Molecular Immunology 76 (2016) 123–133

Contents lists available at ScienceDirect

Molecular Immunology

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

Uncommon structural motifs dominate the antigen binding site in human autoantibodies reactive with basement membrane collagen

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ARTICLE INFO

Article history:
Received 26 March 2016
Received in revised form 1 July 2016
Accepted 5 July 2016
Available online 20 July 2016

Keywords:
Anti-collagen autoimmunity
HCDR3 (heavy chain complementarity determining region 3)
Human monoclonal autoantibody

ABSTRACT

Autoantibodies mediate organ destruction in multiple autoimmune diseases, yet their origins in patients remain poorly understood. To probe the genetic origins and structure of disease-associated autoantibodies, we engrafted immunodeficient mice with human CD34+ hematopoietic stem cells and immunized with the non-collagenous-1 (NC1) domain of the alpha3 chain of type IV collagen. This antigen is expressed in lungs and kidneys and is targeted by autoantibodies in anti-glomerular basement membrane (GBM) nephritis and Goodpasture syndrome (GPS), prototypic human organ-specific autoimmune diseases. Using Epstein Barr virus transformation and cell fusion, six human anti-alpha3(IV)NC1 collagen monoclonal autoantibodies (mAb) were recovered, including subsets reactive with human kidney and with epitopes recognized by patients' IgG. Sequence analysis reveals a long to exceptionally long heavy chain complementarity determining region3 (HCDR3), the major site of antigen binding, in all six mAb. Mean HCDR3 length is 25.5 amino acids (range 20–36), generated from inherently long DH and JH genes and extended regions of non-templated N-nucleotides. Long HCDR3 are suited to forming noncontiguous antigen contacts and to binding recessed, immunologically silent epitopes hidden from conventional antibodies, as seen with self-antigen crossreactive broadly neutralizing anti-HIV Ig (bnAb). The anti-alpha3(IV)NC1 collagen mAb also show preferential use of unmutated variable region genes that are enriched among human chronic lymphocytic leukemia antibodies that share features with natural polyreactive Ig. Our findings suggest unexpected relationships between pathogenic anti-collagen Ig, bnAb, and autoreactive Ig associated with malignancy, all of which arise from B cells expressing unconventional structural elements that may require transient escape from tolerance for successful expansion.

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1. Introduction

Autoimmune diseases affect an estimated 10–20% of the US population, a prevalence that equals that of cancer and heart disease. Autoimmunity often strikes young adults, destroying joints, kidneys, lungs, and other organs and requiring medications that can have devastating long-term effects. Kidney involvement is common and particularly disabling, as it often leads to renal failure requiring dialysis or transplantation. Up to 70–80% of patients with systemic lupus erythematosus or anti-neutrophil cytoplasmic antibody vasculitis develop glomerulonephritis (GN) requiring immunosuppression (Pagnoux et al., 2008). Humoral autoimmunity is often prominent: autoantibodies are key to diagnosis and organ destruction, and their elimination is a major goal of therapy. Adverse side effects of current interventions have spurred efforts to

Abbreviations: Ag, antigen; bnAb, broadly neutralizing anti-HIV Ig; CLL, chronic lymphocytic leukemia; EBV, Epstein Barr virus; GBM, glomerular basement membrane; GN, glomerulonephritis; GP, Goodpasture; GPS, GP syndrome; HC, heavy chain; HCDR3, heavy chain complementarity determining region3; HSC, hematopoietic stem cells; IMGT, ImMunoGeneTics information system; LC, light chain; mAb, monoclonal antibody; NC1, non-collagenous-1 (domain); NSG, NOD-scid-gamma; V, variable.

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http://dx.doi.org/10.1016/j.molimm.2016.07.004
0161-5890/Published by Elsevier Ltd.

develop better targeted, less toxic treatments. These efforts would be aided by insight into the origin and structure of the pathogenic autoantibodies, and of their transmembrane counterparts on the surface of autoreactive B cells. Sequence information could refine our understanding of autoantibody-antigen (Ag) interactions, and provide clues to the origin, regulation, and evolution of the autoimmune response in patients.

Progress on this front has been slow. Antibodies and lymphocyte receptors are structurally complex and extremely diverse, generated by multiple gene rearrangements and enzymatic and somatic processes. This complexity renders isolated proteomic dissection of serum polyclonal antibody mixtures difficult. Proteomic approaches are further complicated by the uniqueness of individual Ig repertoires, particularly the critical heavy chain complementarity determining region 3 (HCDR3) that dominates Ag binding; subject-specific reference libraries are required for Ig proteomics data interpretation. Attempts to isolate Ag-specific Ig sequences from circulating B cells are stymied by the rarity of Ag-specific B cells in peripheral blood (Stevens et al., 1979). Immunization studies reveal a brief circulation half-life for Ag-specific B cells, which rapidly migrate between relatively inaccessible niches in the spleen or other peripheral lymphoid organs and the bone marrow, where they differentiate into resident plasma cells that are the source of the vast majority of serum Ig (Reddy et al., 2010). This may explain difficulties in recovering Ag-specific human monoclonal Ab (mAb) by viral transformation of autoimmune patients' blood cells, and in any case dissociates specificity of serum autoantibodies from that of contemporaneous circulating cells. Moreover, for many autoimmune diseases for which Ab and receptors recognize conformational (non-linear) epitopes, reagents or assays are not yet available that permit capture of Ag-specific B cells for study.

