NEUTROPHILS FROM HEALTHY INDIVIDUALS BUT NOT CELIAC DISEASE PATIENTS SHOW CHEMOTACTIC ACTIVITY TO PT-GLIADIN

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Introduction

- Celiac disease (CD) is caused by the ingestion of gliadin, the immunogenic component of gluten-containing grains

- We have observed that exposure to gliadin induces rapid and massive influx of neutrophils to the murine gut mucosa

Neutrophils

- As part of the innate immune response, neutrophils are recruited early to and activated at sites of injury

Aim

- To study whether gliadin has chemo-attractant properties for human neutrophils

- To study whether neutrophils from health controls (HC) and CD patients respond differently to gliadin

Methods

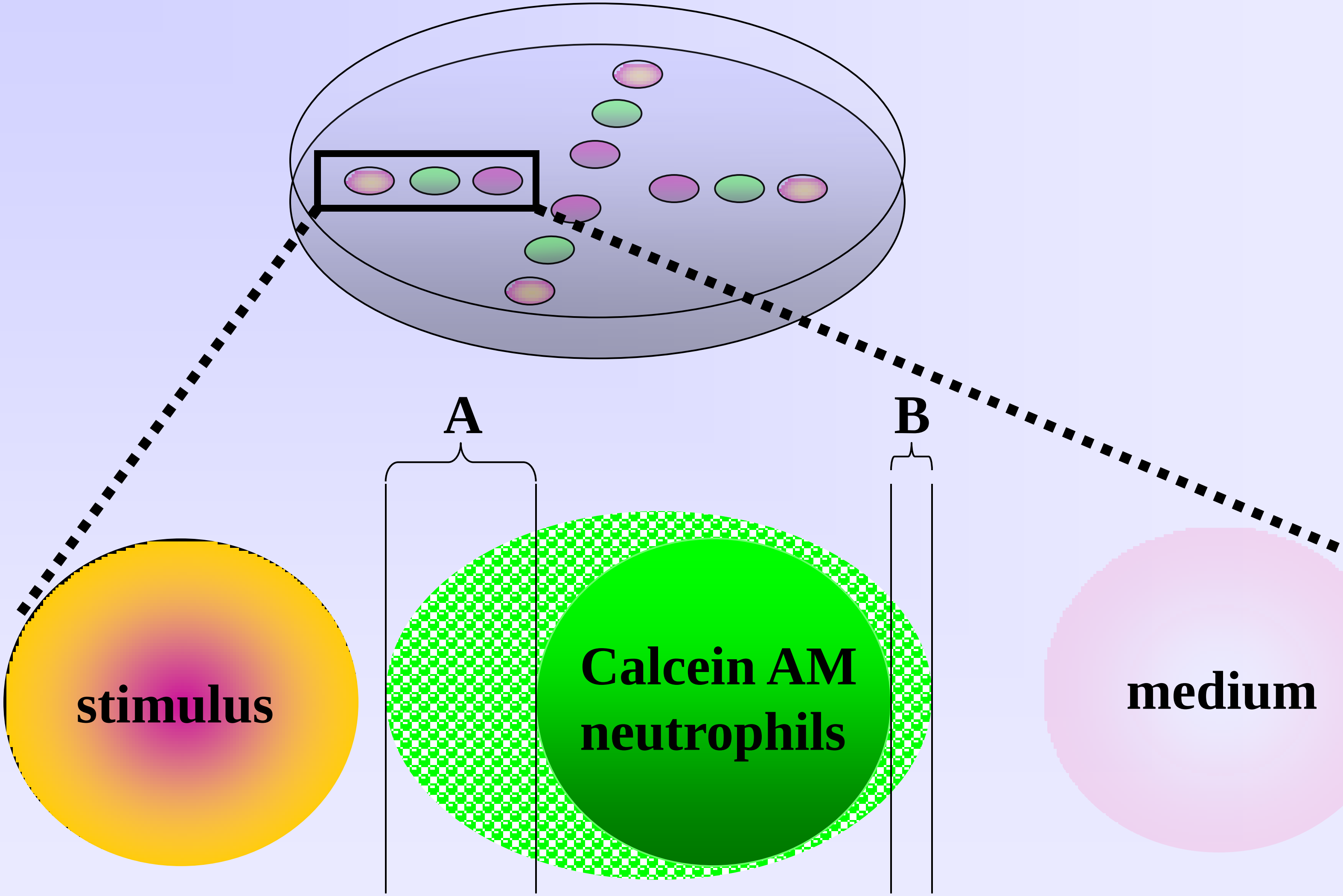
Isolation of Neutrophils

- Heparinized venous blood of HC and CD patients was centrifuged over Ficoll-density gradient; the erythrocyte/granulocyte fraction was collected
- Erythrocytes were lysed with ice cold lysis buffer, suspension was spun down and neutrophils were pelleted, resuspended in HBSS and counted

