Clinical Immunology (2013) 148, 99–109

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Profiling celiac disease antibody repertoire ☆

Sara D'Angelo [a,g], Flavio Mignone [b], Cecilia Deantonio [a], Roberto Di Niro [c], Roberta Bordoni [d], Roberto Marzari [e], Gianluca De Bellis [d], Tarcisio Not [f], Fortunato Ferrara [g], Andrew Bradbury [g], Claudio Santoro [a,*], Daniele Sblattero [a,*]

[a] Department of Health Sciences and Interdisciplinary Research Center on Autoimmune Diseases (IRCAD), University of Eastern Piedmont "Amedeo Avogadro", Novara, Italy
[b] Dipartimento di Scienze e Innovazione Tecnologica, University of Eastern Piedmont "Amedeo Avogadro", Alessandria, Italy
[c] Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06510, USA
[d] Institute of Biomedical Technologies, National Research Council (ITB CNR), Milan, Italy
[e] Department of Life Sciences, University of Trieste, Trieste, Italy
[f] Department of Medical Sciences University of Trieste, Institute of Child Health IRCCS "Burlo Garofolo", Trieste, Italy
[g] Los Alamos National Laboratory, Los Alamos, NM, USA

Received 22 January 2013; accepted with revision 10 April 2013
Available online 22 April 2013

KEYWORDS
Celiac disease;
Autoantibody;
Autoantigen;
Protein microarray;
ORF-display libraries;
Next generation sequencing

Abstract The aim of this study was to dissect the autoantibody response in celiac disease (CD) that remains largely unknown, with the goal of identifying the disease-specific autoantigenic protein pattern or the so called epitome. Sera from CD patients were used to select immunoreactive antigens from a cDNA phage-display library. Candidate genes were identified, the corresponding proteins produced and their immunoreactivity validated with sera from CD patients and controls. Thirteen CD-specific antigens were identified and further validated by protein microarray. The specificity for 6 of these antigens was confirmed by ELISA. Furthermore we showed that this antibody response was not abolished on a gluten free diet and was not shared with other autoimmune diseases. These antigens appear to be CD specific and independent of gluten induction. The utility of this panel extends beyond its diagnostic value and it may drive the attention to new targets for unbiased screens in autoimmunity research.

1. Introduction

The circulating antibody repertoire represents an important source of diagnostic information, serving as a biomarker of the immune state of the body [1]. The identification of disease signatures for known and unknown etiology can be carried out in an unbiased way by performing serum antibody-based

☆ *Grant support*: This work was supported by: Fondazione Cariplo Bando Ricerca Biomedica 2009; EC Marie Curie Research Training Network [contract no. MRTN-CT-20010-289964]; Compagnia San Paolo; MIUR FIRB NG-Lab RBLA03ER38; IRCCS Burlo Garofolo RF 35/07; Regione Piemonte Ricerca Sanitaria and Piattaforma Immonc.
* Corresponding authors at: Università del Piemonte Orientale, Dip. Scienze della Salute, Via Solaroli 17, 28100 Novara, Italy.
E-mail addresses: csantoro@med.unipmn.it (C. Santoro), sblatter@med.unipmn.it (D. Sblattero).

