Molecular Immunology 91 (2017) 49–56

Contents lists available at ScienceDirect

Molecular Immunology

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

A murine Ig light chain transgene reveals IGKV3 gene contributions to anti-collagen types IV and II specificities

Amy G. Clark[a,b], Inge M. Worni-Schudel[a,b], Francesca M. Korte[a], Mary H. Foster[a,b,*]

[a] Department of Medicine, Duke University Health System, Durham, NC, USA
[b] Durham VA Medical Center, Durham, NC, USA

ARTICLE INFO

Keywords:
Collagen
Autoantibody
Kappa light chain
IGKV3

ABSTRACT

A subset of autoimmune diseases result from autoantibodies targeting epitopes on matrix collagen. The most extensively studied are anti-glomerular basement membrane glomerulonephritis (or its systemic counterpart Goodpasture's disease) that destroys kidneys and lungs, and rheumatoid arthritis that leads to disabling arthritis. Autoantibodies in these disorders bind evolutionarily conserved conformational epitopes on the noncollagenous domain 1 (NC1) of the alpha3 chain of type IV [alpha3(IV)NC1] collagen in glomerular and alveolar basement membranes, and on native or citrullinated type II collagen (CII) in joint cartilage, respectively. The genetic origins of pathogenic anti-collagen B cells in these diseases is unknown, but observations from murine models raise the possibility that they overlap despite distinct in vivo immunopathologies. Monoclonal autoantibodies isolated from mice immunized with alpha3(IV)NC1 collagen or CII show a biased use of Ig light chains (LC) encoded by genes of the IGKV3 subgroup (previously Vk21 family), paired with diverse Ig heavy chains. To further explore this relationship and determine if a single murine IGKV3 LC independently predisposes to both anti-collagen responses, we generated a novel transgenic (Tg) C57BL/6 mouse that expresses a productively rearranged IGKV3-encoded LC, termed mLCV3-Tg, in conjunction with endogenously rearranged Ig heavy chains. Tg mice are also genetically deficient in endogenous kappa chains to permit tracking of the mLCV3 transgene. We show that mLCV3-Tg mice are susceptible to humoral autoimmunity against both collagen chains. Anti-alpha3(IV)NC1 collagen, but not anti-CII, mLCV3-encoded Ig are detected in serum of unmanipulated Tg mice, while Toll-like receptor ligands induce secretion of mLCV3-Tg autoantibodies of both collagen specificities from splenocytes ex vivo. This indicates developmental survival of mLCV3-Tg B cells reactive with each antigen, and is consistent with production of the two anti-collagen autoIg from distinct B cell populations. Reduced B cell numbers, low serum Ig kappa levels, low cell surface Ig kappa density, and abundant endogenous lambda chain expression suggest that subsets of IGKV3-encoded B cells are regulated in vivo by mechanisms that include deletion, anergy, and LC editing. These results support the notion that murine IGKV3 LCs contribute structural fitness to antigen binding sites that support diverse anti-collagen autoimmune responses, that these responses are regulated in vivo, and that these cells can nonetheless readily escape immune regulation.

1. Introduction

Autoimmune diseases affect an estimated 50 million Americans, causing extensive morbidity due to spontaneous immune attack on vital organs and tissues. Autoantibodies are prominent in many autoimmune diseases, mediating tissue destruction and serving as biomarkers to facilitate diagnosis, monitor disease, and inform treatment decisions. Therefore, understanding how autoantibodies are generated is key to altering disease pathogenesis and outcomes.

In an important subset of autoimmune diseases, pathogenic autoantibodies target epitopes on matrix collagen (reviewed in (Foster, 2017). Anti-glomerular basement membrane glomerulonephritis (anti-GBM GN), and its systemic counterpart, Goodpasture's disease, rheumatoid arthritis (RA), bronchiolitis obliterans in lung allografts, and epidermolysis bullosa acquisita are mediated in part by anti-collagen Ig that bind epitopes on collagen II, IV, V, and VII respectively (Burkhardt et al., 2002; Burlingham et al., 2007; Lindh et al., 2014; Saus et al., 1988; Woodley et al., 1984). Autoreactivity to collagen V was also recently implicated in atherosclerosis (Dart et al., 2010).

