GLIADIN INDUCES CXCR3-DEPENDENT INTERLEUKIN-8 PRODUCTION IN A SUBPOPULATION OF CELIAC PATIENTS

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Background

- We have recently identified the chemokine receptor, CXCR3, as a receptor for gliadin[1]
- In murine intestinal segments, gliadin induces a MyD88-dependent activation of the zonulin pathway and subsequent increase in intestinal permeability[1]
- This response is induced by binding of gliadin to CXCR3; CXCR3$^{-/-}$ mice fail to respond to exposure to gliadin with zonulin release and increased intestinal permeability, even if the zonulin pathway is intact in these mice[1]

- Gliadin induces TNF-α and IL-8 production in monocyte-derived dendritic cells from healthy volunteers[2,3] and in murine macrophages[4]

- CXCR3 is expressed on various immune cells, including different T cell subsets[5,6] and dendritic cells[7]

Aim

- **To study the role of CXCR3 in the immune response by exposure of gliadin to peripheral blood mononuclear cells (PBMC) from healthy donors and celiac disease patients**

Methods

- Independent ethics Review Board approval was obtained for this study and individuals gave their informed consent
- PBMC from 21 celiac patients on a gluten-free diet and 10 non-celiac volunteers were incubated with PT-gliadin (1 mg/mL) for 24 hours
- In the presence or absence of blocking anti-CXCR3 monoclonal antibody or appropriate isotype antibody control (10 μg/mL)
- TNFα, IL-8, IL-6, IL-10, and IFNγ were measured in culture supernatants by Luminex

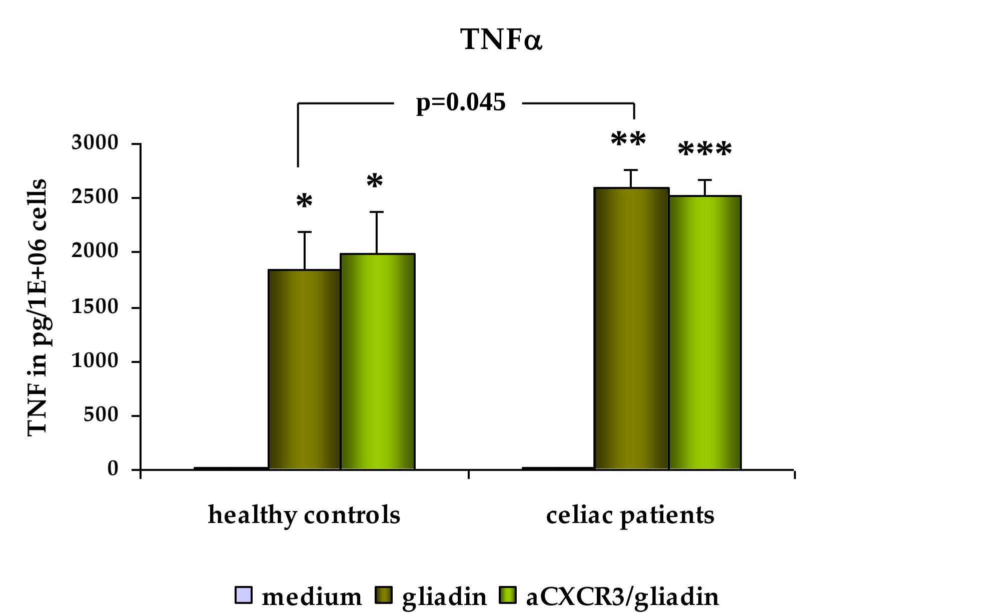
Results

- All cytokines were produced at higher level in celiac patients than in healthy controls

TNFα, IFNγ, IL-6 and IL-10 (figures 1-4)

