Clinical Immunology 168 (2016) 64–71

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

A pre-eclampsia-associated Epstein-Barr virus antibody cross-reacts with placental GPR50

Serra E. Elliott [a,1], Nicholas F. Parchim [b], Rodney E. Kellems [b], Yang Xia [b], Alex R. Soffici [c], Patrick S. Daugherty [a,*]

[a] Department of Chemical Engineering, University of California, Santa Barbara, CA 93106, USA
[b] Departments of Biochemistry and Molecular Biology, University of Texas Medical School at Houston, Houston, TX 77030, USA
[c] Maternal-Fetal Medicine, Cottage Health System, Santa Barbara, CA 93105, USA

ARTICLE INFO

Article history:
Received 15 September 2015
Received in revised form 24 March 2016
accepted with revision 5 May 2016
Available online 12 May 2016

Keywords:
Antibody
Antigen discovery
Peptide
Epitope mapping
Pre-eclampsia
Bacterial display

ABSTRACT

To characterize antibody specificities associated with pre-eclampsia (PE), bacterial displayed peptide library screening and evolution was applied to identify peptide epitopes recognized by plasma antibodies present in women with PE near the time of delivery. Pre-eclamptic women exhibited elevated IgG1 titers towards a peptide epitope KRPSCIGCK within the Epstein-Barr virus nuclear antigen 1 (EBNA-1). EBNA-1 epitope antibodies cross-reacted with a similar epitope within the extracellular N-terminus of the human G protein-coupled receptor, GPR50, expressed in human placental tissue and immortalized placental trophoblast cells. We observed increased antibody binding activity to epitopes from EBNA-1 and GPR50 among women with PE ($n = 42$) compared to healthy-outcome pregnancies ($n = 43$) and nulligravid samples ($n = 21$). The EBNA-1 peptide potently blocked binding of the PE-associated antibody to the GPR50 epitope (IC50 = 58–81 pM). These results reveal the existence of molecular mimicry between EBNA-1 and placental GPR50, supporting a mechanism for IgG1 deposition in the pre-eclamptic placenta.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Autoimmune diseases are thought to involve environmental factors that trigger disease onset, propagate disease, or influence disease severity [1]. However, the definitive identification of specific environmental factors has remained elusive in most cases. Nevertheless, the identification of specific environmental factors that drive pathological immune responses would greatly aid in the development of improved molecular diagnostics and efficacious therapeutic interventions.

Since the immune system archives antigen exposures within the receptor repertoire of B cells, the identification of disease-associated antibodies and their corresponding antigen preferences has been pursued as a means to identify autoantigens and environmental factors. Antigen discovery for antibodies has been pursued using protein microarrays [2,3], fragment libraries from proteins of interest [4,5,6], and cDNA libraries from tissues or cells involved in pathology [7,8]. These methods have enabled identification of both validated antigens [3–4,6] and novel candidate autoantigens [2,3,5,7,8]. However, because such methods rely upon assaying antibody repertoires against predefined, and often incomplete, proteomes they have not enabled an impartial identification of environmental antigens. Next-generation sequencing (NGS) of antibody heavy and light chain encoding genes has proven to be a powerful approach to characterize B-cell repertoires after vaccination [9] and in several diseases including multiple sclerosis [10] and rheumatoid arthritis [11]. Importantly, NGS approaches do not provide a solution to the problem of environmental antigen discovery. Consequently, there remains a distinct need for methods that can identify the environmental antigens giving rise to disease-associated antibodies.

Screening random peptide libraries offers the potential to address this challenge of identifying the targets of disease-associated antibodies and characterize antibody repertoires. The use of random peptide library screening does not require a priori knowledge of disease mechanisms and has been applied to a variety of diseases. In particular, phage display libraries have been applied to inflammatory diseases [12], cancers [13], and autoimmune diseases [14,15,16]. Even so, random peptide libraries have not unambiguously revealed environmental antigens without prior knowledge of the target. The inability to identify specific environmental antigens may be in part due to methodological problems including use of few individual patient specimens [13,16], a single pool dominated by one outlier patient or a single patient-derived antibody for discovery [12,14,15], or insufficient numbers of unique peptides to sample the sequence space of an epitope [14–16]. We previously reported a method for antigen and diagnostic discovery

