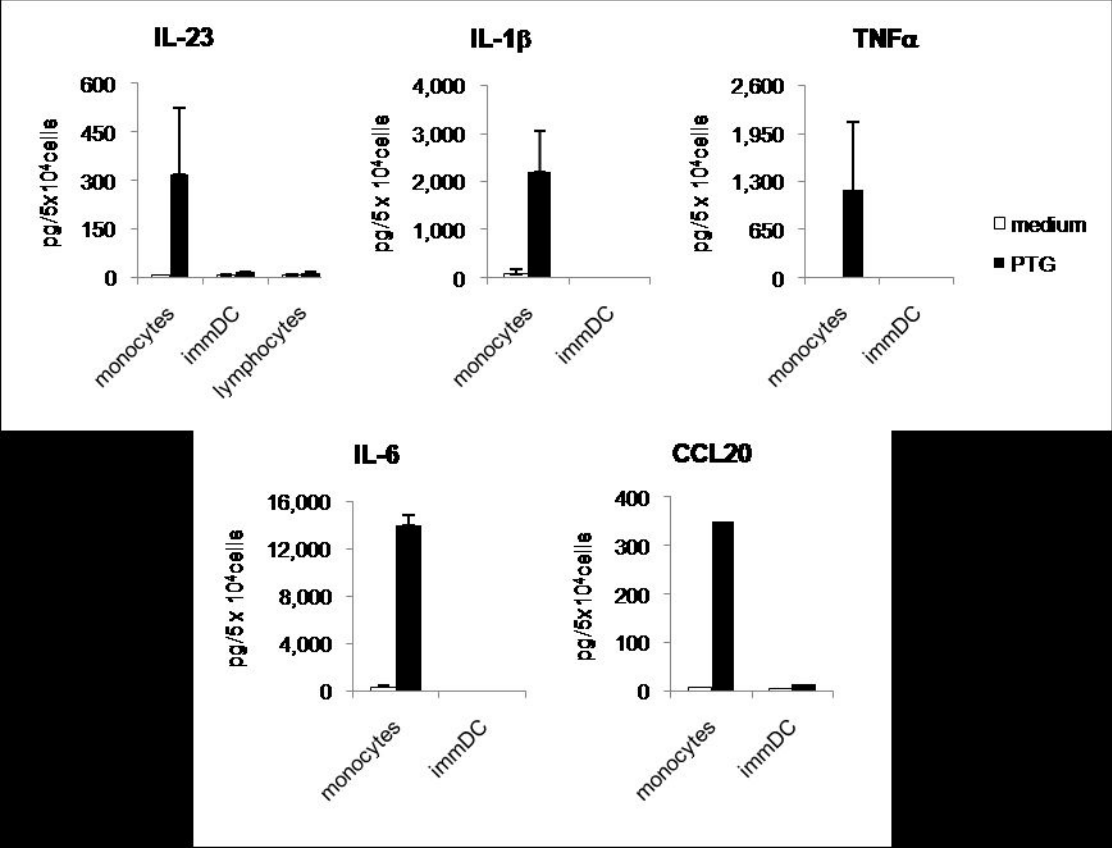
Figure 3 Monocytes are the cell source of IL-23 and related proinflammatory mediators produced in response to *in vitro* gliadin stimulation. Highly purified lymphocytes, monocytes or monocyte-derived immDC were incubated with or without PTG (100μg/ml) for 24h, and supernatants analyzed for production of

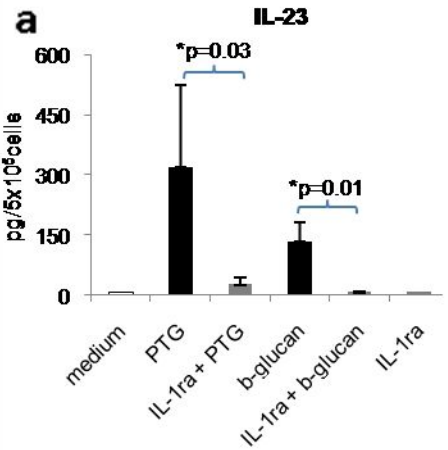
IL-1β, IL-6 IL-23, TNFα and CCL20. PTG directly activates monocytes, and not lymphocytes or immature DC, to secrete IL-23. IL-1β, TNFα, IL-6 and CCL20 responses were also generated by monocytes exposed to PTG, and not their progeny DC. IL-23 data represent the mean of 5 independent experiments. IL-1β, TNFα and IL-6 data represent the means of 3 independent experiments. CCL20 data is one representative of 3 independent experiments. Error bars indicate + s.d.

