# Human IgG+ Plasma Cells in Patients with Systemic Lupus Erythematosus

**vorgelegt von**

**Diplom-Ingenieurin**

**Juliane Kofer**

**aus Pasewalk**

Von der Fakultät III - Prozesswissenschaften

der Technischen Universität Berlin

zur Erlangung des akademischen Grades

Doktor der Ingenieurwissenschaften

- Dr.-Ing. -

genehmigte Dissertation

Promotionsausschuss:

Vorsitzender: Prof. Dipl.-Ing. Dr. Ulf Stahl

Gutachter: Prof. Dr. rer. nat. Roland Lauster

Gutachter: Dr. rer. nat. Hedda Wardemann

Tag der wissenschaftlichen Aussprache: 22.03.2011

Berlin 2011

D 83

Whenever they say it can't be done,
remind them that they make a jellybean that
tastes exactly like popcorn.

*John C. Mayer*

# ABSTRACT

Systemic Lupus Erythematosus (SLE) is a chronic inflammatory autoimmune disease that is associated with a major breakdown in B cell self-tolerance as reflected by elevated serum immunoglobulin G (IgG) levels of predominantly anti-nuclear antibodies (ANAs). Serum antibody titers are maintained by short-lived antibody-secreting plasmablasts and long-lived plasma cells, the latter residing in survival niches of the bone marrow. Experimental evidence from mouse models suggests that treatment-resistant bone marrow plasma cells are the major contributor to serum IgG autoantibodies in SLE. However, the frequency of self-reactive and potentially pathogenic antibodies in the bone marrow plasma cell compartment of SLE patients has not been determined.

The aim of this study was to characterize the human bone marrow IgG+ plasma cell compartment in SLE patients. The Ig genes of 196 bone marrow plasma cells from five SLE patients were cloned and expressed *in vitro* by using a single cell approach that facilitates the unbiased analysis of human plasma cells both on the Ig molecular level and antibody reactivity level. Antibody reactivity testing demonstrated significantly increased frequencies of poly- and self-reactive bone marrow plasma cells in SLE patients with an active disease.

In summary, the data provide direct evidence that the bone marrow harbors high frequencies of self-reactive IgG+ plasma cells that may directly contribute to disease pathogenesis in SLE. Thus, self-reactive IgG+ bone marrow plasma cells in SLE patients may represent an important therapeutic target in SLE.

# ZUSAMMENFASSUNG

Der systemische Lupus erythematodes (SLE) ist eine chronisch entzündliche Autoimmunerkrankung, die durch eine Fehlfunktion der körpereigenen Abwehr verursacht wird. Plasmazellen und Plasmablasten produzieren unter normalen Bedingungen protektive Antikörper, welche einen wesentlichen Bestandteil der Immunantwort auf fremde Erreger darstellen. Charakteristisch für SLE ist die Bildung hochaffiner IgG Antikörper, welche gegen DNA und weitere Bestandteile des Zellkerns im eigenen Organismus gerichtet sind. Diese Autoantikörper werden von kurzlebigen Plasmablasten und langlebigen Plasmazellen sezerniert und führen in Körperorganen wie der Niere zu chronischen Entzündungen. Langlebige Plasmazellen leisten den größten Anteil an IgG Antikörpern im Serum, überleben in Nischen des Knochenmarks für Jahre und sind im Gegensatz zu kurzlebigen Plasmablasten weitgehend therapieresistent. Die Häufigkeit von autoreaktiven und damit möglicherweise pathogenen Antikörpern in der langlebigen Plasmazellpopulation des Knochenmarks wurde in SLE Patienten jedoch bisher nicht analysiert.

Das Ziel dieser Arbeit war daher die Charakterisierung der IgG+ Plasmazellpopulation des Knochenmarks in SLE Patienten. Um die Häufigkeit autoreaktiver Plasmazellen im Knochenmark von SLE Patienten bestimmen zu können, wurde eine Methode verwendet, die es erlaubt einzelne Zellen zu isolieren und ihre jeweiligen IgH und IgL Gene zu klonieren. Mit dieser Information konnten dann Antikörper rekombinant *in vitro* hergestellt werden, welche die gleiche Antigenspezifität aufweisen, wie sie in der ursprünglichen Plasmazelle produziert wurde. Auf diese Weise wurden die Immunglobulingene von 196 Plasmazellen aus Knochenmarkproben von fünf verschiedenen SLE Patienten kloniert und *in vitro* exprimiert. Die Untersuchung der monoklonalen Antikörper zeigte, dass die Anzahl an autoreaktiven Plasmazellen in SLE Patienten mit aktiver Krankheit signifikant erhöht ist und krankheits-assoziierte antinukleäre Antikörper (ANAs) durch somatische Mutation entstanden sind.

Zusammenfassend kann die vorliegende Arbeit zeigen, dass Plasmazellen aus dem Knochenmark von SLE Patienten antinukleäre Antikörper sezernieren und damit direkt zum Krankheitsverlauf beitragen können. Die erhöhte Frequenz von autoreaktiven Plasmazellen im Knochenmark von SLE Patienten unterstreicht zudem die Rolle von langlebigen Plasmazellen als therapeutisches Ziel.

**Eidesstattliche Erklärung**

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertation in allen Teilen selbständig verfasst habe. Bei der Anfertigung der Dissertation wurde keine Hilfe Dritter in Anspruch genommen und genutzte Hilfsmittel vollständig angegeben. Veröffentlichungen von Teilen der vorliegenden Dissertation sind von mir nicht vorgenommen worden. Weiterhin wurde diese Dissertation nicht anderweitig für eine Prüfung oder Promotion zur Beurteilung eingereicht. Ich versichere, dass ich die vorstehenden Angaben nach bestem Wissen vollständig und der Wahrheit entsprechend getätigt habe.

Juliane Kofer Berlin, 30.04.2011

# Table of Contents

# ABBREVIATIONS

| | |
|---|---|
| aa | **a**mino **a**cid(s) |
| ABTS | 2, 2'-**A**zino-bis (3-ethyl**B**enz**T**hiazoline-6-**S**ulfonic acid) |
| AID | **A**ctivation-**I**nduced Cytidine **D**eaminase |
| ANA | **A**nti-**N**uclear **A**ntibody |
| APC | **A**llo**P**hyco**C**yanin |
| BAFF | **B** cell **A**ctivating **F**actor belonging to the TN**F** family |
| B cell | **B**ursal or **B**one marrow derived **cell** |
| BCR | **B C**ell Antigen **R**eceptor |
| bp | **b**ase **p**air |
| CD | **C**luster of **D**ifferentiation |
| CDR | **C**omplementary **D**etermining **R**egion |
| cDNA | **c**omplementary **D**esoxyribo**N**ucleid **A**cid |
| CSR | **C**lass-**S**witch **R**ecombination |
| D | **D**iversity |
| DC | **D**endritic **C**ell |
| DMEM | **D**ulbecco's **M**odified **E**agle **M**edium |
| dNTP | **d**esoxyribo**N**ucleosid **T**ri**P**hospate |
| dsDNA | **d**ouble-**s**tranded **D**esoxyribo**N**ucleid **A**cid |
| DTT | **D**i**T**hio**T**reitol |
| *E.coli* | ***E**scherichia **coli*** |
| EDTA | **E**thylene**D**iamine**T**etraacetic **A**cid |
| ELISA | **E**nzyme-**L**inked **I**mmuno**S**orbent **A**ssay |
| Fab | **F**ragment **a**ntigen **b**inding |
| FACS™ | **F**luorescence **A**ctivated **C**ell **S**orting™ |
| Fc | **F**ragment **c**rystalline |
| FCS | **F**etal **C**alf **S**erum |
| FDC | **F**ollicular **D**endritic **C**ell |
| FSC | **F**orward **Sc**atter |
| FWR | **F**rame**W**ork**R**egion |
| $g_{av}$ | ***av**erage surface **g**ravity* (approx. 9.81 m/s$^2$) |
| GC | **G**erminal **C**enter |
| HEPES | N-2-**H**ydroxy**E**thyl**P**iperazine-N′-2-**E**thane**S**ulfonic Acid |
| HEK 293T | **H**uman **E**mbryonic **K**idney **293** transformed with SV40 large **T**-antigen |
| HRP | **H**orse**R**adish **P**eroxidase |
| IFA | **I**mmuno**F**luorescence **A**ssay(s) |
| IgBLAST | **I**mmunoglobulin **B**asic **L**ocal **A**lignment **S**earch **T**ool |
| Ig | **I**mmuno**g**lobulin |
| IgA,D,E,G,M | **I**mmuno**g**lobulin **A, D, E, G, M** |
| IgH | **I**mmuno**g**lobulin **H**eavy chain |
| Igγ | **I**mmuno**g**lobulin ***g**amma* heavy chain |
| Igκ | **I**mmuno**g**lobulin ***k**appa* light chain |
| Igλ | **I**mmuno**g**lobulin ***l**ambda* light chain |

| | |
|---|---|
| IL | **I**nter**l**eukin |
| IMGT | **I**m**M**uno**G**ene**T**ics information system |
| J | **J**oining |
| kb | **k**ilo **b**ase |
| LB | **L**uria **B**ertani |
| LPS | **L**ipo**P**oly**S**accharide |
| mAb | **m**onoclonal **A**nti**b**ody |
| mRNA | **m**essenger **R**ibo**N**ucleic **A**cid |
| n | **n**umbers |
| NCBI | **N**ational **C**enter for **B**iotechnology **I**nformation |
| NP-40 | **N**onidet **P-40** |
| OD | **O**ptical **D**ensity |
| PBS | **P**hosphate **B**uffered **S**aline |
| PCR | **P**olymerase **C**hain **R**eaction |
| PE | **P**hyco**E**rythrin |
| PEI | **P**oly**E**thylen**I**mine |
| RAG 1 and 2 | **r**ecombination **a**ctivating **g**ene **1** and **2** |
| rpm | **r**ounds **p**er **m**inute |
| RPMI | **R**oosevelt **P**ark **M**emorial **I**nstitute |
| RT | **R**oom **T**emperature |
| RT-PCR | **R**everse **T**ranscription - **P**olymerase **C**hain **R**eaction |
| SHM | **S**omatic **H**yper**M**utation |
| SLE | **S**ystemic **L**upus **E**rythematosus |
| SSC | **S**ide **SC**atter |
| ssDNA | **s**ingle-**s**tranded **D**esoxyribo**N**ucleid **A**cid |
| Taq polymerase | **T**hermus **aq**uaticus polymerase |
| TAE buffer | **T**ris-**A**cetate-**E**DTA buffer |
| TB | **T**errific **B**roth |
| T cell | **T**hymus-derived cell |
| Temp | **Temp**erature |
| TLR | **T**oll-**L**ike **R**eceptor |
| U | **U**nits |
| UV | **U**ltra**V**iolet |
| V | **V**ariable |

# 1 INTRODUCTION

## 1.1 The Immune System

Vertebrates have evolved an elaborate protective immune system consisting of an innate part and an adaptive part to successfully eliminate disease-causing pathogens. Both components provide a dynamic network of cells and molecules that discriminate between self and foreign to efficiently defend the organism against invading pathogens. Innate immune mechanisms are activated immediately during an immune response and preformed, non-specific receptors recognize common surface patterns on pathogens. These innate immune responses activate the adaptive immune system, where lymphocytes detect pathogen-specific epitopes via antigen-specific receptors. The millions of different lymphocyte clones are able to detect virtually all foreign antigens in a highly specific way. Specific antigen recognition is based on clonal selection, where an antigen selectively stimulates those cells that express complementary antigen-specific receptors. Under normal immune conditions, the immune system provides an immediate, specific and efficient immune response against pathogens. However, alterations in this complex network might foster autoimmune reactions, eventually leading to the development of autoimmune diseases.