Under agarose assay

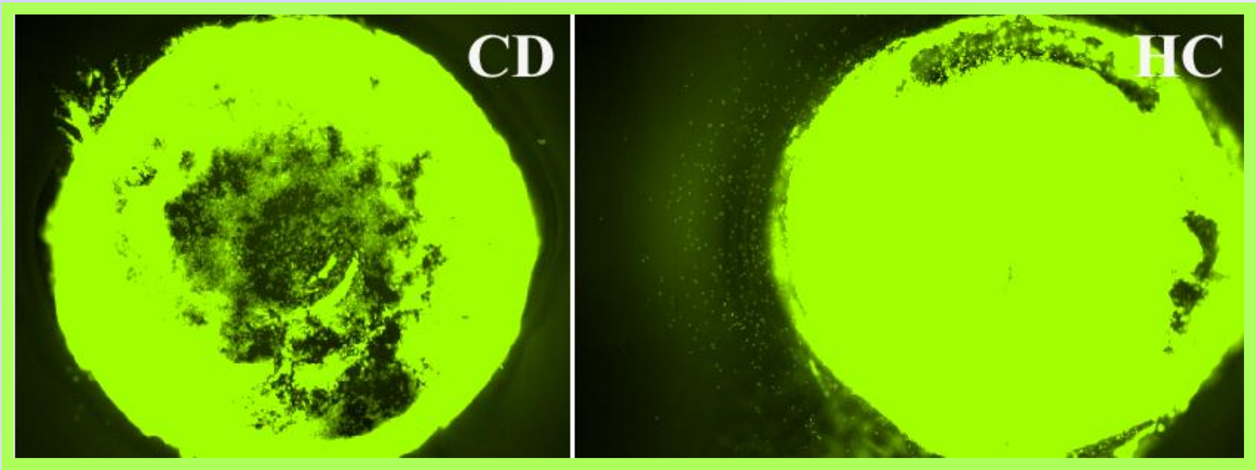
- Neutrophils isolated from HC and CD were labeled with calcein AM and applied to an under-agarose migration assay (***Figure 1***)
- Dishes were incubated with PT-gliadin (20 HC, 16 CD) or N-formyl-methionyl-leucyl-phenylalanine (fMLP) (14 HC, 12 CD) for 2 h at 37°C
- Immediately after incubation, dishes were analyzed using fluorescent microscopy (***Figure 2***)