1521-6616/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.clim.2013.04.009

profiling. The application of this unbiased discovery-driven approach has led to the identification of a large number of novel auto-immune antigens that have been identified using the sera of patients with cancer [2,3] and auto-immune diseases [4–6]. The complex network of the antibody–antigen immune response has recently been investigated by several innovative approaches including bacterial [7], lambda [8,9], T7 [10], and filamentous phage display systems [11]. In particular, phage display provided simple and fast procedures for the identification of novel antigens, allowing the screening of libraries created from appropriate sources directly with immune sera. Furthermore, the implementation of next generation sequencing technologies (NGS) at the screening level resulted in a more comprehensive and faster analysis of the interactions under study [12,13]. By combining the antibody repertoire with cDNA display technologies, thousands of antigen–antibody interactions are simultaneously screened and give, altogether, an extensive analysis of the "immunome" [14]. We developed a robust approach to identify novel autoantigens and to describe the profile of the corresponding autoantibody reactivity. Among autoimmune pathologies, celiac disease (CD) is of particular interest due to its high prevalence (1%) in the general population [15]. CD is an enteropathy developing as a consequence of dietary exposure to wheat gluten and related proteins in barley and rye [16], characterized by the production of antibodies to the autoantigen tissue transglutaminase (TG2) [17]. This depends upon dietary exposure to gluten [18,19] and is found only in genetically predisposed individuals. Although TG2 is the main autoantigen of the disease, with increasing diagnostic importance [20], several other autoantigen targets may exist. The disease association with other autoimmune diseases is well known [21], as well as the fact that these autoimmune disorders developed in unrecognized and/or untreated celiac subjects due to prolonged exposure to gluten [22]. The need for an early and correct diagnosis to prevent morbidity and mortality associated with untreated CD is a growing need. Hence, the identification of other Ab specificities is gaining importance. To date, reactivity to only a limited number of CD specific autoantigens has been clearly defined like actin [23], calreticulin [24], enolase alpha [25], and few others (reviewed in [26,27]). Furthermore only few systematic efforts have been attempted to describe the whole autoantigen repertoire (*i.e.* celiac disease epitome) [7,28]. Here, we used CD sera to select autoantigenic proteins from a large, cDNA library [12] applying an innovative discovery platform where we integrate genomic (NGS) and proteomic (protein microarray) technologies to perform high-throughput large-scale screening for potential autoantigens. We identified a novel panel of 13 CD-associated autoantigens, with a high antibody specificity in patients, that may represent a useful descriptive tool for the understanding of the immunological and pathogenic processes involved in the onset of CD and autoimmunity in general.

2. Materials and methods

2.1. Sera samples

Sera were obtained from IRCCS Burlo Garofolo, Italy; informed consent was obtained from all donors. Celiac patients underwent a complete small intestine mucosal biopsy and test for antitissue transglutaminase antibodies. Healthy controls tested negative for anti-TG2 and were asymptomatic. Sample details are summarized in Table 1. Briefly, the sera used for antigen selections were obtained from untreated CD patients, all confirmed by histological analysis of the intestinal mucosa biopsy. The microarray screening was performed with 46 sera from CD patients and 45 sera from healthy donors. The ELISA screening was performed with 3 different sets of patients' sera: the first comprised 46 CD patients, the second (termed pre-GFD–gluten free diet) consisted of 31 CD patients, and the third (termed post-GFD) consisted of the same set of 31 pre-GFD patients, whose sera were collected after 3 to 9 months of GFD; 44 healthy sera were used as controls. The ELISA was also performed with sera derived from patients affected by other autoimmune diseases: 28 type 1 diabetes (T1D) and 30 multiple sclerosis (MS) patients. All sera were tested for both IgG and IgA TG2 reactivity (Eurospital Eu-tTg kit).

Table 1 Sera used for autoantigen discovery platform. Set of sera for phage selection: pools H and J were used for library selections, pool C for the negative selection. Discovery set of sera for microarray: CD patient and healthy control sera used to screen antigen microarrays. Validation set of sera for ELISA: CD and healthy control sera for antigen validation after microarray screening (F, female; M, male).

	No.	Sex	Average age
Selection set for phage selection			
CD pool H	5	M 3 F 2	11.8 (8–21)
CD pool J	5	M 2 F 3	13.8 (5–28)
Healthy pool C	5	M 3 F 2	14.3 (5–28)
Discovery set for microarray test			
CD	46	M 17 F 29	11.2 (1–37)
Healthy	45	M 25 F 20	23.7 (17–50)
Validation set for ELISA test			
CD	46	M 10 F 36	7.5 (2–27)
Pre-GFD	31	M 12 F 19	5.8 (1–14)
Post-GFD	31	M 12 F 19	6.5 (2–15)
Healthy	44	M 20 F 24	9.2 (7–10)
Autoimmune set for ELISA test			
T1D	28	M 18 F 10	8.6 (3–14)
MS	30	M 16 F 14	43.4 (32–62)
Healthy	30	M 18 F 12	13.3 (5–28)