## 1.2 Immunoglobulins

Immunoglobulins (Igs) are glycoproteins that are synthesized and expressed by B lymphocytes either on their cell surface as part of the B cell antigen receptor (BCR) or in a soluble form secreted by plasma cells as so-called antibodies. Each B cell has approximately $10^5$ such BCRs in its plasma membrane (Alberts et al., 2002) and all Igs expressed by one B lymphocyte have the same antigen specificity (Nossal and Lederberg, 1958). Membrane-bound Igs form the antigen-binding unit of the BCR and are associated with the transmembrane signaling proteins Ig$\alpha$ and Ig$\beta$ (Reth, 1995).

The basic structural unit of an Ig consists of two identical heavy (H) chains that are covalently linked by disulfide bonds to two identical light (L) chains. Each heavy and light chain can be divided into a variable region (V) that determines the antigen specificity and a constant region that mediates distinctive effector functions (Figure 1). The diversity in the V regions is mostly attributed to three hypervariable, complementarity-determining regions (CDRs) in each chain, while the framework regions (FWRs) provide the structural backbone. CDR3 is the most

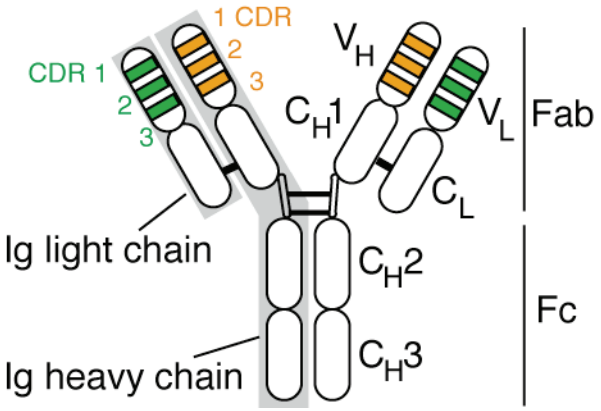



**Figure 1. Schematic Structure of an IgG Antibody.**
Immunoglobulins are composed of two identical heavy and light chains, which are linked by disulfide bonds. The Fc portion of the antibody bears the constant region (C-region), thus determining the effector function of the molecule. The antigen binding portion (Fab) is linked to the Fc portion by a hinge region and contains a variable region (V region). The complementarity-determining regions (CDRs) contribute to the antigen binding site (modified, Tiller, 2009).

variable domain among the CDRs and constitutes a dominant part in antibody specificity (Parhami-Seren and Margolies, 1996). The human germline encoded constant region can be classified into five major isotypes (IgM, IgD, IgG, IgE, and IgA) that determine the molecular structure of an individual Ig and the biological response following antigen binding (Janeway et al., 2008). In addition to five classes of Ig heavy chains, higher vertebrates have two types of Ig light chains, κ and λ, which do not seem to mediate functional properties other than its antigen specificity.

## 1.3 Generation of Immunoglobulin Diversity

The enormous diversity of potentially dangerous molecules requires a virtually unlimited antibody repertoire to efficiently protect the individual against infections. The main mechanisms of immunoglobulin diversity occur early in lymphocyte development in the fetal liver and adult bone marrow. The combinatorial joining of separate gene segments in a process called somatic recombination enable the mammalian immune system to generate an almost unlimited number of different heavy and light chains in a highly economic manner (Tonegawa, 1983).

Three separate chromosomes carry the gene segments for IgH, Igκ and Igλ chain assembly and differ slightly in their organization (Lefranc and Lefranc, 2001). The assembly of different combinations of inherited variable (V), diversity (D), and joining (J) gene elements at one allele allows for combinatorial diversity and is accomplished by an orderly sequence of site-

specific rearrangement mediated by the recombination activating gene (RAG) encoded enzymes RAG1 and RAG2 (Oettinger et al., 1990). The IgH chain locus on chromosome 14 contains about 46 functional variable (V) region gene segments, 27 diversity (D) gene segments and 6 joining (J) gene segments allowing 7,452 different functional IgH chain rearrangements. The Igκ locus is mapped to chromosome 2 and harbours approximately 37 functional V gene segments and 5 J gene segments facilitating 185 different functional Igκ chain recombinations. The Igλ locus on chromosome 22 contains about 35 functional V gene segments and 4 J gene segments facilitating 140 different functional Igλ chain rearrangements. The pairing of a functional Ig heavy chain with any functional Ig light chain thus allows the generation of about 2.4 million different BCRs during early B cell development. Ig gene rearrangement starts at the heavy chain locus and is terminated as soon as a productive IgH

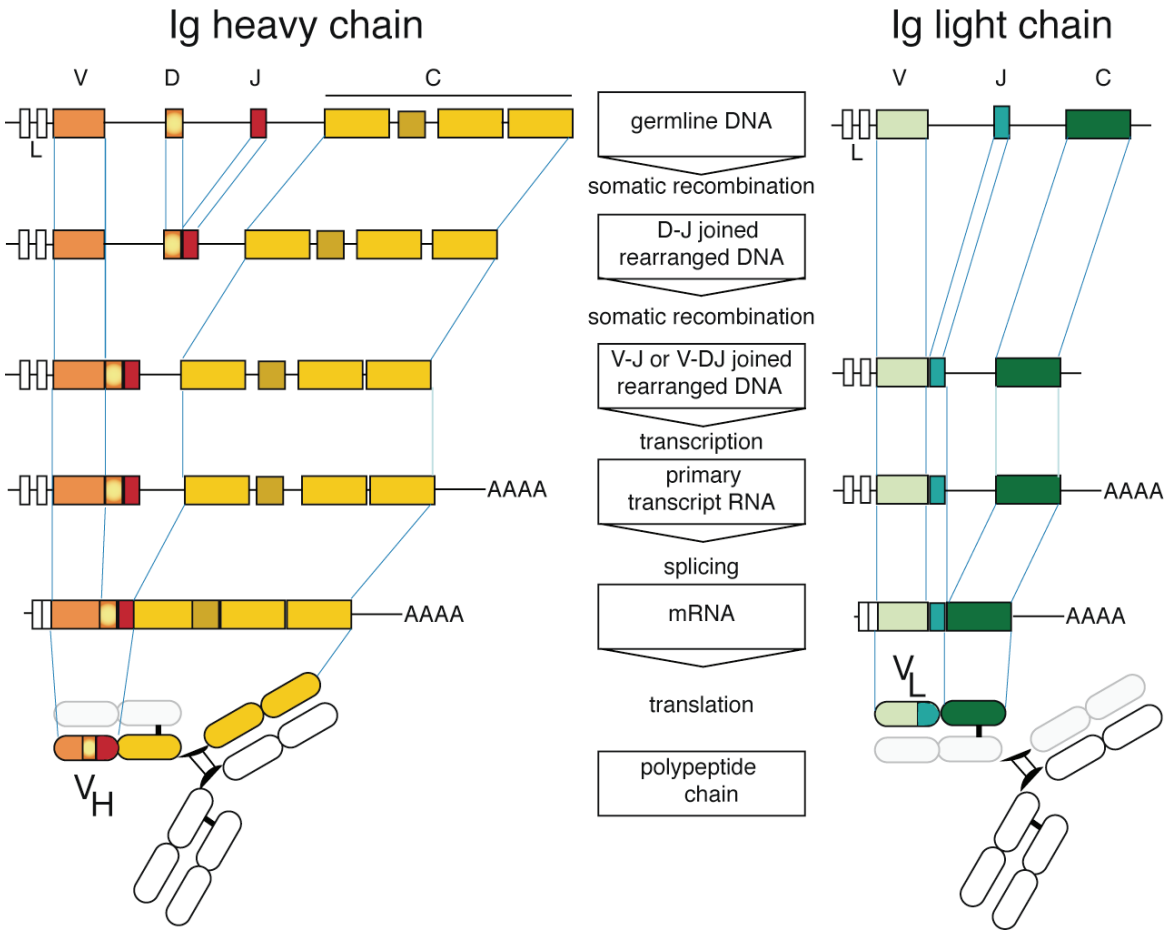



**Figure 2. Assembly of an Immunoglobulin.**
The heavy chain V region ($V_H$) consists of three gene segments. After linking a D element to a J part, the DJ gene segment is fused to the V gene element. The C regions, encoded by one or more exons, are joined to the $V_H$ or $V_L$ exon by RNA splicing. To assemble a complete light chain gene ($V_L$) from genomic DNA, a V gene segment is combined with a J gene portion (modified, Janeway et al., 2008).

chain is expressed (Figure 2; Alt et al., 1984). IgL chain rearrangement usually occurs first at a κ allele and if that fails it either takes place at the second κ allele or at a λ allele (Hieter et al., 1980; Hieter et al., 1981a ; Hieter et al., 1981b; Korsmeyer et al., 1981; Nemazee and Weigert, 2000; van der Burg et al., 2001). During V(D)J recombination, recombination signal sequences flanking each gene segment ensure that only appropriate gene segments combine (Tonegawa, 1983). Additional diversity is provided by imprecise joining due to random loss and gain of nucleotides at the gene segment joining sites. This junctional diversification facilitates a high level of diversity in heavy and light chain CDR3s, which has evolved to be especially important in antibody specificity.

## 1.4 Immunological Memory and Plasma Cells

The most important biological consequence of adaptive immunity is the establishment of immunological memory (Sallusto et al., 2010). Immunological memory is generated by both lymphocyte differentiation and clonal expansion, where activated lymphocytes establish a tailor-made immune response to a particular pathogen and mount an accelerated and enhanced response upon antigen reencounter (Nossal et al., 1965).

When a naïve B cell binds to a foreign antigen and receives an additional co-stimulatory signal in a peripheral lymphoid organ, it either proliferates and differentiates into a short-lived plasma cell secreting low-affinity antibodies or is recruited into a germinal center (GC; Figure 3; Shapiro-Shelef and Calame, 2005). Germinal center B cells undergo antigen-dependent proliferation in response to antigen presentation by follicular dendritic cells (FDCs) and co-stimulatory signals by T helper cells. During the GC reaction, the enzyme activation-induced cytidine deaminase (AID) alters the B cell receptor affinity for an antigen by introducing somatic hypermutations (SHM; Berek and Milstein, 1988). This process is termed affinity maturation and enables positive selection based on the BCR affinity for the respective antigen. However, affinity maturation increases the BCR affinity only in a few B cells. These few B cell clones with higher affinity are preferentially stimulated by an antigen, proliferate and eventually differentiate either into memory B cells or into plasma cells (Klein and Dalla-Favera, 2008). Class-switch recombination (CSR) of the IgH chain, again mediated by AID, changes the Ig isotype and enables a more distinctive effector function of the secreted antibody while retaining its specificity (Muramatsu et al., 2000).

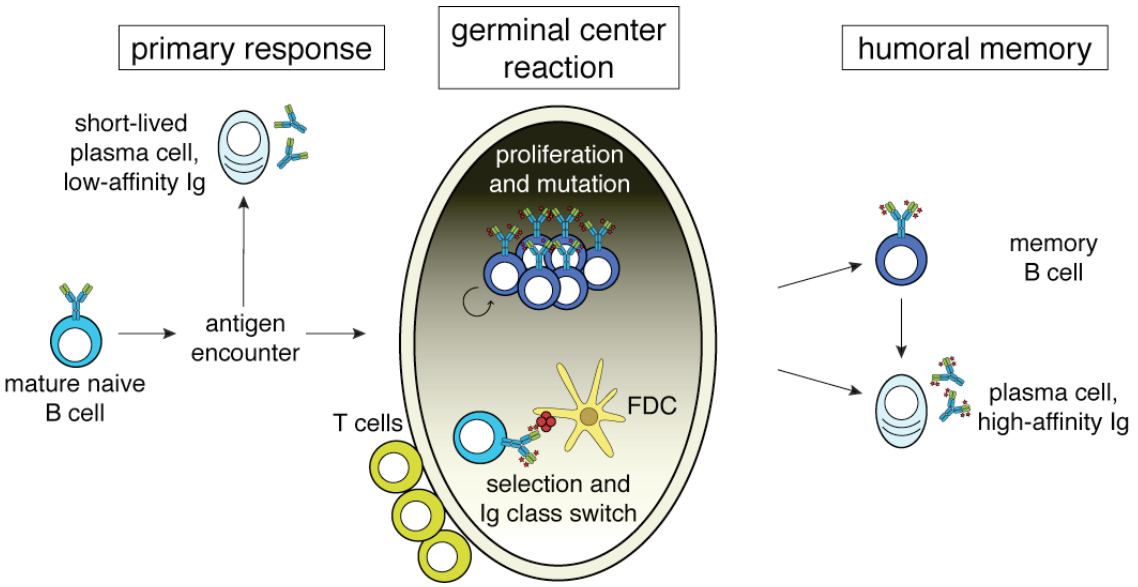



**Figure 3. Overview of B cell Activation.**
After antigen encounter, mature naïve B cells either differentiate into short-lived plasma cells, secreting low-affinity Ig, or undergo affinity maturation in germinal centers. During germinal center reactions, B cells can acquire somatic hypermutations (red stars) and undergo Ig class switch recombination when help by follicular dendritic cells (FDCs) and T cells is provided. As a result of positive selection during affinity maturation, B cells are selected into the memory B cell pool or the long-lived plasma cell pool, secreting high-affinity Igs (modified, Shapiro-Shelef and Calame, 2005).