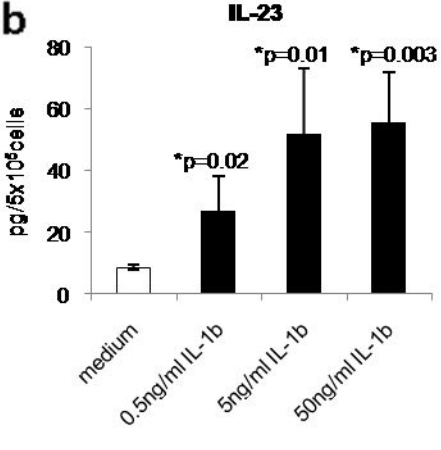
Figure 4 The IL-1 system regulates IL-23 production in human monocytes. **(a)** IL-1ra significantly inhibited IL-23 responses from monocytes exposed to PTG and the positive control, β-glucan. Highly purified monocytes were incubated with or without 0.5μg/ml IL-1ra prior to addition of PTG or β-glucan for 20h. These results represent the means of 5 independent experiments. Error bars indicate + s.d. **(b)** IL-1β alone directly activates monocytes to secrete IL-23 in a dose dependent manner, however its capacity to do so is greatly reduced (~10-fold) compared to that of PTG or β-glucan. Purified monocytes were treated with and without 0.5, 5 or 50ng/ml rhIL-1β for 20h, and culture supernatants were analyzed for IL-23 production. These results represent the means of 5 independent experiments. P values compare IL-1β data sets to medium alone. Error bars indicate + s.d.


a
IL-23
IL-1β
TNFα
pg/10⁵PBMC
*p=0.01
*p=0.03
*p=0.02
medium
PTG
HLA-DQ2+ HD (6)
CD patients (7)
IL-1ra
IL-6
p=0.2
*p=0.001
p=0.07
*p=0.04
b
cRPMI
25ug/ml PTG
100ug/ml PTG
250ug/ml PTG
500ug/ml PTG
25ug/ml b-glucan
100ug/ml b-glucan
250ug/ml b-glucan
500ug/ml b-glucan


a
IL-23
pg/10⁶PBMC
300
225
150
75
0
**p=0.0002
**p=0.0006
medium
PTG
IL-1ra + PTG
b-glucan
IL-1ra + b-glucan
IL-1ra
IL-1β
4,000
3,000
2,000
1,000
0
**p=0.000006
**p=0.000006
b
IL-23
40
30
20
10
0
**p=0.0006
5ng/ml IL-1b


IL-23
pg/5x10⁴cells
600
450
300
150
0
monocytes
immDC
lymphocytes
IL-1β
pg/5 x 10⁴cells
4,000
3,000
2,000
1,000
0
monocytes
immDC
TNFα
pg/5 x 10⁴cells
2,600
1,950
1,300
650
0
monocytes
immDC
medium
PTG
IL-6
pg/5 x 10⁴cells
16,000
12,000
8,000
4,000
0
monocytes
immDC
CCL20
pg/5x10⁴cells
400
300
200
100
0
monocytes
immDC


a
IL-23
*p=0.03
*p=0.01
pg/5x10^5cells
600
450
300
150
0
medium
PTG
IL-1ra + PTG
b-glucan
IL-1ra + b-glucan
IL-1ra


b
IL-23
*p=0.02
*p=0.01
*p=0.003
pg/5x10^5cells
80
60
40
20
0
medium
0.5ng/ml IL-1b
5ng/ml IL-1b
50ng/ml IL-1b