* Corresponding author at: Engineering II, Rm 3357, University of California, Santa Barbara, Santa Barbara, CA 93106-5080., USA.
E-mail addresses: serraelliott@engr.ucsb.edu (S.E. Elliott), nicholas.f.parchim@uth.tmc.edu (N.F. Parchim), rodney.e.kellems@uth.tmc.edu (R.E. Kellems), yang.xia@uth.tmc.edu (Y. Xia), alex@greatbaby.com (A.R. Soffici), psd@engr.ucsb.edu (P.S. Daugherty).
[1] Present address: Division of Immunology and Rheumatology, Stanford University, Stanford, CA 94305, USA.

http://dx.doi.org/10.1016/j.clim.2016.05.002
1521-6616/© 2016 Elsevier Inc. All rights reserved.

termed antibody diagnostics via evolution of peptides (ADEPt). ADEPt was validated by demonstrating the capability to unambiguously identify the environmental antigen (gliadin peptides in wheat, barley, and rye) associated with celiac disease, without reliance upon prior knowledge to bias discovery [17]. Here, we sought to determine whether ADEPt could enable the identification of environmental antigens for diseases where the antigens are unknown.

Pre-eclampsia (PE), which affects 5–8% of pregnancies [18], was selected for ADEPt application since several different lines of evidence suggest an immune component to etiology. Most prominently, PE patients produce IgG that act as agonists of the angiotensin II type 1 receptor (AT1-AAs) [19] as early as 18 weeks' gestation [20]. Notably, AT1-AAs have been shown to induce hypertension and proteinuria [21] and promote complement protein C3 deposition in the placenta and kidney [22] in a mouse model of PE. Similarly, AT1-AAs were identified in a transgenic PE rat model in which hypertension, proteinuria, and complement deposition also occur [23]. Furthermore, women with PE exhibit elevated levels of the pro-inflammatory cytokines IL-6 and IL-8 compared to pregnant and non-pregnant control patients [24] and significantly reduced levels of circulating [25] and placental [26] $CD4^+ CD25^+ FoxP3^+$ regulatory T cells, a finding that can promote autoantibody production [27]. Moreover, circulating $CD19^+ CD5^+$ B cells, a class associated with the production of autoreactive antibodies [28], are elevated in PE patients [29], and produce AT1-AAs when cultured in the presence of serum from women with PE. Taken together, these prior studies support an important role for the immune system in the pathogenesis of PE. Patients with PE were recently reported to possess additional disease-associated antibodies distinct from reported AT1-AAs present in 70% [30] to 95% [31] of PE cases. However, these antibodies could not be conclusively related to particular antigens [32]. Here, we sought to identify antigens recognized by PE-associated antibodies, and thereby elucidate the immune mechanisms of pathogenesis. Our results point to a molecular mimicry mechanism operating in women with PE, wherein a viral antigen specific IgG1 antibody cross-reacts with a placental antigen. More generally, our study further supports the idea that the present method may be useful to identify environmental factors involved in human disease in an unbiased fashion.

2. Materials and methods

2.1. Patient samples

Blood samples were obtained from pregnant women with PE or healthy-outcome pregnancy (HOP) as aliquots of samples taken for routine blood work during clinical assessments at the Santa Barbara Cottage Hospital [32]. Additional plasma from PE, HOP and nulligravid women and tissue samples were provided from the University of Texas Medical School at Houston. All blood samples were obtained near the time of delivery, where applicable. Clinical details are described in Supplementary material. All subjects provided informed consent, and samples were collected according to institutional guidelines.