Anti-GBM GN and RA are the most extensively studied diseases

* Corresponding author at: Dept of Medicine, Duke University Health System, Box 103015, Durham, NC, 27710, USA.
E-mail addresses: amy.clark@duke.edu (A.G. Clark), ingeschudel@gmail.com (I.M. Worni-Schudel), fkorte@wellesley.edu (F.M. Korte), mary.h.foster@dm.duke.edu, mhfoster@duke.edu (M.H. Foster).

http://dx.doi.org/10.1016/j.molimm.2017.08.015
Received 30 May 2017; Received in revised form 30 July 2017; Accepted 16 August 2017
0161-5890/ Published by Elsevier Ltd.

linked to anti-collagen reactivity. In anti-GBM GN, pathogenic anti-GBM IgG recognize conformational epitopes on the noncollagenous domain 1 (NC1) of the alpha3 chain of type IV collagen [hereafter alpha3(IV)NC1] that are expressed in capillary and alveolar basement membranes in kidneys and lungs, respectively (Borza and Hudson, 2003; Lerner et al., 1967; Saus et al., 1988). Autoantibody deposition can precipitate acute inflammation leading to kidney failure and life-threatening lung hemorrhage. In RA, a chronic autoinflammatory disease characterized by peripheral joint destruction, patients develop autoantibody responses to triple-helical fibrillar type II collagen (CII), a major component of hyaline and articular cartilage, as well as to citrullinated proteins, including citrullinated CII, and immunoglobulin (rheumatoid factor) (Burkhardt et al., 2002; Lindh et al., 2014). IgG and B cells reactive with native CII are frequently recovered from inflamed joints but not blood of RA patients, an enrichment that suggests that anti-CII IgG production predominantly occurs locally, rather than in secondary lymphoid organs (Lindh et al., 2014; Ronnelid et al., 1994; Tarkowski et al., 1989). Direct pathogenicity of both sets of anti-collagen autoantibodies is suggested by transfer experiments; patient-derived anti-alpha3(IV)NC1 IgG induce nephritis in squirrel monkeys (Lerner et al., 1967), and mouse anti-CII IgG that crossreact with CII epitopes recognized by RA patients' IgG transfer an RA-like severe erosive polyarthritis to naïve mice (Holmdahl et al., 1990; Nandakumar and Holmdahl, 2005; Nandakumar et al., 2003; Stuart and Dixon, 1983; Terato et al., 1992). Anti-CII IgG and B cells also appear to participate in a joint-destructive amplification cycle that promotes formation and pathogenicity of their anti-citrullinated-CII counterparts (Uysal et al., 2009).

The genetic origins of pathogenic anti-collagen B cells and IgG in anti-GBM GN and RA, and their potential as therapeutic targets, remain unknown. Efforts to sequence patients' anti-collagen IgG have been unsuccessful to date, frustrated in part by limitations of current technologies to sequence patients' polyclonal IgG mixtures or to capture B cells that recognize conformational epitopes. However, restricted epitope specificity and shared idiotypes among anti-collagen Ig are reported for both anti-GBM GN and RA (Lindh et al., 2014; Meyers et al., 1998; Netzer et al., 1999). Thus within each disease, pathogenic Ig may arise from a limited subset of B cells bearing conformationally-related Ig receptors.

Observations from mouse models raise the possibility that these anti-collagen autoimmune diseases overlap in immune origins, despite their distinct disease manifestations, target Ag distributions, and immunopathologies. We previously reported sequence analysis of anti-alpha3(IV)NC1 collagen monoclonal autoantibodies (mAb) isolated from immunized C57BL/6 and SJL mice (Sackey et al., 2008). The anti-alpha3(IV)NC1 mAb exclusively use light chains (LC) encoded by genes of the IGKV3 subgroup (previously termed Vk21 family), paired with diverse Ig heavy chains. Preferential use of IGKV3 genes is notable; this subgroup is uncommon in the expressed repertoire of healthy adult mice, despite its being one of the larger IGKV subgroups with respect to number of functional genes (Kaushik et al., 1989). A strikingly biased use of IGKV3 genes was also observed among anti-CII IgG mAb isolated from mice with collagen-induced arthritis (CIA), a commonly used model of RA (Mo et al., 1993; Mo and Holmdahl, 1996). Up to 68% of anti-CII IgG mAb recovered from DBA/1 mice with CIA use IGKV3-encoded LCs, many of which are minimally mutated (Mo et al., 1993; Mo and Holmdahl, 1996). The IGKV3 LCs are paired with diverse VH, DH, and JH genes, and with HCDR3 of varied sequence and length. IGKV3-encoded LCs are also used by the 6 C1 epitope-specific anti-CII mAb recovered from immunized B10.Q mice bearing an anti-CII $V_HD_HJ_H$ knock-in (Cao et al., 2011).