Following vaccination or infection, persistent levels of specific antibodies are detectable in human serum for decades (Hammarlund et al., 2003; Manz et al., 2005; Amanna et al., 2007). These serum antibody levels are sustained by memory B cells and plasma cells that were positively selected during germinal center reactions. How selection into the memory cell or plasma cell compartment takes place, is still under investigation. A current model proposes that long-lived memory B cells are not as rigidly selected as long-lived plasma cells (Tarlinton, 2008; Amanna and Slifka, 2010). Experimental evidence from studies in mice demonstrated that the differentiation choice to become a plasma cell requires a positive selective step based on high affinity and the strength of interaction between antigen and BCR (Smith et al., 1997; Takahashi et al., 1998; Smith et al., 2000; Paus et al., 2006; Phan et al., 2006). Memory B cells are long-lived and express membrane-bound Igs but are quiescent in the absence of antigen. Upon activation, they readily proliferate and give rise to more memory B cells and antibody-secreting plasma cells with the same antigen-specificity (Lanzavecchia and Sallusto, 2009). The majority of GC-derived plasma cells migrate to the bone marrow (Smith et al., 1997; Smith et al., 2000). The bone marrow is the principal site of antibody production (MacMillan et al., 1972) although less than 0.5 % of mononuclear cells in human bone marrow

from healthy individuals are plasma cells (Terstappen et al., 1990; Hiepe et al., 2011). Terminally differentiated plasma cells selected in germinal center reactions synthesize and secrete up 30,000 antibody molecules per second (Hibi and Dosch, 1986), but do not proliferate, and lose the ability to sense antigenic changes or T cells in their environment (Amanna and Slifka, 2010). Moreover, human terminally differentiated plasma cells are demonstrated to be long-lived (Hammarlund et al., 2003; Amanna et al., 2007) and protect from infection through the production of high affinity antigen-specific serum antibodies (Manz et al., 2005; Radbruch et al., 2006).

### 1.5 Central and Peripheral B Cell Tolerance

The enormous diversity of the Ig repertoire acquired by random somatic V(D)J recombination and affinity maturation also generates BCRs that recognize self-antigens from the individual and have the potential of becoming pathogenic (Burnet, 1972). In healthy individuals, a series of checkpoints purge autoreactive B cells from the repertoire, both centrally in the bone marrow during B cell development and in peripheral lymphoid tissues (Goodnow et al., 1995). The outcome of these selection processes is a marked narrowing of the Ig repertoire (Melchers et al., 1995; Loder et al., 1999).

Central tolerance is established in the fetal liver and adult bone marrow prior to the first antigen encounter, where immature B cells are censored for reactivity with ubiquitous membrane-bound self-antigens. To prevent autoimmunity, self-reactive B cells are induced to either undergo clonal deletion, BCR editing or anergy. Clonal deletion is characterized by self-antigen induced apoptosis of autoreactive B cells (Nemazee and Burki, 1989). B cell receptor editing is the main mechanism of central tolerance (Retter and Nemazee, 1998; Casellas et al., 2001; Halverson et al., 2004) and is conducted by secondary Ig gene rearrangements that generate a new antigen receptor with an innocuous specificity (Gay et al., 1993; Radic et al., 1993; Tiegs et al., 1993). Immature B cells can become functionally unresponsive to antigens due to recurrent exposure to an antigen (Nossal and Pike, 1980; Goodnow et al., 1988). These anergic B cells can emigrate from the bone marrow into T cell zones of secondary lymphoid tissues (Cornall et al., 1995).

The processes of central tolerance remain incomplete, allowing some self-reactive B cells to escape into the periphery (Nemazee and Sato, 1983; Souroujon et al., 1988; Shlomchik et al., 1993). Further, affinity maturation in germinal centers generates BCRs with

the potential to recognize self-antigens (Rajewsky, 1996) and thus mechanisms censoring autoreactive BCRs in germinal centers are essential for the establishment of tolerance. Peripheral tolerance mechanisms are less well defined, but further purge self-reactive B cells from the normal B cell repertoire (Meffre and Wardemann, 2008). One cardinal feature of peripheral checkpoints is the balance between stimulatory and inhibitory signals that regulate the activation and expansion of B cells (McGaha et al., 2005). To become activated in a peripheral lymphoid organ, a B cell must receive at least two different signals, i.e. binding to its antigen and an additional co-stimulatory signal (Goodnow et al., 2010).

## 1.6 Autoimmunity

A low level of autoreactivity is physiological and crucial to normal immune function (Dighiero and Rose, 1999; Wardemann et al., 2003; Tiller et al., 2007). However, autoimmunity develops when multiple tolerance checkpoints are eventually bypassed (Goodnow, 2007). A breakdown in tolerance to self-antigens results in the development of autoantibodies that lead to a variety of pathological outcomes (McGaha et al., 2005). Before B cells become sufficiently dysregulated to give rise to autoimmunity, a cooperation between T cell and B cell defects is required (Lipsky, 2001). Epitope spreading, i.e. an increase in the number of autoantigens targeted by lymphocytes, is often observed as an autoimmune disease progresses from initial activation to a chronic inflammatory state (Davidson and Diamond, 2001). Chronic inflammation provides a constantly replenished supply of antigens and can trigger polyclonal activation (Goodnow, 2007). Activated autoreactive B cells then act as antigen-presenting cells and present novel peptides of autoantigens together with costimulatory molecules (Foreman et al., 2007). Genetic factors are often crucial determinants of susceptibility to autoimmune disease and work in concert to generate an abnormal phenotype (Davidson and Diamond, 2001). However, some environmental trigger is usually the prerequisite for developing an autoimmune disease, although the trigger is not known for most autoimmune diseases.

## 1.7 Systemic Lupus Erythematosus and Autoantibodies

### 1.7.1 Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease with a prevalence of 10 in 40,000 among Northern Europeans that predominantly affects young

women of reproductive age (Kotzin, 1996; Rahman and Isenberg, 2008). Lupus usually fluctuates over time with a relapsing-remitting nature (Davidson and Diamond, 2001) and is driven by an immune response directed against ubiquitous, mostly intranuclear, self-antigens resulting in an inflammation of affected organs (Sherer et al., 2004; Crispín et al., 2010). High-affinity IgG autoantibodies are a hallmark of the disease and affect various organs directly with kidney and skin being studied most intensively (Rahman and Isenberg, 2008). Diagnosis is usually based on laboratory and clinical abnormalities summarized in the classification criteria from the American College of Rheumatology, where any four of eleven criteria should be fulfilled (Table 1; Tan et al., 1982; Hochberg et al., 1997). To assess disease activity, the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) is employed, where organ manifestations of the last 10 days are evaluated. SLEDAI scores vary between 0 and 105, with values above 10 characterizing high disease activity (Petri, 2007).

The development of SLE depends on both genetic predisposition and environmental triggers (Rahman and Isenberg, 2008). Amplification loops comprising components of the innate and the adaptive immune system drive the activation of autoreactive B cells and the production of autoantibodies, which in turn sustain inflammatory cascades (Harley et al., 2009). Experimental evidence shows that lupus pathogenesis develops as an aberrant response to cell death (Pisetsky and Ronnblom, 2009), where deficiency in complement may be an important reason for the poor waste disposal observed (Rahman and Isenberg, 2008).

**Table 1. Classification Criteria for the Diagnosis of Systemic Lupus Erythematosus** (Tan et al., 1982; Hochberg, 1997).

| # | Criterion |
|---|---|
| 1 | Malar Rash (Rash on Cheeks) |
| 2 | Discoid Rash (Circular Raised Patches) |
| 3 | Photosensitvity |
| 4 | Oral Ulcers |
| 5 | Arthritis (Nonerosive Arthritis at Two or More Peripheral Joints) |
| 6 | Serositis (Pleuritis or Pericarditis as Documented by Electrocardiography) |
| 7 | Renal Disorder (Proteinurea) |
| 8 | Neurologic Disorder (Seizures or Psychosis) |
| 9 | Hematologic Disorder (Hemolytic Anemia or Leukopenia or Lymphopenia or Thrombocytopenia) |
| 10 | Immunologic Disorder (Positive for anti-dsDNA, anti-Sm and/or antiphospholipid Antibodies) |
| 11 | Antinuclear Antibodies (Abnormal Titer as Determined by IFA or an Equivalent Assay) |

Moreover, T cell help is crucial in antigen-driven affinity maturation and facilitates the generation of class-switched high-affinity autoantibodies that are closely linked to tissue damage in SLE. Standard treatment in SLE is aimed at preventing flares and avoiding end-organ damage, which is mainly achieved by corticosteroids and anti-malarial drugs (Crispín et al., 2010). However, high serum ANA titers are present before the onset of clinical symptoms (Arbuckle et al., 2003; Rahman and Isenberg, 2008) and pathogenic serum ANAs frequently persist during treatment induced remission of clinical symptoms.

### 1.7.2 The Role of B cells in SLE

Genetic, immunological and clinical observations place B cells at the center of lupus pathogenesis as initiating, amplifying and effector cells (Sanz and Lee, 2010), as SLE fails to develop in the absence of B cells (Shlomchik et al., 1994). B cells in SLE produce an array of pathogenic IgG autoantibodies constituting the main immunological events in lupus pathogenesis (Lipsky, 2001; Zouali, 1997). In particular, high affinity anti-DNA IgG antibodies are highly specific for SLE and are frequently linked to glomerulonephritis in SLE patients (Hahn, 1998).

The production of autoantibodies might occur through a two-stage mechanism where the preactivation of the overall Ig repertoire is preceded by the conversion of nonpathogenic BCRs into pathogenic autoantibodies due to affinity maturation (Zouali, 1997). Analysis of monoclonal antibodies cloned from single B cells at different stages during their maturation revealed that the central tolerance checkpoints are violated in SLE (Figure 4; Wardemann et al., 2003; Meffre et al., 2004; Yurasov et al., 2005). Further, autoreactive B cells are not excluded from germinal center reactions in SLE patients (Cappione et al., 2005) and thus enable the generation of autoreactive memory B cells and plasma cells. However, frequencies of autoreactive IgG+ memory B cells in SLE patients are similar to those of healthy donors (Figure 4; Tiller et al., 2007; Mietzner et al., 2008). As long-lived plasma cells are the major contibutor to serum IgG levels (Benner et al., 1981), their role in human SLE pathogenesis should be further elucidated.

