

Identification of target antigens of antibodies in inflammatory bowel disease

Protein microarray experiments identify Phla1 as a target antigen

Nathalie Vermeulen, Severine Vermeire, Paul Rutgeerts, Xavier Bossuyt

Departments of Gastroenterology and laboratory Medicine, University Hospital Gasthuisberg (Leuven, Belgium)



Background

Several antibodies exist in patients with IBD. These antibodies are directed against microbial antigens [e.g. antibodies against yeast (ASCA)] or against self antigens [e.g. perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) and anti-pancreas antibodies (PAB)]. The target antigens of pANCA and PAB are still unknown, which limit their potential for diagnosis and prognosis in IBD. Potentially, antibodies in IBD could be an important non-invasive diagnostic tool.

Hypothesis

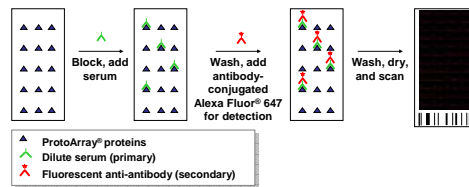
The development of sensitive and specific assays would clarify the utility of serological markers in IBD. However, this needs the identification of the target antigens of antibodies in IBD.

Aim

To identify novel target antigens in IBD, using the Protein Microarray.

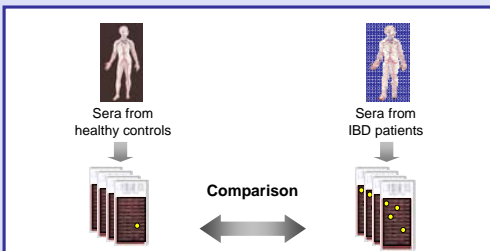
Experimental design : Protein Microarray

- Innovative technology for biomarker discovery
- ProtoArray@Human Protein Microarray v4.0 (Invitrogen)
- ~8,000 human proteins arrayed on a microscope slide
- All clones fully sequenced
- Expressed in Sf9 insect cells



Study cohort (n=30)

- 10 ulcerative colitis patients (UC); Age: 45,3 (29-72); F/M: 4/6
 - ◆ Strong pANCA positive ($\geq 1/80$)
 - ◆ Left-sided or pancolitis
- 15 Crohn's disease patients (CD); Age: 43,4 (26-66); F/M: 9/6
 - ◆ 10 ASCA-positive
 - ◆ 5 PAB-positive
 - ◆ Ileum and colon involvement
- 5 Healthy controls (HC); Age: 33,8 (29-40); F/M: 3/2



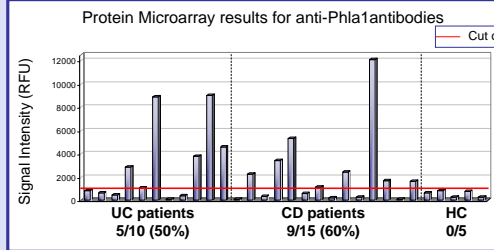
Results

Comparative analysis of the antibody profiles in IBD patients to the profiles in healthy controls :

- 75 target antigens in IBD patients
- 88 target antigens in controls

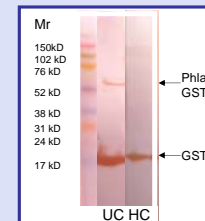
First target antigen for validation:

Phla1 (Pleckstrin homology-like domain family A member 1)

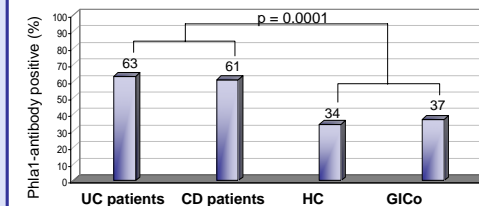


Validation by Western blotting in second cohort (n=40)

- 100 UC patients
- 100 CD patients
- 100 Healthy controls
- 100 non-IBD gastrointestinal controls (GIC)



Western blotting results for anti-Phla1 antibodies



Conclusions

- Protein microarray is a novel technology to identify antibodies
- Several antibodies exist in patients with IBD
- UC patients and CD patients show a different antibody profile
- The detection of anti-Phla1 antibodies by Western blotting validates the microarray results and confirms Phla1 as an autoantigen in IBD

Future Work

- Further study anti-Phla1 antibodies in IBD
- Explore more proteins

Contact Information

Nathalie.vermeulen@uz.kuleuven.be

Xavier.Bossuyt@uz.kuleuven.be

Phone : +32 16 34 79 25