Biased use of minimally mutated IGKV3 genes in both anti-alpha3(IV)NC1 and anti-CII murine anti-collagen responses indicates that a germline-encoded LC conformation can predispose to multiple anti-collagen autoreactivities. This suggests an unexpected relationship between distinct anti-collagen B cell populations, and raises questions about their mechanisms of regulation in healthy animals and of activation in disease. In this regard, we previously generated an IgM autoantibody transgenic mouse expressing the IGKV3-encoded LC and IGHV1-encoded HC of a murine mAb that targets an alpha3(IV)NC1 collagen epitope recognized by IgG from patients with anti-GBM GN (Zhang et al., 2008). In this model, HC + LC Ig Tg B cells are deleted in the bone marrow unless rescued by secondary rearrangements on the endogenous light and/or heavy chain alleles. Whereas this B cell fate was attributed in large part to expression of a dominant anti-alpha3(IV) NC1 HC, the findings described above raise the possibility that the IGKV3-encoded LC plays a prominent role in determining B cell fate in this model.

To test the hypothesis that the murine IGKV3 LC independently predisposes to autoimmunity that is regulated in vivo, we generated a novel transgenic mouse that expresses the IGKV3-encoded LC in conjunction with endogenously rearranged Ig HCs, using superimposed genetic deficiency of endogenous kappa chains to facilitate monitoring of the LC Tg. We show that subsets of B cells expressing a single IGKV3 LC Tg can react with alpha3(IV)NC1 collagen or CII and survive in peripheral lymphoid organs of LC Tg mice. Anti-alpha3(IV)NC1 collagen but not anti-CII IGKV3 LC-encoded Ig are detected in serum of unmanipulated Tg mice, and Toll-like receptor (TLR) ligands induce distinct patterns of anti-collagen Tg Ig secretion from splenocytes ex vivo, consistent with origin of the two anti-collagen autoIg from distinct B cell populations. These results support the notion that murine IGKV3 LCs contribute structural fitness to the Ag binding site for generating diverse anti-collagen autoimmune responses. Nonetheless, reduced B cell numbers, low cell surface Ig kappa density, and endogenous lambda chain expression suggest that subsets of IGKV3-encoded B cells are regulated in vivo by mechanisms that include deletion, anergy, and LC editing.

2. Materials and methods

2.1. Generation of IGKV3 LC Tg mice with endogenous kappa knockout

Isolation and characterization of a murine prototypic anti-α3(IV) NC1 mAb derived from an immunized B6 mouse and production of a DNA construct expressing the mAb IGKV3-2*01(former Vk21C)/Jk2 kappa light chain (hereafter mLCV3-Tg) was described previously (Sackey et al., 2008; Zhang et al., 2008). C57BL/6NHsd founders bearing the mLCV3-Tg were created by the Duke University Transgenic Mouse Core Facility. Four founder lines were bred with C57BL/6J (B6) mice lacking endogenous kappa chains (kKO, Jackson Laboratory stock #002400, Bar Harbor, ME, USA) (Zou et al., 1993), to facilitate tracking of mLCV3-Tg expression in vivo and in vitro. All data presented herein are from mice bearing the mLCV3-Tg and homozygous for endogenous kappa knockout (mLCV3-Tg/kKO; mean age 8.7 months, range 1.5–18 mo), or controls including non-transgenic kKO (non-Tg/kKO, devoid of kappa) and non-transgenic kappa sufficient (k+) littermates (mean age 6.2 months, range 2–12 mo), of either sex, reared under conventional specific pathogen-free conditions. Kappa$^+$ controls include homozygous wildtype k+/+ or heterozygous k+/− genotypes, depending on availability. The care and use of all experimental animals were in accordance with institutional guidelines, and all studies and procedures were approved by the Animal Care and Use Committee of Duke University. Blood was collected and serum stored −20C until assay. Spleens were removed into culture media for processing as described below. Bone marrow was flushed from the leg bones using sterile PBS.

2.2. Flow cytometry

Single cell suspensions of 0.5–1.0 × x10^6 bone marrow or red blood cell-depleted spleen cells were incubated with fluorescently labeled antibodies and fixed in 1% paraformaldehyde prior to analysis by flow cytometry. Fluorescent stains include (NIF antibody registry number,