Immunobiology 217 (2012) 111–117

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.de/imbio

Naturally occurring V region connected antibodies inhibit anti-dsDNA antibody reactivity with dsDNA

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ARTICLE INFO

Article history:
Received 7 June 2011
Received in revised form 14 July 2011
Accepted 19 July 2011

Keywords:
Natural antibodies
Systemic lupus erythematosus
IgG-reactive antibodies
Autoantibodies

ABSTRACT

The production of autoantibodies against a vast array of self antigens, most notably double stranded (ds) DNA, characterized systemic lupus erythematosus (SLE). The purpose of this work is to study specific Ig fractions isolated from normal human serum (NHS) and their effect on the binding of anti-double-stranded deoxyribonucleic acid (dsDNA) antibodies (Abs) to dsDNA. A fraction named immunoglobulin G (IgG)-reactive IgG was purified from total NHS IgG by absorption onto (CNBr)-activated Sepharose 4B linked to intact IgG molecules (IgG-Sepharose column). IgG-reactive IgG was co-incubated with systemic lupus erythematosus (SLE) patient's serum and binding of the anti-dsDNA Abs to dsDNA was measured by enzyme-linked immunosorbent assay (ELISA). Co-incubation of SLE patient's serum with IgG-reactive IgG resulted in a dose-dependent reduction in binding of anti-dsDNA Abs to dsDNA. A reduction greater than 70% was observed at a concentration of 300 μg of IgG-reactive IgG per mL of a 400-fold diluted SLE patient's serum whereas total NHS IgG, at the same concentration, resulted in a 10% reduction in binding. The purification process used to isolate IgG-reactive IgG was based on interactions between intact Ig rather than on interactions between $F(ab')_2$ portions. IgG_2 is the predominant immunoglobulin (Ig) subclass in IgG-reactive IgG. Thus, IgG_2 might have an important role in the connectivity characteristics of NHS IgG. The capacity of IgG-reactive IgG to inhibit anti-DNA Ab binding to dsDNA may have potential application in the treatment of SLE. This targeted biological approach may provide an alternative strategy to immunosuppressants.

Introduction

Despite a rigorous selection process during B cell development in the bone marrow a where B lymphocytes with potentially dangerous self-reactivity are eliminated a significant number of self-reactive B cell survives to enter the peripheral compartment (Monroe and Dorshkind 2007). In healthy individuals, a major point at which reduction in the number of self-reactive B cells takes place is during transition from IgM naive, to IgM memory B cells before the onset of somatic hypermutation. In patients with SLE, this progressive decline in self-reactive cells does not occur, suggesting that SLE is associated with a failure to establish self-tolerance during early B cell development which leads to increased numbers of autoreactive mature naive B cells (Yurasov et al. 2005).

SLE, the most serious of the lupus disorders, is characterized by the production of a number of autoantibodies which involves any B cell subset including B1 cells, marginal zone (MZ) B cells, short-lived plasma cells or germinal centre-matured long-lived plasma cells (Jacobi and Diamond 2005). The most common autoantibody seen in lupus is the antinuclear antibody (ANA). The type of ANA pattern helps to determine if SLE or a related connective tissue disease is present and anti-dsDNA antibodies are the most frequently detected antibodies in SLE. Current diagnostic criteria for

Abbreviations: Abs, antibodies; Ag, antigen; BF, binding buffer; CDR, complementary determining regions; CNBr, cyanogen bromide; CRI, cross-reactive idiotypes; dsDNA, double-stranded deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; FR, framework; GB, GammaBind G Sepharose; GBF, GammaBind G Sepharose flowthrough; Id, idiotype; Ig, immunoglobulins; MZ, marginal zone; NHS, normal human serum; ORG, ORGENTEC; PBS, phosphate buffered saline; PS, polysaccharides; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; RF, rheumatoid factor.

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doi:10.1016/j.imbio.2011.07.026

SLE should include 4 of the ten symptoms to be present at any time during a patient's history among which is the dsDNA antibody reacting with double stranded deoxyribonucleic acid (DNA) (Pearson and Lightfoot 1981). The processes that govern the generation of pathogenic anti-dsDNA Abs in SLE are largely unknown. Autoantibodies may appear many years before the diagnosis of SLE with subsequent progressive accumulation of specific autoantibodies during the development of this syndrome (Arbuckle et al. 2003). Anti-dsDNA Abs may arise as a consequence of polyclonal antigen (Ag)-nonspecific disturbances of B and of T cells which leads to an extensive diversity of anti-DNA Abs, or as a consequence of Ag-driven responses being promoted by polyclonal activation (Dziarski 1988; Klinman et al. 1994; Singh et al. 1998). The high ratio of replacement to silent mutations in the complementary determining regions (CDR) suggests Ag-driven expansion (Shlomchik et al. 1987; Eilat et al. 1989). Back mutations of SLE patient's anti-DNA-IgG demonstrates that some germline configuration of these Abs have no specificity for DNA suggesting that at least some anti-DNA autoantibodies are generated from non-DNA reactive B cells during the normal immune response (Wellmann et al. 2005). Basic amino acids introduced by somatic mutations may contribute to anti-DNA Abs binding to DNA through electrostatic interactions (Eilat et al. 1988) supporting Ag-driven positive selection of somatic mutations in the gene elements that frequently encode multi-reactive IgM autoantibodies (Van Es et al. 1991). Furthermore, the reversion of somatically mutated anti-dsDNA-IgM, from the same patient, to germline sequence, suggests that both self and non-self Ags are involved in B cell activation (Zhang et al. 2008).