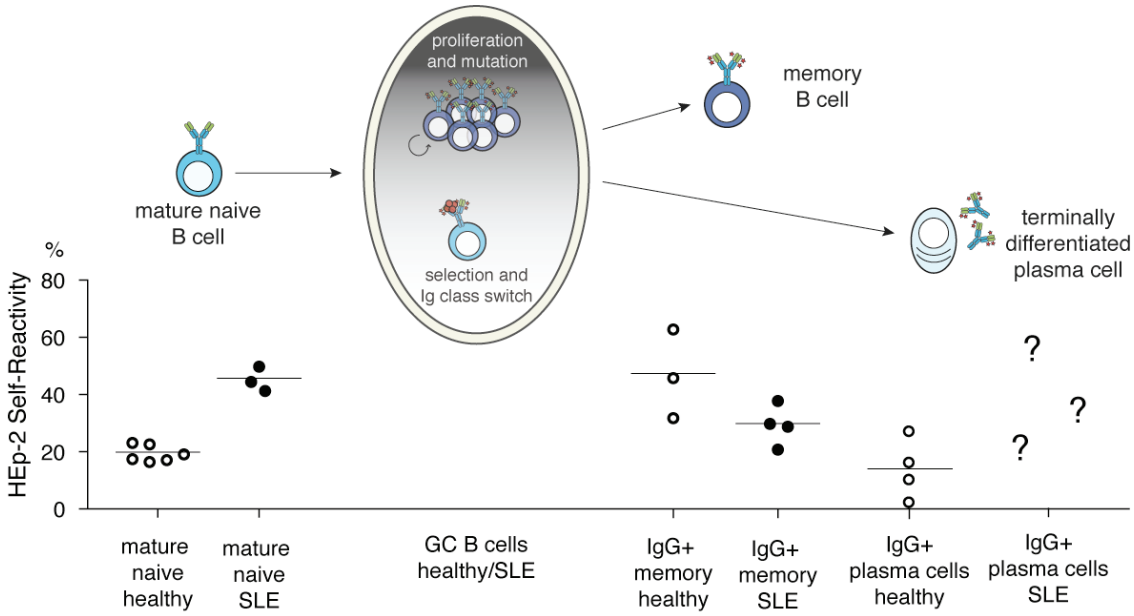



**Figure 4. B Cell Tolerance Checkpoints in Healthy Humans and SLE Patients.**
Recombinant monoclonal antibodies isolated during different B cell stages were tested for self-reactivity with HEp-2 cells by ELISA and IFA. Dots represent individual donors (white, healthy donor; black, SLE patient), horizontal bars indicate averages (modified, Meffre and Wardemann, 2008; Scheid et al., 2011).

# 2 AIM

Long-term humoral immunity is sustained by the formation of memory B cells and long-lived antibody-secreting plasma cells that have undergone antigen-mediated selection during germinal center responses. Previous single cell studies have elegantly dissected the immunoglobulin (Ig) gene repertoire and antibody reactivity profile of circulating IgG+ memory B cells and have demonstrated an enrichment for self-reactive antibodies in this compartment that mainly arise from non-reactive or polyreactive precursors by somatic mutations. Immunological niches for human long-lived plasma cells are primarily located in the bone marrow, where plasma cells survive for decades and continuously secrete large amounts of protective antibodies. The Ig gene molecular features and the frequency of self-reactivity of this B cell compartment were recently assessed in healthy individuals. The data suggest that in contrast to the development of memory B cells, entry into the bone marrow plasma cell compartment is tightly controlled by self-tolerance checkpoints that thereby prevent the production of self- and polyreactive serum IgG antibodies.

Antibody secreting plasma cells may be directly involved in the pathogenesis of systemic lupus erythematosus (SLE) by secreting high affinity self-reactive antibodies, but little is known about the molecular features and antibody reactivity profiles of long-lived plasma cells in SLE patients. The aim of this work was to analyze the Ig gene repertoire and the extent of self-reactivity in the IgG+ bone marrow plasma cell compartment in SLE patients. Antibodies from single IgG+ bone marrow plasma cells of five patients were cloned and expressed to assess the reactivity profiles. To evaluate the contribution of somatic hypermutation to antibody reactivity, poly- and self-reactive antibodies from one patient were reverted into their Ig germline configuration and their reactivity was compared to the mutated counterparts. The results provide new insights into the properties of terminally differentiated IgG+ plasma cells in SLE and might foster further understanding of the disease.

# 3 METHODS

## 3.1 Strategy Outline

To analyze the Ig gene features and antibody reactivity profiles of human plasma cells, a library of recombinant monoclonal antibodies was generated from single isolated primary IgG+ plasma cells from bone marrow of five SLE patients. Single bone marrow plasma cells were sorted by flow cytometry according to cell surface markers (Figure 5). For each individually sorted plasma cell, IgH and IgL chain variable region genes were amplified by nested RT-PCR with primer mixes (Figure S4). Human Ig gene sequences were analyzed and classified according to the international ImMunoGeneTics nomenclature by using the National Center for Biotechnology IgBLAST database. Defined restriction sites were introduced by PCR for IgG+ plasma cells to allow convenient ligation of the obtained IgH and IgL PCR products into

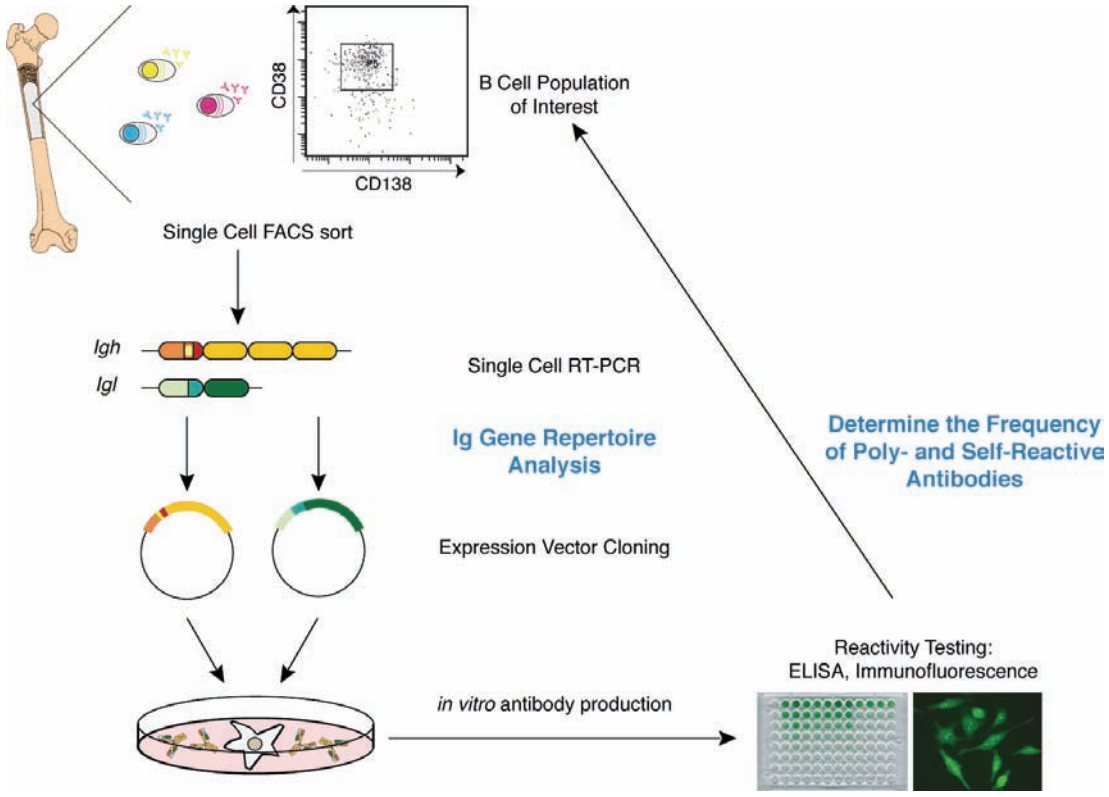



**Figure 5. Experimental Strategy.** Single plasma cells from human bone marrow were isolated by fluorescence activated cell sorting (FACS). IgH chain and corresponding IgL chain transcripts were amplified for each individual plasma cell by nested RT-PCR, classified by Ig gene sequence analysis and cloned into eukaryotic expression vectors. The vectors were co-transfected into HEK 293T cells to produce monoclonal antibodies of the same specificity *in vitro*. Recombinant antibodies were tested for reactivity with diverse self- and non-self antigens by ELISA and IFA experiments and the frequency of poly- and self-reactive antibodies was determined (Tiller et al., 2008).

eukaryotic expression vectors containing the appropriate human immunoglobulin constant regions. After amplification of expression vectors in *Escherichia coli* and subsequent plasmid DNA purification, the plasmids encoding for the Igγ chain and the corresponding Igκ or λ light chains were transiently co-transfected into human embryonic kidney 293T cells (HEK 293T). Cell culture supernatants containing the recombinant human immunoglobulins were collected and the antibodies were purified. To test for the reactivity of the monoclonal antibodies, binding analyses such as enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assays (IFAs) were conducted.

## 3.2 Fluorescence-Activated Cell Sorting (FACS)

Bone marrow aspirate from five SLE patients was collected after signed informed consent in accordance with protocols reviewed by the Institutional Review Board of the Charité University Medical Center. Mononuclear Cells were purified from bone marrow by Ficoll-Paque® density gradient centrifugation and plasma cells from patients SLE1 and SLE2 were pre-enriched using CD138 magnetic beads (Miltenyi Biotec). Due to small sample sizes and low absolute mononuclear counts, CD138-pre-enrichment was not conducted for patients SLE3-SLE5. Bone marrow mononuclear cells or pre-enriched CD138+ bone marrow cells were stained on ice with anti-CD19-PECy7, anti-CD27-FITC, anti-CD38-APC and anti-CD138-PE antibodies (Becton Dickinson) according to standard staining protocols. Single plasma cells were sorted into 96-well PCR plates containing 4 µl lysis solution (0.5 x PBS containing 10 mM DTT, 8 U RNAsin (Promega)) using a FACSVantage cell sorter with Diva configuration (Becton Dickinson). PCR plates were sealed with Microseal 'F' film (Bio-Rad), immediately frozen on dry ice and stored at -80 °C until further processing.

## 3.3 Reverse Transciption Polymerase Chain Reaction (PCR)

### 3.3.1 cDNA Synthesis

Total RNA from single cells was reverse transcribed in nuclease-free water (Eppendorf) using 150 ng random hexamer primer (pd(N)6, Roche Applied Science), 0.5 mM dNTP (Invitrogen), 10 mM DTT, 0.5 % v/v Igepal CA-630 (Sigma), 14 U RNAsin (Promega) and 50 U Superscript® III Reverse Transcriptase (Invitrogen). cDNA was synthesized in a total

volume of 14 µl in the original 96-well sorting plate. Reverse transcription reaction was performed for 60 min at 50 °C.

### 3.3.2 Nested PCR for Amplification of Human Ig Gene Transcripts

Human Igγ, Igκ and Igλ gene transcripts were amplified independently by nested Polymerase Chain Reaction (PCR) starting from 3 µl cDNA as template (Figure S4). All PCR reactions were performed in 96-well plates in a total volume of 40 µl per well containing 7 pmol of each primer or primer mix (see page 76, Primer Sequences for Amplification of Human Ig Genes), 12 nmol of each dNTP (Invitrogen) and 1.2 U HotStar® Taq DNA polymerase (Qiagen) in nuclease-free water. All nested PCR reactions with gene-specific primers or primer mixes were performed with 3.5 µl of unpurified first PCR product. Each round of PCR was conducted for 50 cycles to assure sensitivity with a hot start reaction at 94 °C that is necessary to activate the enzyme (Table 2).