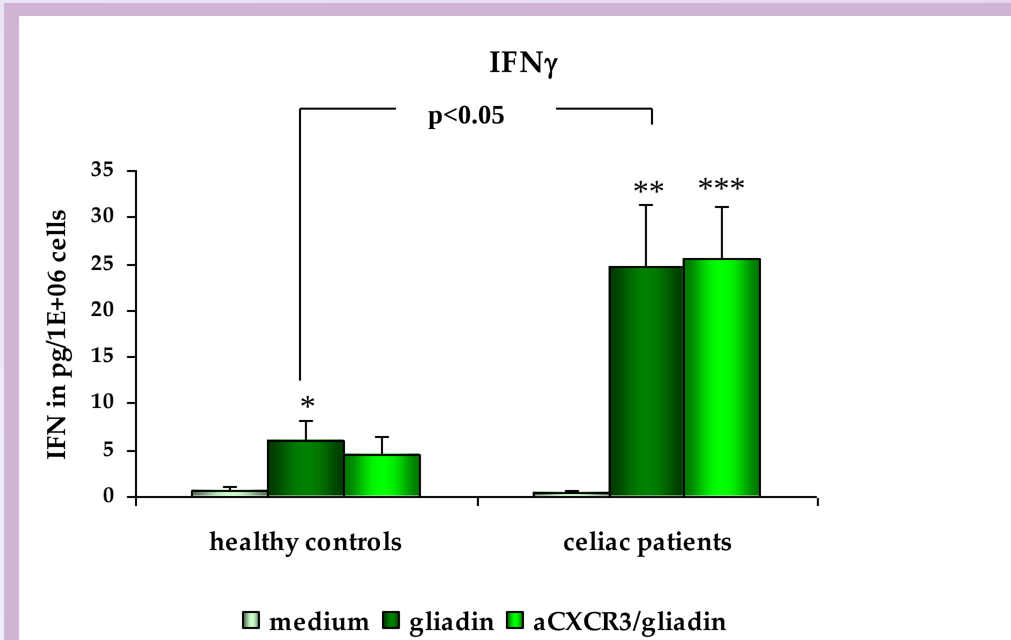
- TNFα, IL-6, IL-10, and IFNγ were produced by PBMC of all individuals after gliadin stimulation
- Production of TNFα and IFNγ was not influenced by blocking the CXCR3 receptor;
- Production of IL-6 and IL-10 was partially decreased and increased, respectively, by blocking of CXCR3

Figure 1



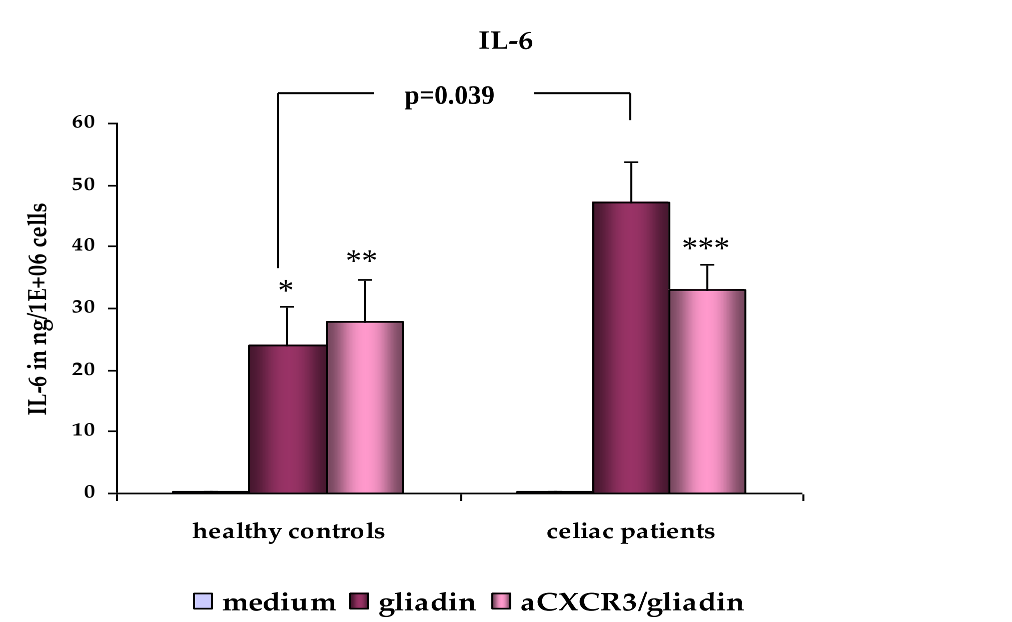
*   p<0.01 compared to medium
** p<1.3xE-12 compared to medium
***p<2xE-13 compared to medium