2.2. Screening a random peptide library against dilute plasma

A 15-mer random *Escherichia coli* display peptide library [33] was screened using plasma depleted of *E. coli* binders to remove peptides binding HOP antibodies using magnetic selection and enrich for peptides that bind PE antibodies with fluorescence-activated cell sorting (FACS). Groups of 3 PE and 3–4 HOP plasma samples were pooled together to create 6 pools of each type. After incubating with either PE or HOP plasma diluted (1:100 or 1:200) in phosphate buffered saline (PBS), cells were labeled with a biotinylated anti-human IgG specific secondary (Jackson ImmunoResearch) diluted (1:500) in PBS with 0.1% bovine serum albumin (BSA). Streptavidin-conjugated magnetic beads were used for magnetic depletion while streptavidin conjugated to *R*-phycoerythrin (SA-PE) (Invitrogen) diluted (1:333) in PBS (0.1% BSA) fluorescently labeled cells for FACS. Incubations with plasma or labeling reagents were conducted at 4 °C. PE and HOP pools were assayed for binding to the library population at each round of FACS to determine which pool to use for enrichment or subsequent depletion. Screening was performed in duplicate using the same sample pools in different order of depletion/enrichment. Bacterial colonies (~130) were randomly selected for sequencing from different rounds of the duplicate screens. Peptide sequences from bacterial colony plasmids were identified using the Geneious software package. Peptide binding motifs were determined by inspection of the unique sequences. To evolve the antibody binding peptide motif, a focused bacterial displayed peptide library of the form xxxKxxxC[VIL]GCxxxx was constructed. Screening against this library used 3 new pools of PE and HOP using diluted plasma (1:200 and 1:500). From the focused library, ~100 clones were selected for sequencing from screening rounds (primarily from the final round).

2.3. Identifying the native antigen corresponding to the peptide motif

Unbiased searches using ScanProsite identified a panel of candidate antigens and the corresponding source organism (additional information in Supplementary material). Individual 15-mer epitopes derived from these proteins containing the motif were moved, using recombinant DNA, to the N-terminus of the eCPX display scaffold along with a C-terminal peptide tag (P2x) that binds a fluorescent reporter (YPet-Mona) of scaffold expression [34]. These cell-surface expressed antigen epitopes and library-isolated peptides were evaluated for significantly increased PE binding over HOP and dynamic range for at least 16 PE and 16 HOP. After incubating with diluted plasma (1:200), cells were washed 2× with cold PBS, and resuspended with biotinylated anti-human IgG diluted (1:500) in PBS with 0.1% BSA for secondary labeling. Subsequently, the cells were washed with cold PBS and resuspended in SA-PE diluted (1:333) in PBS with 0.1% BSA for fluorescent labeling and flow cytometric analysis. The fluorescent intensity measured for each peptide was divided by the background intensity of the negative control scaffold without an N-terminal peptide. Epitopes with a statistically significant ($p < 0.05$) difference between PE and HOP and highest dynamic range were down-selected for further analysis against a total of 42 PE, 43 HOP, and 21 nulligravid samples.

2.4. Analyzing full-length EBNA-1 protein activity

Samples from PE cases ($n = 36$) and HOP controls ($n = 39$) were assayed in duplicate for antibody binding to full-length EBNA-1 using a commercial IgG ELISA (GenBio ImmunoWell) following the manufacturer's protocol. PE and HOP ELISA activity distributions and the correlation between the ELISA and binding activity of bacterial displayed peptides were assessed. To confirm a relationship between EBNA-1 fragment (EB15) activity and the ELISA, EB15 binding antibodies were depleted from 10 reactive PE samples, 1 reactive HOP, and 9 nonreactive HOP samples and subsequently evaluated by ELISA. Depletions were carried out in duplicate by incubating plasma samples diluted (1:50) in the specimen diluent provided in the commercial kit with ~1×10^7 cells/μL. The depleted supernatant was retained after centrifugation and evaluated for ELISA activity. As a control, the eCPX scaffold with the C-terminal tag peptide was similarly used to “deplete” 5 reactive PE and 5 nonreactive HOP samples.

2.5. Antibody blocking activity of the EBNA-1 synthetic peptide

A 15-mer peptide spanning the PE specific epitope of EBNA-1 (EB15; RPQKRPSCIGCKGTH) was synthesized with a disulfide bond. An unrelated synthetic peptide (NCP) was used as a negative control. After pre-incubating pools of 3 PE patients (selected for high binding activity to GPR50) for 1 h at room temperature with varied concentrations

Download English Version:

https://daneshyari.com/en/article/6087031

Download Persian Version:

https://daneshyari.com/article/6087031

Daneshyari.com