**Table 2.** PCR program for amplification of human Ig gene transcripts.

| **Step** | **Temperature** | **Duration** |
|---|---|---|
| Activation of HotStar Taq® | 94 °C | 15 min |
| | 50 cycles | |
| Denaturation | 94 °C | 30 s |
| Annealing | 58 °C (Igγ, Igκ)<br>60 °C (Igλ) | 30 s |
| Elongation | 72 °C | 55 s (1st PCR)<br>45 s (2nd PCR) |
| Final Elongation | 72 °C | 10 min |
| Hold | 4 °C | ∞ |

To identify amplification products of appropriate size, three microliter aliquots of the second PCR products were mixed with an equal amount of 60 % sucrose loading dye and were run on a 2 % agarose gel in TAE buffer for 25 min at 120 V. Gels were stained in aqueous ethidium bromide solution for 10 min and DNA bands were visualized under UV light. Expected PCR product sizes for amplified Ig gene transcripts are 450 bp for Igγ, 510 bp for Igκ and 405 bp for Igλ.

### 3.3.3 Reversion Strategy for Mutated Ig Heavy and Light Chain Genes

An overlap-PCR strategy was applied to revert mutated Ig heavy and light chain transcripts into their unmutated germline counterparts (Figure S6; Tiller et al., 2008). Unmutated germline V genes were amplified from previously cloned unmutated templates with gene-specific forward primers containing the AgeI restriction site and individual gene-specific reverse primers that anneal to the 3' end of the FWR3. Mutated CDR3-J sequences were reverted independently by PCR using individual primers containing a minimal complementarity of 10 nucleotides to the germline V gene transcript. Reverse J gene-specific primers included the respective restriction sites as indicated (Figure S6). PCRs were performed at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 45 s for 30 cycles (see also Table 2; according to conditions for γ,κ and λ, 2nd PCR). Equal amounts of the reverted V and CDR3-J gene PCR products were fused under the same conditions in a 20-cycle overlap PCR under the same conditions. Full-length reverted V(D)J gene PCR products were gel extracted before digestion and purified before cloning into the respective expression vectors. Gene identitiy and the successful reversion of somatic mutations were confirmed by sequence analysis of the cloned products, as described below.

## 3.4 Ig Gene Sequence Analysis

All samples were sequenced by Eurofins MWG Operon (http://www.eurofinsdna.com). Four microliter aliquots of the Igγ, Igκ and Igλ chain second PCR products were sequenced in a final volume of 20 µl. Respective reverse primer for sequencing (see page 76, Primer Sequences for Amplification of Human Ig Genes) were provided at a concentration of 10 µM according to the instructions of Eurofins MWG Operon. Obtained human Ig gene sequences were analyzed for Ig gene usage and CDR3 analysis, the number of V gene mutations by IgBLAST comparison with Genbank (http://www.ncbi.nlm.nih.gov/igblast/) and the IgG isotype subclass (http://imgt.cines.fr). IgH CDR3 length was determined as indicated in the IgBLAST result by counting the amino acid residues following framework region (FWR)3 up to the conserved tryptophan-glycine motif in all JH segments or up to the conserved phenylalanin-glycine motif in Jκ and Jλ segments (Kabat and Wu, 1991). In addition, the number of positively charged (histidine,

arginine, lysine) and negatively charged (aspartate, glutamate) amino acids were determined for each Ig heavy and Ig light chain CDR3.

## 3.5 Directional Cloning into Expression Vectors

### 3.5.1 Preparation of Eukaryotic Expression Vectors

Igγ, Igκ, or Igλ expression vectors (Figure S5) with human Ig heavy chain gamma1 (γ1), Ig light chain kappa (κ) or Ig light chain lambda (λ) constant regions, respectively, and an ampicillin resistance gene were cloned in *Escherichia coli* (*E. coli*) DH10B bacteria. Vectors were prepared using the NucleoBond Xtra Maxi Kit from Macherey-Nagel. The manufacturer's instructions were followed. After plasmid purification, the DNA concentration was determined by NanoDrop spectrophotometer and 25 µg of circular vector DNA were linearized using the appropriate restriction enzymes.

### 3.5.2 Restriction Endonuclease Digestion

Second PCR products for Igλ genes contained restriction sites allowing direct cloning into expression vectors (Tiller et al., 2008). For Igγ and Igκ genes, restriction sites were introduced after sequencing by gene-specific primers and first PCR products as template. Purified PCR products or circular vector DNA were digested in two successive steps in a total volume of 40 µl with restriction enzymes (NEB) under optimal buffer conditions for the indicated duration (Table 3).

**Table 3.** Conditions for Endonuclease Digestion.

| | 1st Enzyme | Temp | Duration | 2nd Enzyme | Temp | Duration |
|---|---|---|---|---|---|---|
| γHeavy Chain | Age I | 37 °C | 2 h | Sal I | 37 °C | 2 h |
| κLight Chain | Age I | 37 °C | 2 h | BsiW I | 55 °C | 2 h |
| λLight Chain | Age I | 37 °C | 2 h | Xho I | 37 °C | 2 h |

To test for successful and complete linearization of the digested expression vectors, gel electrophoresis was performed using an 0.8 % agarose gel. Linearized expression vectors and digested PCR products were purified by using the NucleoSpin Extract II Kit (Macherey-Nagel) to obtain pure DNA as basic requirement for an efficient ligation reaction.

### 3.5.3 Ligation

The ligation was conducted for 90 min at room temperature in 96-well plates in a total volume of 10 µl per well with 200 U T4 DNA ligase (NEB), 8 µl of digested, purified PCR product and 25 ng linearized expression vector. Ligation products were immediately transformed into competent *E. coli* DH10B or stored at -20 °C.

### 3.5.4 Preparation of Competent Bacteria

The *E.coli* strain DH10B (Invitrogen) was used for transformation of recombinant expression vector constructs. Competent bacteria were prepared by inoculating one freshly grown *E.coli* DH10B colony in 5 ml Luria Bertani (LB) medium and were allowed to grow in a bacteria shaker incubator overnight at 37 °C and 240 rpm. This preculture was transferred into 500 ml LB medium and bacteria were cultivated to an optical density ($OD_{600}$) between 0.6 and 0.8, where *E.coli* bacteria are in the exponential growth phase. Bacteria were kept on ice for 30 min and after 10 min of centrifugation at 1500 $g_{av}$, bacterial pellets were resuspended in ice-cold sterile 180 ml 0.1 M $CaCl_2$ solution. The cells were centrifuged again for 10 min at 1500 $g_{av}$ and resuspended in 5 ml 0.1 M $CaCl_2$ containing 15% glycerol. Aliquots of competent bacteria suspensions were stored at -80 °C. Transformation efficiency of bacteria was determined by calculating how many colonies were grown per µg of added DNA. Bacteria were transformed with different amounts of DNA in the range from 0.1 ng to 20 ng following the protocol described below. The number of obtained colonies was divided by the amount of DNA (µg) and multiplied with the ratio of the final volume (ml) at recovery and the volume (ml) plated. Typical transformation efficiencies ranged from $4 \times 10^5$ to $10^6$ colonies per µg DNA.

### 3.5.5 Transformation of Competent Bacteria

Five microliter of competent *E. coli* DH10B bacteria were transformed with 3 µl of the ligation product in 96-well plates. After 30 min incubation on ice, a heat shock was performed at 42 °C for 45 seconds and bacteria were allowed to grow in 200 µl LB medium for 30 min under moderate shaking at 37 °C. 100 µl were plated on LB plates containing 100 µg/ml ampicillin and plates were incubated overnight at 37 °C.

### 3.5.6 Screening Bacterial Colonies by PCR

Bacterial colonies were screened for the presence of appropriately sized inserts by PCR (Table 4). The 5' oligonucleotide Ab sense used for all insert check reactions is complementary to a sequence in the vector upstream of the insert site. The 3' oligonucleotide for heavy chain inserts (IgG internal) binds to the constant γ region of the plasmid. For Igκ and Igλ light chain insert amplification, the 3' oligonucleotides were 3' Cκ494 and 3' Cλ, binding to the human constant κ or λ region of the vector, respectively. The PCR products were analyzed by 2 % agarose gel electrophoresis to confirm the expected sizes (650 bp for Igγ1, 700 bp for Igκ and 590 bp for Igλ). Amplicons of the correct size were sequenced to confirm identity with the original PCR products.

**Table 4.** PCR program for Screening Bacterial Colonies.

| **Step** | **Temperature** | **Duration** |
|---|---|---|
| Activation of HotStar Taq® | 94 °C | 15 min |
| | 27 cycles | |
| Denaturation | 94 °C | 30 s |
| Annealing | 58 °C | 30 s |
| Elongation | 72 °C | 60 s |
| Final Elongation | 72 °C | 10 min |
| Hold | 4 °C | ∞ |

### 3.5.7 Purification of Plasmid DNA

Plasmid DNA was isolated using the commercially available NucleoSpin® Plasmid Kit (Macherey-Nagel). All steps were carried out as instructed by the manufacturer. Briefly, single colonies were grown overnight at 37 °C, 200 rpm in 4 ml terrific broth (TB) media containing 100 µg/ml ampicillin. After alkaline lysis of the cells and neutralization, the sample fluid was loaded on silica-gel membrane resins and washed with the supplied washing buffer. From 2 ml bacteria cultures, on average 25 µg plasmid DNA was recovered after elution with 75 µl elution buffer. The DNA concentration was determined by NanoDrop spectrophotometer. Plasmids were stored at -20 °C.

## 3.6 Recombinant Antibody Production

### 3.6.1 Cell Culture

Human embryonic kidney (HEK) 293T (ATCC, No. CRL-11268) cells were cultured in 150 mm culture plates (Becton Dickinson) under standard conditions (37 °C, 5 % CO2) in Dulbecco's Modified Eagle's Medium (D-MEM; GibcoBRL) supplemented with 10 % heat-inactivated fetal calf serum (FCS; Biochrom), 100 µg/ml streptomycin, 100 U/ml penicillin G and 0.25 µg/ml amphotericin (100x Antibiotic-Antimycotic mix; GibcoBRL).

Transient transfections of exponentially growing cells were performed at 80% cell confluency by cationic polymer polyethylenimine (PEI) transfection (Boussif et al., 1995) with equal amounts (10–15 µg each) of IgH and corresponding IgL chain encoding plasmid DNA.

### 3.6.2 Polyethylenimine- Mediated DNA Transfection of HEK 293T Cells

For PEI-mediated transfection, HEK 293T cells were washed with 10 ml serum-free DMEM and 25 ml DMEM supplemented with 1% Nutridoma-SP (Roche) was added. Equal amounts of IgH and IgL chain expression vector DNA were mixed in 150 mM sterile $NaCl_2$ solution. The 3-fold weight amount of PEI was added to the plasmid solution and the mixture was immediately vortexed for 10 s. A subsequent incubation step of 10 min at room temperature allowed formation of DNA-polymer complexes. The mixture was added dropwise to the culture dish. Cells were cultured for 4 days in 25 ml DMEM supplemented with 1 % Nutridoma-SP (Roche) before supernatants were harvested and stored with 0.01 % sodium azide at 4 °C until further use.

## 3.7 Enzyme-Linked Immunofluorescence Assays

### 3.7.1 Determination of Recombinant Ig Concentrations by ELISA

The concentration of recombinant IgG molecules in the harvested supernatants and purified antibody eluates were determined by antibody sandwich ELISA using a goat anti-human IgG Fcγ fragment antibody (Jackson ImmunoResearch) as capture antibody. High-binding capacity microtiter plates (Costar) were coated with 100 ng capture antibody in 50 µl PBS. Plates were sealed with Parafilm® and incubated over night at room temperature. The plates were then washed three times with deionized water and incubated with 200 µl/well

blocking buffer for 1 h to reduce unspecific binding sites. After another three washing steps, each well was incubated with 50 µl of the supernatants with unknown IgG concentrations at eight serial 1:3 dilutions in PBS starting with 1:3 dilutions for the supernatants and 1:100 dilutions for the purified antibody eluates. A human monoclonal IgG1 antibody (Sigma-Aldrich) was used as standard in serial dilutions beginning with a concentration of 5 µg/ml. After 2 h of incubation, the plates were washed three times to remove unbound antibodies and per well 50 ng HRP-conjugated goat anti human IgG Fcγ fragment antibody (Jackson ImmunoResearch) in blocking buffer were added and incubated for 1.5 h. After washing, brief blocking with blocking solution and three additional washing steps, the samples were developed by adding 100 µl of a 2,2′-Azino-di-[3-ethylbenzthiazoline sulfonate (6)] diammonium salt (ABTS) solution (Roche) as HRP substrate and analyzed using a microplate reader at a wavelength of 405 nm. Antibody concentrations were calculated using SoftMax ® software (Molecular Devices).