Figure 1

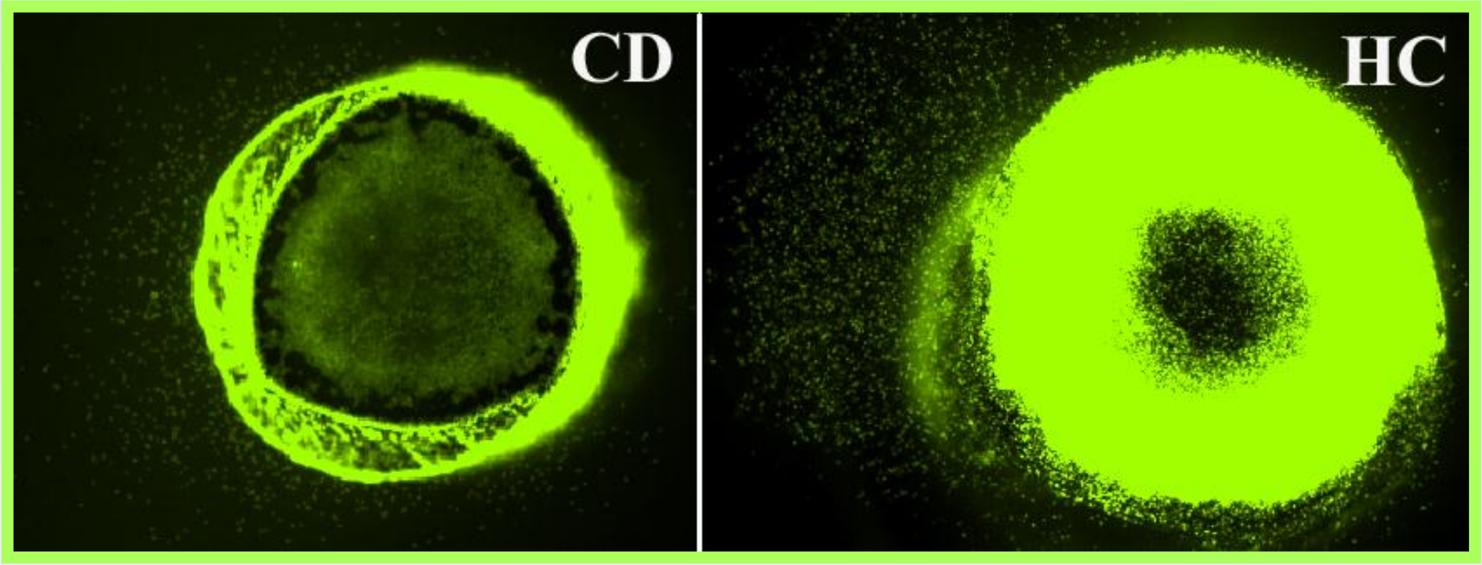


Net neutrophil migration = A-B

Figure 2



PT-gliadin



fMLP

Taxi-scan assay

- Neutrophils from HC were applied to the Taxi-scan assay, an *in vitro* model that allows real-time monitoring of chemotaxis to PT-gliadin or fMLP as a positive control (***Figure 3***)

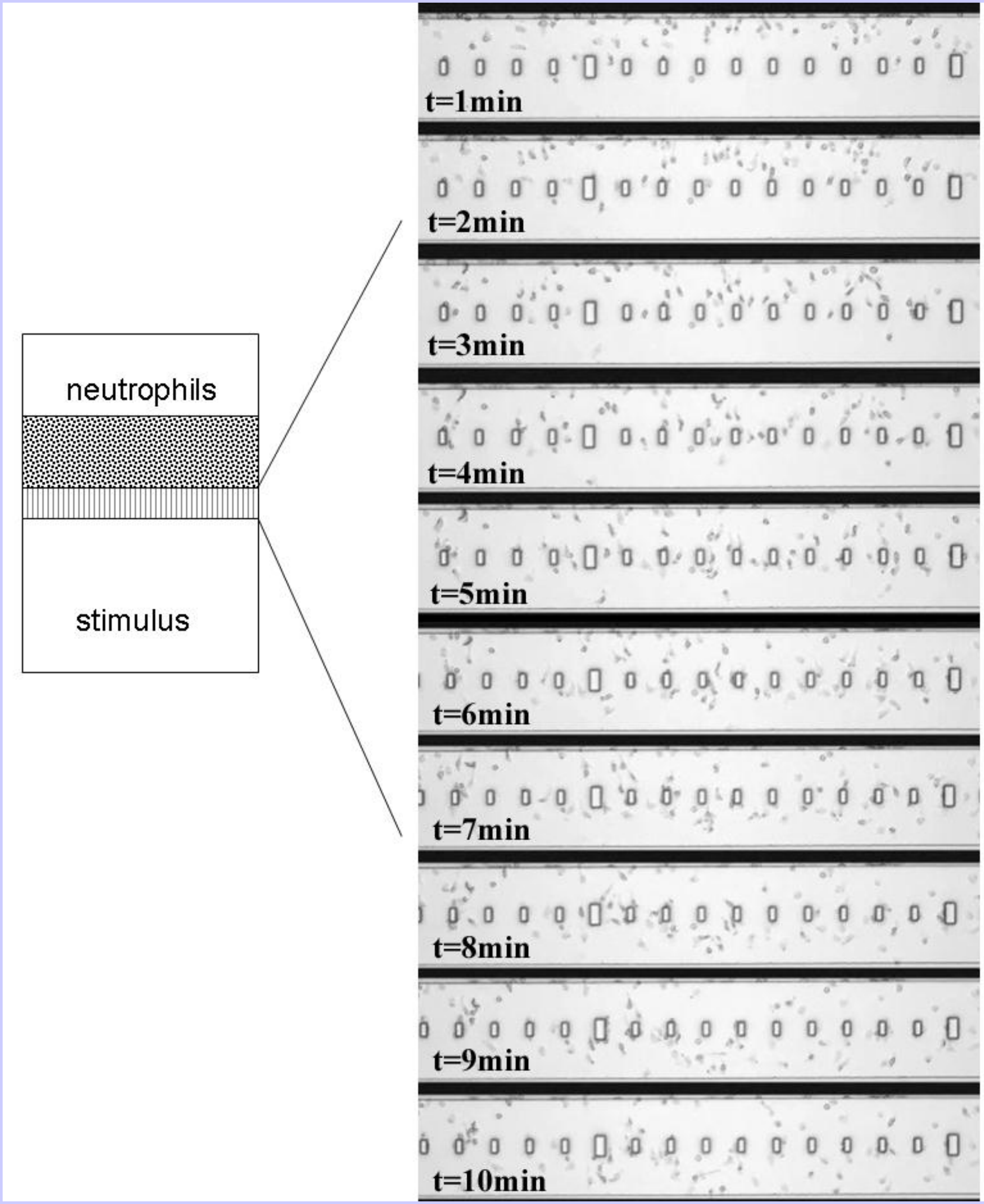

neutrophils
stimulus
t=1min
t=2min
t=3min
t=4min
t=5min
t=6min
t=7min
t=8min
t=9min
t=10min