Figure 2



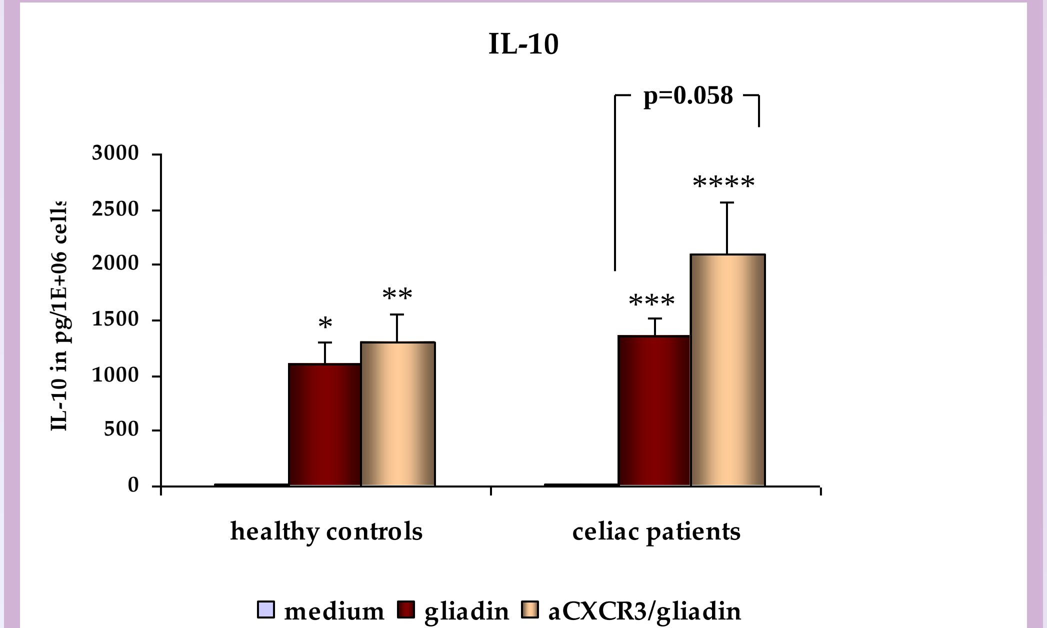
*   p=0.01 compared to medium
** p<0.001 compared to medium
***p<0.001 compared to medium

Figure 3



*   p=0.004 compared to medium
** p=0.003 compared to medium
***p=0.015 compared to gliadin alone