### 3.7.2 Purification of Recombinant Antibodies

Recombinant antibodies were purified from the supernatants with Protein G beads (GE Healthcare) according to the manufacturer's instructions. In brief, 25 ml cell culture supernatant were incubated with 25 µl Protein G beads under agitation for at least 14 h at 4 °C. After centrifugation at 800 $g_{av}$ for 10 min at 4 °C, supernatants were removed and the beads were transferred to chromatography spin columns (Bio-Rad) that were equilibrated with PBS before use. After two rounds of washing with PBS, the antibodies were eluted in three fractions (200 µl each) with 0.1 M glycine (pH 3.0) and collected in tubes containing 20 µl 1 M Tris (pH 8.0) with 0.1 % sodium azide.

### 3.7.3 Determination of Polyreactive Antibodies by ELISA

ELISAs were performed as described above (3.7.1) except high-binding capacity microtiter plates were coated with 250 ng (insulin) or 500 ng (dsDNA, ssDNA, lipopolysaccharide (LPS)) of individual antigens in PBS. ssDNA was prepared from salmon sperm dsDNA (Sigma-Aldrich) by boiling at 95 °C for 30 min and aliquots were immediately frozen at –20 °C. Human recombinant insulin solution (Sigma-Aldrich) and LPS from *E. coli* Serotype 055:B5 (Sigma-Aldrich) were stored at 4 °C. Antibody concentrations in supernatants were adjusted to 1 µg/ml and three consecutive 1:4 dilutions in PBS were prepared. Polyreactivity controls were the recombinant human monoclonal antibodies mGO53

(negative; Wardemann et al., 2003), eiJB40 (low polyreactive; Wardemann et al., 2003), and ED38 (highly polyreactive, Meffre et al., 2004) that were included on each plate. Bound antibodies were detected using ABTS (Roche) as substrate. The cut-off $OD_{405}$ at which antibodies were considered reactive was determined for each experiment based on the $OD_{405}$ minus 2x the standard deviation for the low positive control antibody eiJB40 at a concentration of 0.25 µg/ml. A minimum of 3 controls was included in each experiment to allow calculation of standard deviations. Antibodies were considered polyreactive, when binding to at least two structurally different types of tested antigens and if positive reactivity was confirmed in at least two independent experiments.

### 3.8 Immunofluorescence Assays

HEp-2 cell coated slides (Bios) were incubated in a moist chamber at room temperature with 20 µl purified antibodies for 30 min at a concentration of 100 µg/ml, washed twice in PBS and incubated for additonal 30 min with Cy3-labeled goat anti-human IgG (Jackson ImmunoResearch) according to the manufacturer's instructions. Slides were washed again twice in PBS before mounting with FluoromountG (Southern Biotech). Samples were examined on a Zeiss Axioplan 2 fluorescence microscope. Control stainings with PBS, ANA-negative and ANA-positive control sera were performed as suggested by the manufacturer and were included in all experiments. Positive staining was determined by comparison to the controls at equal exposure times.

### 3.9 Statistical Analysis

P-values for Ig gene repertoire analysis, analysis of positive charges in IgH CDR3, and antibody reactivity were calculated by Fisher Exact test or Chi Square Test. P-values for IgH CDR3 length and V gene mutations were calculated by non-paired two-tailed Student's *t* test using the GraphPad Prism Software.

# 4 RESULTS

## 4.1 Clinical Features of Five Analyzed SLE Patients

Bone marrow aspirates were drawn from five lymphopenic SLE patients, one male (age 31) and four female individuals (age 36-69). Clinical features such as clinical course, disease activity, treatment, and serology were diverse (Table 5), as reflected in Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores varying between 1 and 13. Two patients suffered from lupus nephritis, four patients showed low levels of complement components. Patients were selected due to elevated serum IgG anti-nuclear antibody (ANA) titers as determined by immunofluorescence indicating the presence of autoreactive antibody secreting cells.

**Table 5. Patient Characteristics**.

| Patient | SLE1 | SLE2 | SLE3 | SLE4 | SLE5 |
|---|---|---|---|---|---|
| Gender | Male | Female | Female | Female | Female |
| Age/ Age at Diagnosis | 31/31 | 41/31 | 69/43 | 50/22 | 38/13 |
| Clinical Course | Relapse | Relapse | Relapse | Remission | Relapse |
| SLEDAI | 8 | 13 | 4 | 1 | 5 |
| Renal Involvement | No | Lupus Nephritis WHO 5 | No | No | Lupus Nephritis WHO 4 |
| Treatment* | pred, aza | pred | pred, aza, cph | pred, hcq | pred, hcq |
| Hematology** | L,T | Leukocytopenia, T | L, T | Leukocytopenia, T | L, T |
| ANA IgG | positive | positive | positive | positive | positive |
| HEp-2 Nuclear Staining Pattern | Homogeneous, fine dense granular | Nuclear dots | Homogeneous, fine dense granular | Homogeneous, fine granular | Homogeneous, fine dense granular |
| Serology*** | D | D, Nuc, Sm | Coombs pos | Neg | D, APSA |
| Complement | Low C3 and C4 | Low C3 | Low C3 | Norm | Low C3 |

* aza, Azathioprine; cph, cyclophosphamide; hcq, hydroxychloroquine; pred, prednisolone;

** L, lymphopenia; T, thrombocytopenia

*** APSA, anti-phosphatidylserine antibodies; D, anti–double-stranded DNA antibody (IgG); Nuc, anti-nucleosome antibody; P, antiphospholipid antibody; Sm, anti-Smith antibody.

## 4.2 Ig Gene Features of Human IgG+ Bone Marrow Plasma Cells from SLE Patients

To characterize the immunoglobulin gene repertoire of human IgG+ plasma cells from SLE patients, single CD138+CD38+CD27+CD19+/- mononuclear cells were isolated from human bone marrow of five patients with SLE (Figure 6; SLE1-SLE5; Terstappen et al., 1990; Odendahl et al., 2000; Ellyard et al., 2004). The matching Ig heavy (Igh) and Ig light (Igl) chain genes of 305 IgG+ bone marrow plasma cells were cloned and sequenced (Table S1-

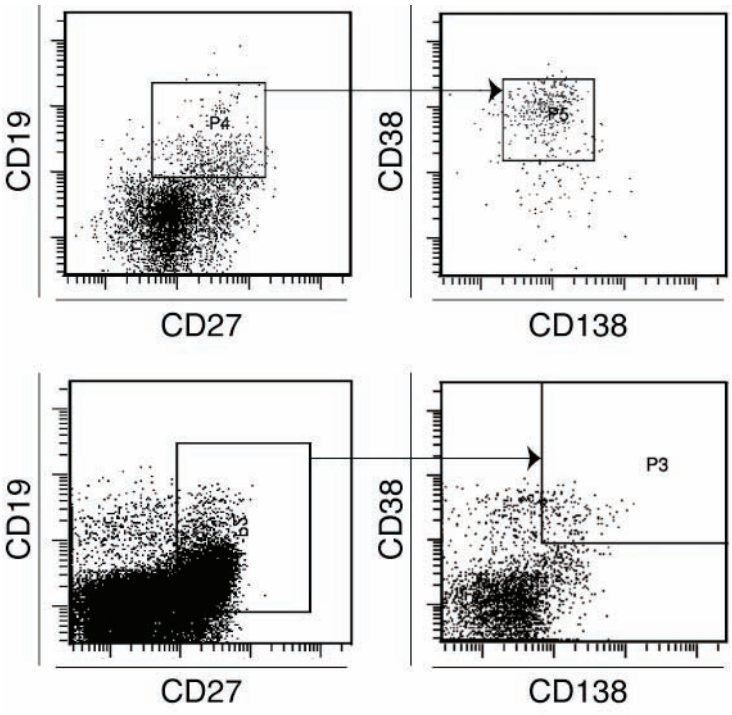



**Figure 6. Representative FACS Profiles of Sorted Plasma Cells from SLE Patients.**
FACS plot shows representative sort gates for the isolation of CD138+CD38+CD27+CD19+/- single mononuclear bone marrow cells from SLE patients that were either CD138-preenriched (upper panel; SLE1, SLE2) or were sorted without CD138 preenrichment (lower panel; SLE3, SLE4, SLE5).

Table S5) and compared to data from the IgG+ plasma cell compartment of four healthy donors (HD; Scheid et al., 2011). Sequence analysis showed that all antibodies were unique in their V(D)J rearrangement, and heavy and light chain pairings, and thus originated from non-clonally related plasma cells (Table S1-Table S5).

Class-switch recombination and an increased number of V gene mutations indicate antigen-mediated selection in germinal centers (Shimizu et al., 1984; Berek et al. 1991; Jacob et al., 1991; Kuppers et al., 1993; Feuillard et al., 1995; Rajewsky, 1996; Takahashi et al., 1998). Bone marrow plasma cells from SLE patients displayed an IgG subclass distribution reflecting that of human serum IgG antibodies with IgG1>IgG2>IgG3>IgG4 on average (Figure 7A, Figure S7A; Plebani et al., 1989). However, in contrast to single cell data from the IgG+ plasma cell compartment of healthy donors, IgG3 and IgG4 were on average more frequently expressed in SLE patients ($P$=0.0005; Figure 7A). Individual patients showed abnormalities in the IgG subclass distribution of bone marrow plasma cells like reduced IgG2 plasma cell frequencies in SLE3 and SLE5 (Figure S7A). However, altered IgG subclass distribution was not a general feature in all patients.

In agreement with their origin from germinal center reactions, nearly all IgH and IgL chain genes from IgG+ bone marrow plasma cells in SLE patients were somatically mutated (Figure 7B, Figure S7B). V gene mutation analysis showed mutation frequencies similar to healthy donors for *VH* genes (23.8 in SLE vs. 23.9 in HD), whereas *VL* genes in SLE patients carried on average lower numbers of mutations than in healthy donors (12.7 vs. 13.5 for *VK* and 11.3 vs. 13.3 for *VL*). Variation in load of somatic mutations among the patients was high (Figure S7B) and low numbers of somatic mutations in *VL* genes were not a universal feature of all patients.

Ig gene repertoire and Ig heavy (IgH) chain characteristics such as CDR3 length and positive amino acid charges have been associated with autoreactivity (Ichiyoshi and Casali, 1994; Radic and Weigert, 1994; Crouzier et al., 1995; Barbas et al., 1995; Wardemann et al., 2003). Ig gene sequence analysis showed no significant differences between SLE patients and healthy donors in IgH and Igκ variable (V) and joining (J) gene usage and IgH complementary determining region 3 (CDR3) features, such as length and number of positive charges (Figure 8A, B, Figure S7C,D). However, Igλ chain V gene usage was significantly different in SLE patients, as reflected in an underrepresentation of Vλ1 gene segments in IgG+ bone marrow plasma cells of SLE patients compared to healthy donors that was associated with increased Vλ3 to Vλ9 usage ($P$=0.002; Figure 8C, Figure S7E).