In addition to alteration in mutational activity, SLE patients show alteration of Ig repertoire. A bias toward V_H3, V_H4-34, and $V_\kappa1$ gene family usage was shown, however the Ig gene repertoire is not predictive of a self-reactive antibody selection bias in SLE (Yurasov et al. 2005). No particular family of germline V genes has been demonstrated to encode anti-DNA Abs, for more than twenty apparently different DNA antibody idiotypes have been identified, some of which tend to be public or cross-reactive (CRI) (Isenberg et al. 1990). The idiotypic cross-reactions of Ig from unrelated patients suggest that in SLE autoantibodies are derived from related families of germline genes (Shoenfeld et al. 1983). The precise nature of Ags, self or foreign, triggering the production of anti-dsDNA Abs is not defined yet. Anti-dsDNA Abs can bind to a wide variety of antigens, including nucleic acids, nucleoproteins, cell membranes, and phospholipids. There is evidence for idiotypic and reactivity relationship between anti-DNA Abs and anti-bacterial polysaccharides (PS) (Grayzel et al. 1991; Spellerberg et al. 1995).

Anti-idiotypic Abs reactive with anti-DNA Abs were detected in sera of healthy individuals as well as in SLE patients in remission implying that autoreactive Abs may be manipulated by a heterogeneous population of anti-idiotypic Abs (Williams and Isenberg 1998; Silvestris et al. 1987; Muryoi et al. 1990).

The aims of this study were to separate naturally occurring V region connected Abs from NHS by exploiting their reactivity with total intact NHS IgG, and to test their reactivity against anti-dsDNA Abs present in the sera of SLE patients.

Materials and methods

Patients' sera

Sera from five Serbian patients with a confirmed diagnosis of SLE according to the American College of Rheumatology (ACR) revised criteria were studied. Patients (four female and one male, with a mean age at 46.2 ± 13.22 years) had active disease and high serum levels of anti-dsDNA Abs. The anti-dsDNA Ab screen ORG 604S, which detect IgG, IgM and IgA isotypes, was used to measure anti-dsDNA Ab level. Serum was collected from patients by peripheral venepuncture according to standard operating procedures. The Institution's Ethics Committee approved the study. Full informed consent was obtained from all patients.

Healthy donors' sera

Sera of 30 healthy Serbian blood donors with no history of SLE or other autoimmune disorders were studied. Serum was collected by peripheral venepuncture according to standard operating procedures. For each experiment, the pooled serum contained an equal volume of serum from each donor. Usually 3 mL from each donor were used. Prior to affinity chromatography procedures, serum was heat inactivated at 56 °C for 30 min.

Purification of NHS IgG and of the GammaBind flowthrough fraction

Two millilitres of NHS, containing about 18.0 mg of IgG, was added to 10 mL of binding buffer (BF) and then dialysed by centrifugation (MW limit 50 kDa). Alternatively, 2 mL of NHS was added to 2 mL of 2× BF. Prior to loading to a 2.0 mL GammaBind G (GB) column the mixture was filtered through a 0.2 μm-pore size Millipore filter. The GB column capacity was 18 mg of human IgG per 1 mL of drained gel. After elution IgG containing fractions were collected and dialysed by centrifugation and thus represents NHS IgG. However, the GB flow through fraction (GBF) contains unbound IgG. This fraction was dialysed and concentrated (to original serum volume) by centrifugation in phosphate buffered saline (PBS) prior to determining Ig class concentration by capture ELISA. GBF fractions with IgG concentrations of at least 10 μg per mL were used to separate IgG-reactive GBF Abs on IgG-Sepharose column.

Purification of IgG-reactive IgG and of IgG-reactive GBF Abs

Separation of IgG-reactive IgG from total NHS IgG affinity purified on GB, and of IgG-reactive GBF Abs was performed using an IgG-Sepharose column (NHS IgG-coupled to CNBr-activated Sepharose 4B). Samples of affinity purified IgG-reactive IgG and of IgG-reactive GBF Abs obtained from six independent runs were concentrated by ultrafiltration (YM-50, cut-off MW 50,000). The Ig concentration present in IgG-reactive IgG obtained from total NHS IgG and in IgG-reactive GBF Abs was adjusted to 100 μg per mL.

Detection of rheumatoid factor activity

In order to determine the rheumatoid factor (RF) activity of IgG-reactive IgG and of IgG-reactive GBF Abs, the quantitative measurement of autoantibodies reactive with the Fc portion was performed using the RF screen ORGENTEC (ORG) 522S (*Diagnostika GmbH* – GTI, Brookfield, WI). This ELISA kit is used for the quantitative measurement of IgG, IgM and IgA RF in serum or plasma. The cut off was 25 U per mL. The RF level was determined at Ig concentrations of 100 μg (IgG-reactive IgG) and of 50 μg (IgG-reactive GBF Abs) per mL. The serum of a rheumatoid arthritis (RA) patient (which contained about 50 μg per mL of IgG at 200-fold dilution, assuming a serum IgG concentration of 10 mg per mL) was tested at 200-fold dilution.

Detection of anti-dsDNA Abs in SLE patients' serum

The quantitative measurement of autoantibodies reactive with dsDNA was performed using the ELISA kit ORG 604S anti-dsDNA