Figure 4



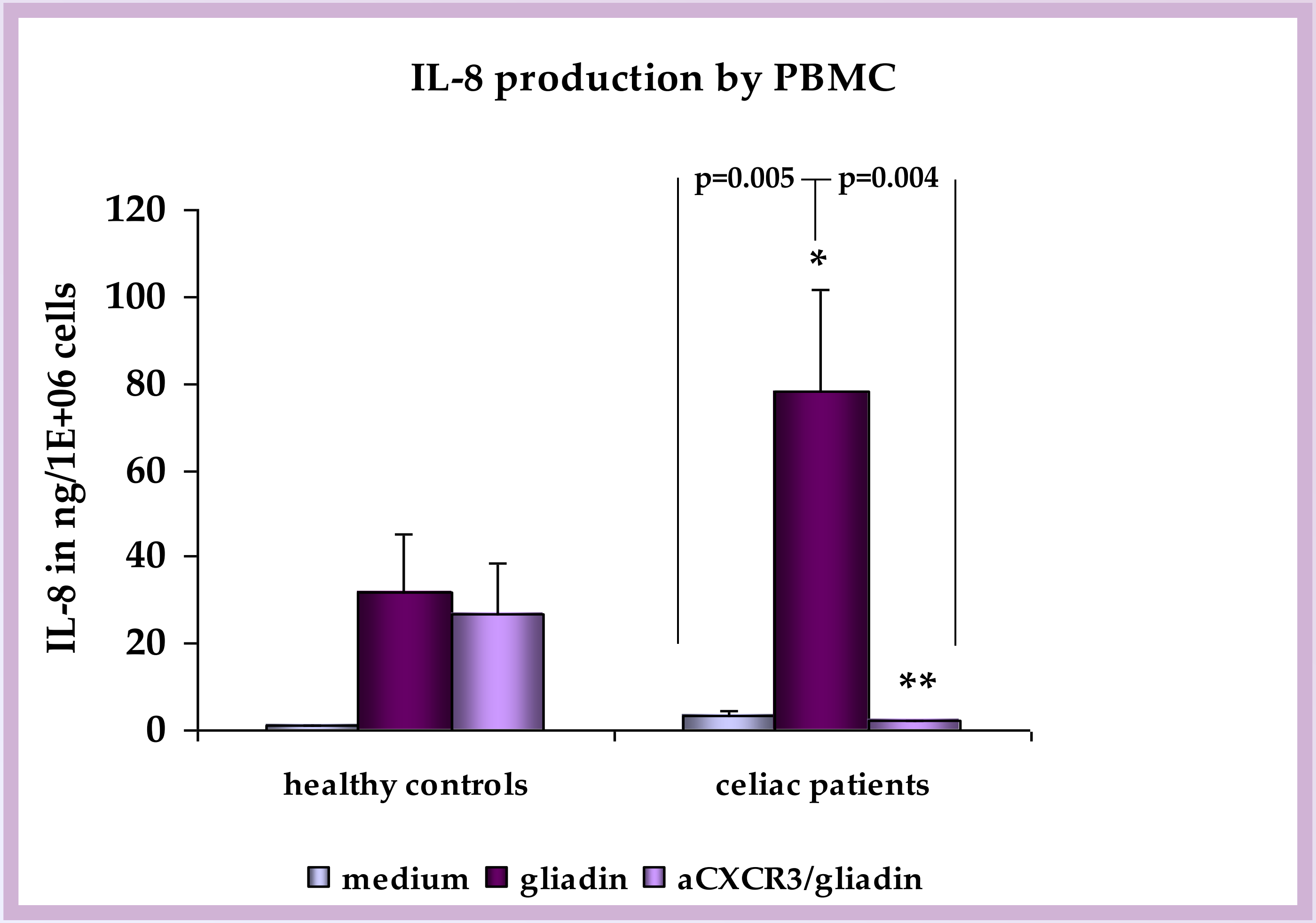
*   p<0.001 compared to medium
**   p<0.001 compared to medium
*** p=8.6E-09 compared to medium
****p<0.001 compared to medium

IL-8 (figure 5)

- Gliadin induced IL-8 production only in a subgroup of individuals; 30% of the healthy controls and 42.8% of the celiac patients was IL-8 responder

- In celiac patients, the IL-8 response was completely abrogated when CXCR3 was blocked prior to gliadin stimulation in the celiac group but ***not*** in the control group

Figure 5



IL-8 production by PBMC
IL-8 in ng/1E+06 cells
120
100
80
60
40
20
0
p=0.005
p=0.004
*
**
healthy controls
celiac patients
medium
gliadin
aCXCR3/gliadin

Summary

Cytokine production

- Gliadin induces cytokine production in both healthy individuals and celiac patients

- Cytokine levels are higher in celiac patients

- Gliadin-induced production of TNFα, IFNγ, IL-6 and IL-10 occurs in all individuals, but production of IL-8 is restricted to a subgroup of individuals

Role for CXCR3

In healthy individuals;

- cytokine production is *not* CXCR3-mediated

In celiac patients;

- IL-8 production appeared to be CXCR3-dependent, since blocking of this receptor inhibited completely IL-8 production
- Partial decrease of IL-6 and a partial increase of IL-10 production was observed after blocking of CXCR3

Discussion

- These results show that gliadin provokes strong immune responses in all individuals regardless the existence of celiac disease

- These results suggest the importance of different regulatory mechanisms at intestinal level in order to prevent gliadin-induced tissue damage;

  I. an **intact intestinal barrier function** that forms the first defense and prevents the passage of gliadin from lumen into mucosa

II. An **immune system** that has strong and intact **regulatory potential** and protects the body from invading antigens by preventing a skew of the immune response in the direction of autoimmune pathogenesis, in particular in individuals with a genetic profile that predisposes to autoimmunity

References

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