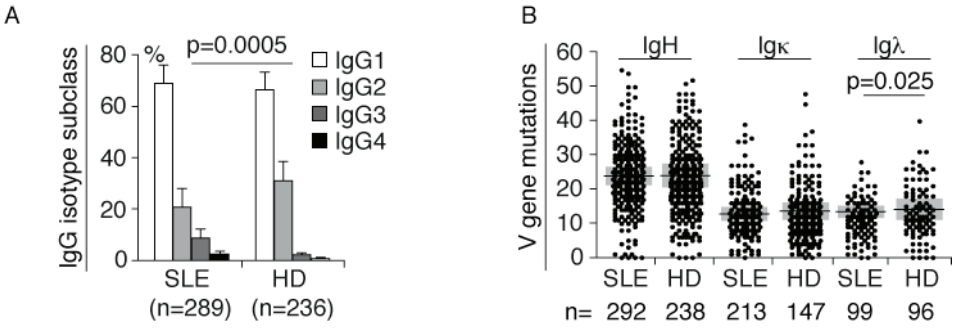



**Figure 7. Affinity Maturation-Associated Gene Features of IgG+ Bone Marrow Plasma Cells from SLE Patients.**

Ig gene subclass distribution and Ig gene V gene mutations of IgG+ plasma cell antibodies from five SLE patients shown in comparison to IgG+ plasma cell antibodies from four healthy donors (HD). (A) IgG subclass distribution. Bar graphs summarize the data for SLE patients (n=289) in comparison to healthy donors (n=236). Errors bars indicate standard deviation of means. (B) Dots represent the absolute number of somatic mutations (nucleotide exchanges compared to the nearest germline Ig gene segment) in individual *Igh* and *Igl* Vκ and Vλ gene segments (FWR1-FWR3) in IgG+ plasma cell antibodies from SLE patients and healthy donors. Horizontal lines indicate averages, gray boxes indicate standard deviation of means.

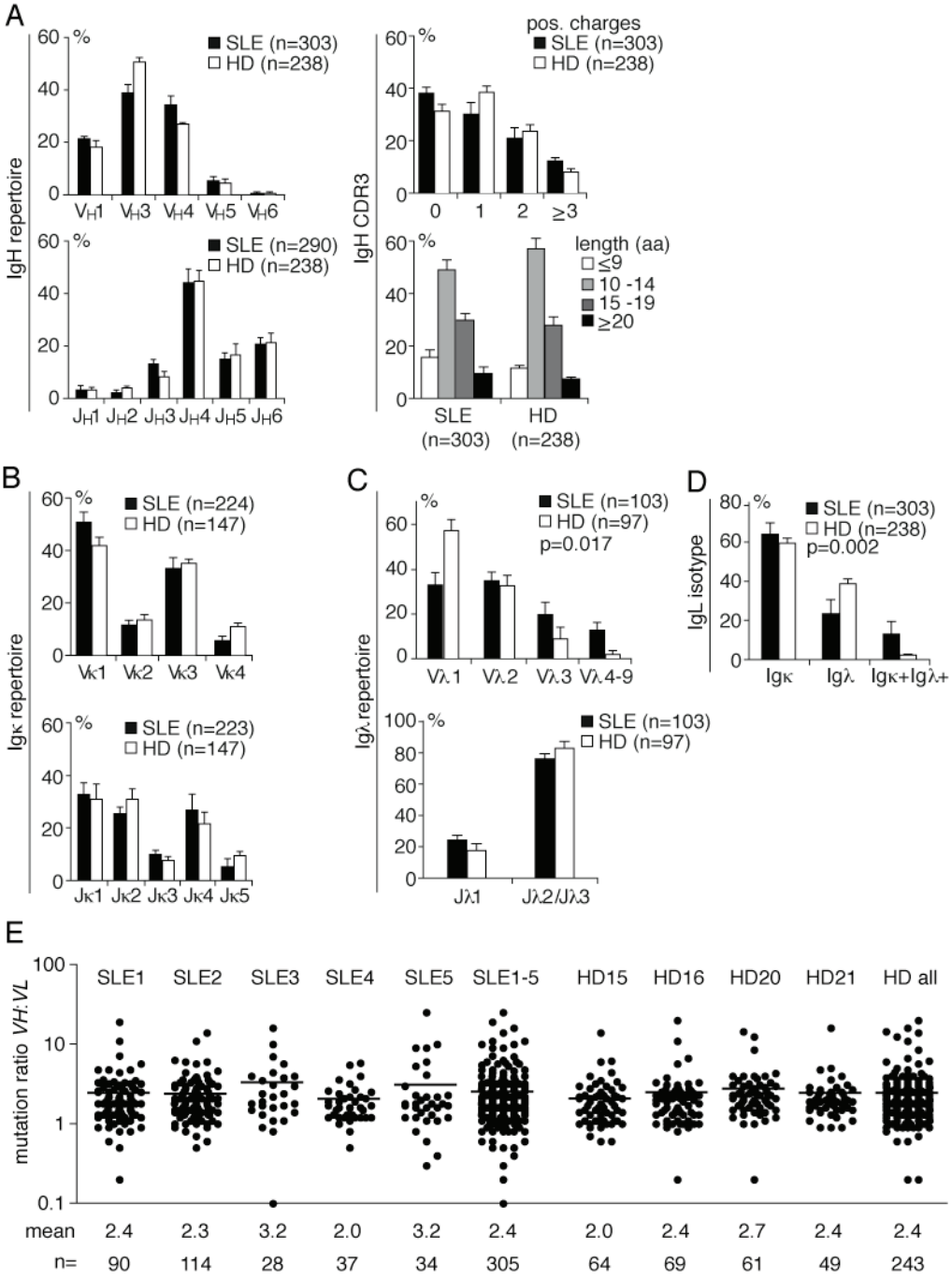



**Figure 8. Ig Gene Features of IgG+ Plasma Cells from SLE Patients.**

Ig gene features of IgG+ plasma cell antibodies from five SLE patients are shown in comparison to IgG+ plasma cell antibodies from four healthy donors (HD). VH/JH gene family usage and IgH CDR3 positive charges and length (A), and Vκ/Jκ (B) and Vλ/Jλ (C) gene family usage are shown. Bar graphs display the data obtained from lupus patients (γ, n=305; κ, n=226; λ, n=103) in comparison to healthy donors (γ, n=238; κ, n=147; λ, n=97). (E) Ratio of *VH* to *VL* somatic mutations in IgG+ plasma cells from SLE patients. Dots represent individual antibodies from SLE patients (SLE1-SLE5) and healthy donors (HD15, HD16, HD20, HD21) and pooled data (SLE1-5, HD). *VL* mutation values were set from 0 to 1, in cases where mutated IgH chain transcripts were paired with unmutated IgL chains. (A)-(D) Error bars indicate standard deviation of means.

Alterations in Igλ light chain recombination were also detected in the Ig light chain isotype distribution, as IgG+ plasma cells displayed on average a significantly lower frequency of Igλ functional transcripts in SLE patients (Figure 8D, Figure S7E). Elevated levels of Igκ and Igλ light chain double positive plasma cells were observed in all SLE patients ($P$=0.002; Figure 8D, Figure S7F).

The average number of mutations is typically higher in *VH* than in *VL* gene segments, both in healthy donors and SLE patients (de Wildt et al., 1999; de Wildt et al., 2000; Mietzner et al., 2008). The mutation ratio of IgG+ bone marrow plasma cells from three SLE patients was comparable to healthy donors, whereas patients with large numbers of Igκ and Igλ light chain included functional transcripts showed elevated ratios of *VH* to *VL* somatic mutations (3.2 in SLE3 and SLE5 as compared to 2.0- 2.4 in the other SLE patients and 2.0- 2.7 in HD; Figure E).

Thus in summary, SLE is associated with alterations in subclass distribution, Vλ gene usage and Ig light chain isotype expression in IgG+ bone marrow plasma cells.

### 4.3 Polyreactive IgG+ Bone Marrow Plasma Cells in SLE Patients

Entry into the bone marrow plasma cell compartment appears to be strongly selective and requires high antigen specificity (Smith et al., 1997; Smith et al., 2000; Takahashi et al., 1998; Phan et al., 2000; Scheid et al., 2011). In SLE patients however, pathogenic autoantibodies are frequently polyreactive, which might be important in disease pathogenesis (Spatz et al., 1997; Sabbaga et al., 1990). Between 2-16 % of IgG+ bone marrow plasma cells from healthy donors express polyreactive antibodies that react with structurally diverse self- and non-self antigens (Scheid et al., 2011). To determine if SLE is associated with an impaired exclusion of polyreactive plasma cells, the cloned IgG and IgL genes of 196 bone marrow plasma cells were recombinantly expressed *in vitro* and the frequency of polyreactive antibodies in the IgG+ bone marrow plasma cell compartment of SLE patients was measured. The recombinant monoclonal antibodies were thus tested by ELISA for binding to a small panel of self- and non-self antigens comprising single-stranded and double-stranded DNA (ssDNA and dsDNA), lipopolysaccharide (LPS), and insulin (Figure 9; Table S1-Table S5). Between 3-30 % of IgG+ bone marrow plasma cells reacted with at least two structurally distinct antigens

in these assays (Figure 9B). A high degree of variation in the frequency of polyreactivity was also observed for healthy donors (2-16 %; Figure 9C). However on average, SLE patients showed significantly higher frequencies of polyreactive antibodies than healthy donors (17.3 % for SLE vs. 9.0 % for HD; Figure 9C). Interestingly, polyreactivity levels above those measured in healthy donors were found only in two SLE patients with active disease (SLE1 and SLE5)

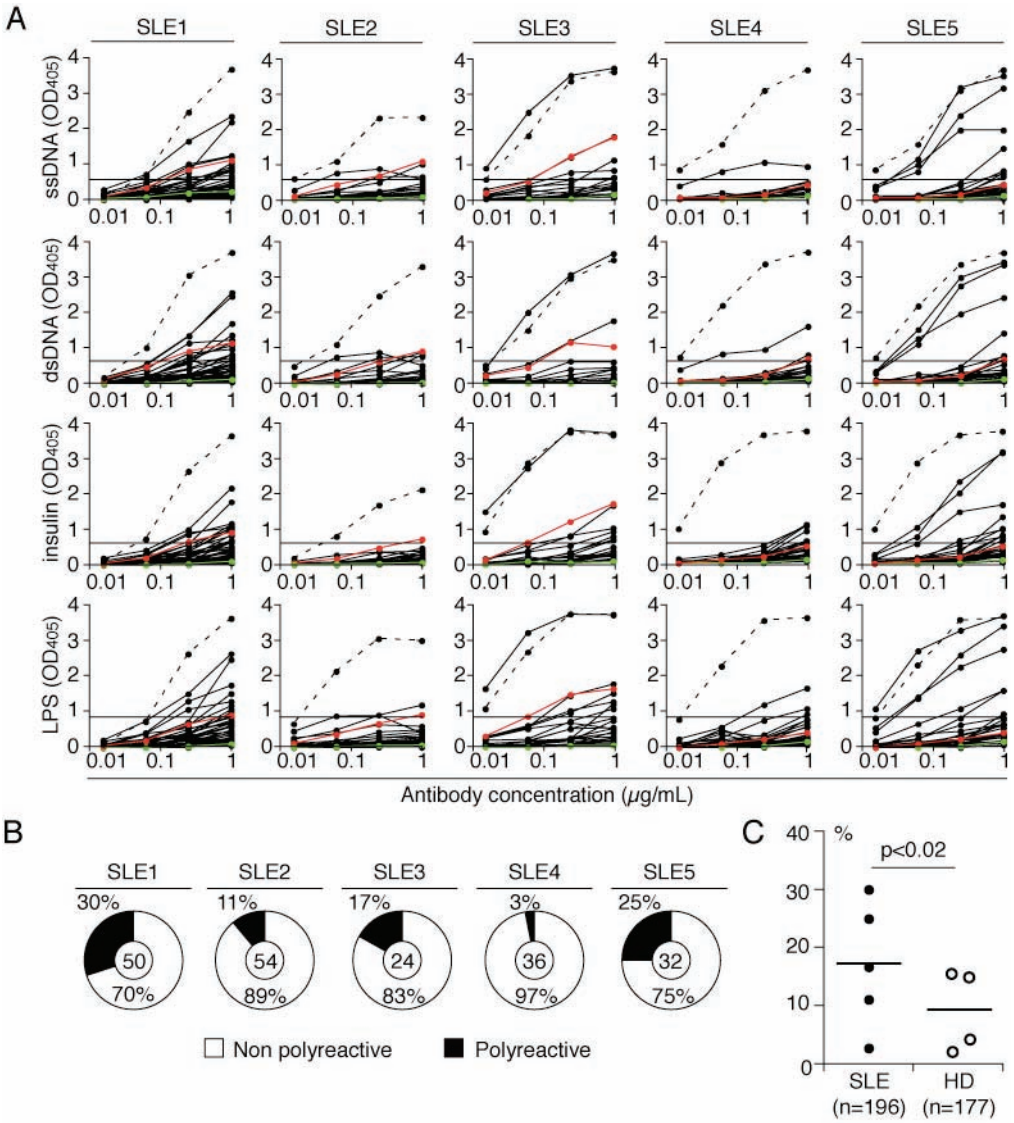



**Figure 9. Polyreactive Antibodies in the IgG+ Plasma Cell Compartment of SLE Patients.**
(A) IgG plasma cell antibodies (black lines) from individual SLE patients (SLE1-5) were tested for polyreactivity by ELISA with ss/dsDNA, insulin, and LPS. Dashed lines represent the high positive control antibody ED38. Horizontal lines show cut-off $OD_{405}$ for positive reactivity as determined by comparison to the negative control antibody mGO53 (green line) and low positive control eiJB40 (red line). (B) Pie charts summarize the frequency of polyreactive clones for individual donors. The numbers of tested antibodies are displayed in the pie chart centers. (C) Dot plots compare the frequency of polyreactive plasma cell antibodies in individual SLE patients to individual healthy donors (HD). Horizontal lines represent mean values of reactivity for all donors; n indicates the number of tested antibodies.