To circumvent these obstacles, we turned to a humanized model as a platform to probe origins of human pathogenic autoimmunity at the molecular level, and to sample and capture human Ag-reactive cells and autoantibodies. We focus on anti-glomerular basement membrane (GBM) nephritis, and its systemic counterpart, Goodpasture Syndrome (GPS), because they have long been recognized as prototypic organ-specific autoimmune diseases. The autoantigen, alpha3(IV)NC1 collagen, is well characterized and autoantibodies play a prominent role in organ injury (Borza and Hudson, 2003). Patient IgG can transfer nephritis to susceptible animal hosts, such as squirrel monkeys, in whom pathogenic epitopes are accessible in vivo (Lerner et al., 1967; Luo et al., 2010), and glomerulonephritis rapidly recurs in transplant recipients with persistent circulating anti-GBM IgG (Wilson and Dixon, 1973). Linear Ig deposits along the glomerular and/or alveolar basement membranes are a hallmark of disease, and either this histopathological finding or detection of circulating anti-alpha3(IV)NC1 collagen Ig is required for diagnosis. Serum autoantibody levels guide treatment decisions, including duration of plasmapheresis and timing of transplantation (Hellmark and Segelmark, 2014).

Herein, we report isolation and sequence analysis of six human mAb reactive with alpha3(IV)NC1 collagen, subsets of which bind human kidney GBM and alpha3(IV)NC1 collagen epitopes recognized by nephritis patients' IgG. These mAb derive from mice in which a human immune system was established by engraftment with cord blood-derived human CD34+ hematopoietic stem cells (HSC), followed by immunization with purified alpha3(IV)NC1 collagen. Sequence analysis reveals unusual features shared by the six mAbs, including strikingly long HCDR3s reminiscent of those reported among protective broadly neutralizing mAb (bnAb) reactive with human immunodeficiency virus (HIV), another Ab specificity rarely encountered in human serum, even among HIV-infected individuals. The results provide previously unattainable insight into structural motifs of human autoreactivity with basement membrane collagen, and clues into origins and regulation of enigmatic B cells autoreactive with GBM antigen.

2. Materials and methods

2.1. Mouse studies and immunizations

Female NOD-scid-gamma (NSG) immunodeficient mice were purchased from the Duke Cancer Center Isolation Facility Breeding Core and housed under specific pathogen free conditions. Hu-HSC mice were generated essentially as described (Worni-Schudel et al., 2015), with the following modifications; 3–4 h after irradiation (100 cGy, X-RAD 320, Precision X-ray, North Branford, CT) 9.5–10 week old NSG recipients were intravenously injected with purified human CD34+ HSC (AllCells LLC, Alameda, CA), using $2.5–10 \times 10^4$ CD34+ HSC per mouse (Pearson et al., 2008; Shultz et al., 2005). All studies were approved by the Duke University Institutional Review Board and Animal Care and Use Committee and conform to institutional standards and to the National Institutes of Health guide for the care and use of laboratory animals. 14 weeks after HSC injection, Hu-HSC mice were immunized with 25 μg bovine alpha3(IV)NC1 collagen (Eurodiagnostica, Malmo, Sweden) emulsified in Freund's Complete Adjuvant, followed by boost 3 weeks later with antigen in Freund's Incomplete Adjuvant. Tissue was harvested at 1.5–3 weeks following this boost (19–20 weeks post-engraftment).

2.2. Flow cytometry

Single-cell suspensions of cells were analyzed by flow cytometry as described (Clark et al., 2011; Worni-Schudel et al., 2015), using fluorescent-conjugated antibodies (Becton Dickinson-Pharmingen, San Jose, CA). Cells were analyzed on a FACSCalibur or BD CANTO apparatus (Becton Dickinson-Pharmingen) and results analyzed using FlowJo (Treestar, Ashland, OR, USA), with live cells gated on forward and side scatter. Chimerism, or the level of human lymphocyte engraftment, in Hu-HSC mouse tissue was calculated as follows: % Chimerism = (%humanCD45+ cells × 100)/(%humanCD45+ cells + %mouseCD45+ cells).

2.3. Cell culture, Epstein Barr virus (EBV) transformation, and hybridoma generation

Harvested human B cells were transformed and fused essentially as described (Bonsignori et al., 2011; Worni-Schudel et al., 2015). Briefly, cells were cultured in bulk overnight in complete medium supplemented with Cyclosporin A, CpG oligonucleotides, checkpoint 2 kinase inhibitor, and EBV B95-8 virus suspension (ATCC, Manassas, VA, USA), and then seeded into 96-well plates and refed weekly, with cyclosporin A and CpG oligonucleotides withdrawal at two weeks. Transformed B cell lines for which supernatants tested consistently positive for reactivity with antigen were electrofused with HMMA2.5 mouse-human chimera myeloma cells (gift of Lisa Cavacini, Beth Israel Deaconess Medical Center, Boston) (Posner et al., 1987) using a PA-4000/PA-101S apparatus and hypoxanthine, aminopterin, thymidine and ouabain selection, as described (Yu et al., 2008). Antigen-reactive lines were subcloned using a standard limiting dilution method.

2.4. Enzyme linked immunosorbent assay (ELISA)

Human immunoglobulin (Ig) class, light chain type, Ig concentration, and anti-alpha3(IV)NC1 collagen Ab in serum and cell supernatants were detected by ELISA, as described (Worni-Schudel et al., 2015; Zhang et al., 2008). Results for binding to antigen [bovine alpha3(IV)NC1 collagen (Eurodiagnostica, Malmo,