Figure 3

Results

Taxi-scan assay showed that PT-gliadin has chemoattractant property for human neutrophils (***Figure 3***)

Under agarose assay

PT-gliadin

- HC neutrophils showed chemotactic response to PT-gliadin (5.772 ± 1.338 net neutrophil migration)

- CD neutrophil migration was markedly reduced compared to HC (0.403 ± 0.259 net neutrophil migration, *P*=0.0005) (***Figure 4***)

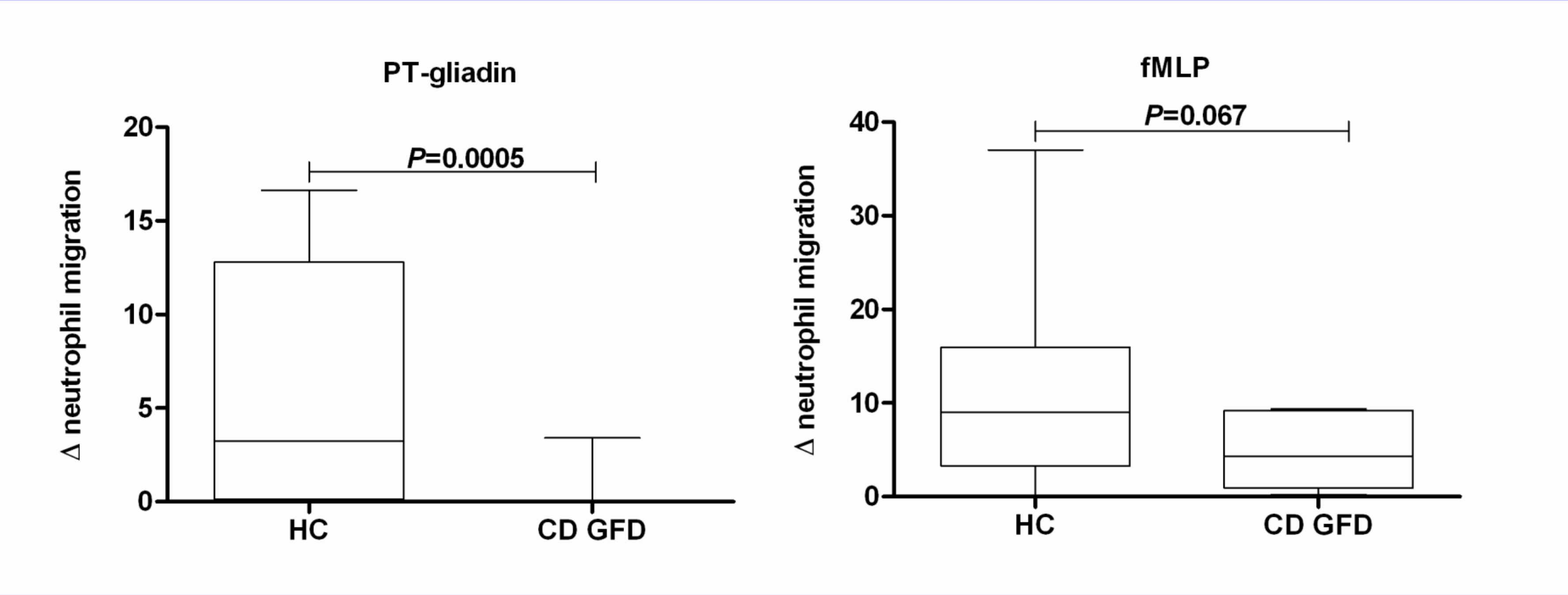


Figure 4

fMLP

- A similar, albeit non-significant difference was also observed in the migration to fMLP
- HC neutrophils showed strong response to fMLP (11.93 ± 2.886 net neutrophil migration)
- CD neutrophils had impaired response to fMLP albeit that this difference did not reach significancy (4.790 ± 1.080 net neutrophil migration, P=0.067) (***Figure 4***)

Conclusions

- We show that PT-gliadin has chemoattractant properties for human neutrophils
- We also show that CD neutrophils show a trend toward reduced responsiveness to fMLP when compared to HC neutrophils

- We show that the chemotactic response of CD neutrophils to PT-gliadin is strongly and significantly impaired when compared to HC neutrophils

Discussion

- We hypothesize that suboptimal, innate immune response results in a delayed arrival of neutrophils at the site of gluten-promoted inflammation and that this may critically contribute to the autoimmune pathogenesis of CD