and the lowest level of polyreactivity was observed in patient SLE4 with low disease activity in clinical remission. Thus, exclusion of polyreactive IgG+ plasma cells from the bone marrow compartment is impaired in some but not all SLE patients.

### 4.4 Self-Reactive IgG+ Bone Marrow Plasma Cells in SLE Patients

Self-reactive IgG+ bone marrow plasma cells are efficiently excluded from the bone marrow compartment of healthy donors (Scheid et al., 2011). In SLE patients, self-reactive IgG serum antibodies display a cardinal feature of the disease and are a diagnostic marker (Kotzin, 1996). To determine if SLE is associated with a defect in self-tolerance that allows self-reactive plasma cells to accumulate in the bone marrow, all antibodies were tested for self-reactivity with the human larynx carcinoma cell line HEp-2 that is used as a diagnostic test for autoantibody detection (Figure 10; Egner, 2000). To discriminate between anti-nuclear antibodies (ANAs) that were detected in the patients' sera and other autoantibodies, indirect immunofluorescence assays (IFA) on fixed HEp-2 cells were performed. HEp-2 cell reactive antibodies from all SLE patients showed diverse staining patterns with cytoplasmic, cytoplasmic plus nuclear, and nuclear reactivity as previously observed for healthy donors (Figure 10A,B; Table S1-Table S5; Scheid et al., 2011). However, IgG+ plasma cells from SLE patients showed on average significantly elevated frequencies of HEp-2 self-reactive antibodies with an enrichment for anti-nuclear antibodies in SLE patients with active disease ($P$=0.004, Figure 10C,D). Interestingly, patient SLE4 being in clinical remission showed normal levels of HEp-2 reactivity and a low level of polyreactivity as compared to healthy donors.

ANAs with homogeneous chromatin staining patterns as judged by Hoechst co-staining were exclusively found in SLE patients and were devoid of the bone marrow plasma cell compartment in healthy donors (SLE2-27L, SLE3-11, SLE5-90; Figure 11A; Scheid et al., 2011). Two of the three patients with chromatin-reactive bone marrow plasma cells showed homogenous, fine dense granular ANA serum patterns and anti-dsDNA IgG titers (Table 5). All chromatin-reactive monoclonal plasma cell antibodies were thus tested for specificity to dsDNA by IFA with the flagellate *Crithidia luciliae* (Figure 11B). Antibody SLE2-27L but not antibodies SLE3-11 and SLE5-90 showed *Crithidia luciliae* kinetoplast staining indicating specificity for native dsDNA. However despite their chromatin staining patterns, antibodies

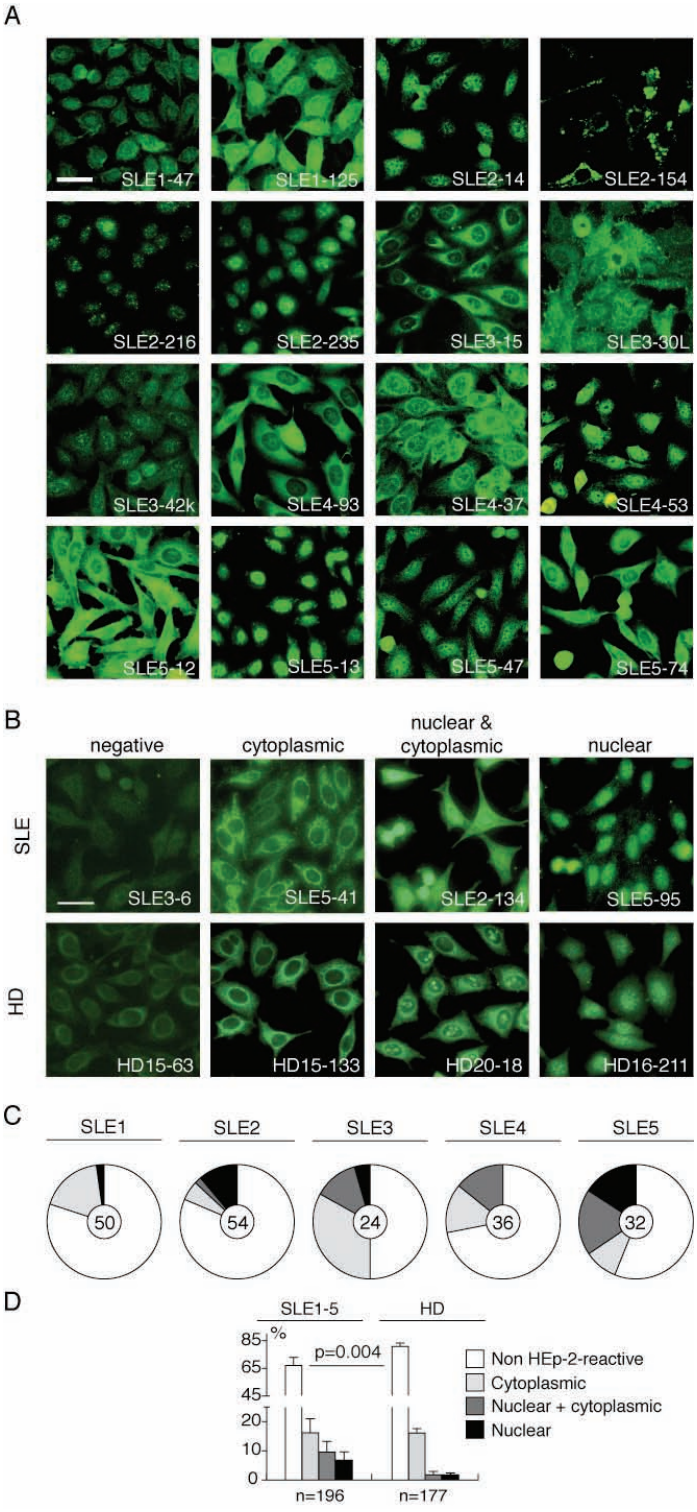



**Figure 10. Self-Reactive Antibodies in the IgG+ Plasma Cell Compartment of SLE Patients.**
IgG+ plasma cell antibodies from five SLE patients were tested for self-reactivity with HEp-2 cells. (A) Diverse HEp-2 cell immunofluorescence staining patterns of IgG+ plasma cell antibodies. Scale bar depicts 50 µm. (B) Representative antibodies cloned from IgG+ plasma cells of SLE patients are shown in comparison to IgG+ plasma cells from healthy donors and classified into negative, cytoplasmic, nuclear plus cytoplasmic, and nuclear. Scale bar represents 50 µm. (C) Pie charts summarize the frequency of non-reactive (white) and HEp-2 self-reactive IgG+ plasma cell antibodies in individual SLE patients with cytoplasmic (light gray), nuclear plus cytoplasmic (dark gray) and nuclear (black) IFA staining patterns. The number of tested antibodies is indicated in the pie charts center. (D) Bar graphs compare the frequency of distinct HEp-2 cell staining patterns of all SLE patients in comparison to all healthy donors. Error bars indicate standard deviation of means.

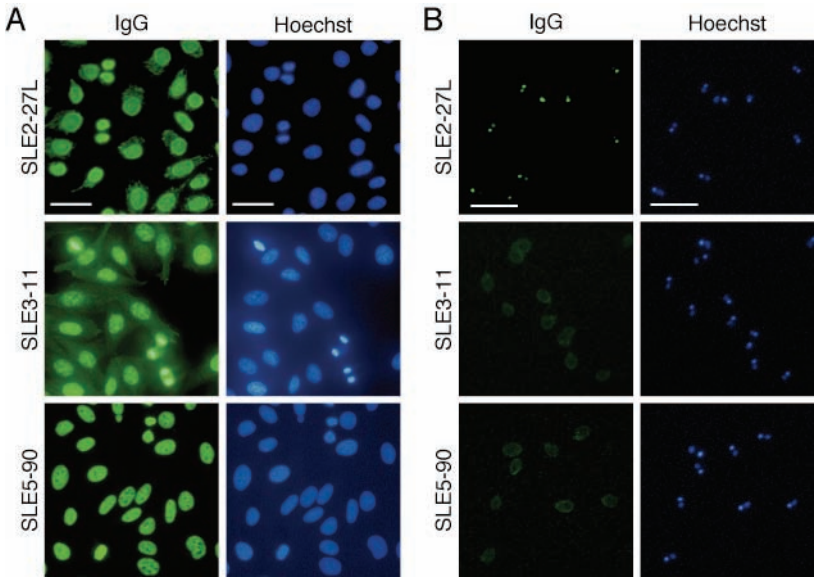



**Figure 11. Chromatin-Reactive Antibodies in the IgG+ Plasma Cell Compartment of SLE Patients.**
IgG+ plasma cell antibodies from five lupus patients were tested for dsDNA reactivity with HEp-2 and *Crithidia luciliae* cells. (A) Strong homogenous nuclear HEp-2 staining patterns of IgG+ plasma cell antibodies SLE2-27L, SLE3-11, and SLE5-90. Nuclei are visualized by Hoechst staining. Scale bar represents 50 µm. (B) *Crithidia luciliae* IFA staining pattern of the same antibodies as in A. Nuclei and kinetoplasts are visualized by Hoechst staining. Scale bar represents 20 µm.

SLE3-11 and SLE5-90 lacked reactivity with nucleosomes and histones as determined by ELISA (data not shown).

Anti-nuclear antibody SLE2-27 originated from an Igκ and Igλ light chain included plasma cell (Table S2). The anti-nuclear reactivity of SLE2-27 was independent of whether the IgH chain was co-expressed with the Igκ light chain, Igλ light chain or with both light chains (Figure 12A). However, anti-dsDNA specificity as determined by IFA with the flagellate *Crithidia luciliae* was dependent on sole expression of the Igλ light chain and was not detected when the Igκ light chain was co-expressed (Figure 12B). However, analyzing the antibody repertoire of all SLE patients did not show a correlation between Igλ isotype inclusion and antibody reactivity, as double Ig light chain positive plasma cells were identified where one, both or none of the two IgL chains mediated antibody self-reactivity when expressed with the respective IgH chain (Table S1- Table S5).

In summary, the bone marrow compartment in SLE patients with active disease is enriched for self-reactive IgG+ plasma cells and disease-associated IgG+ ANAs including anti-chromatin specific plasma cells fail to be excluded.

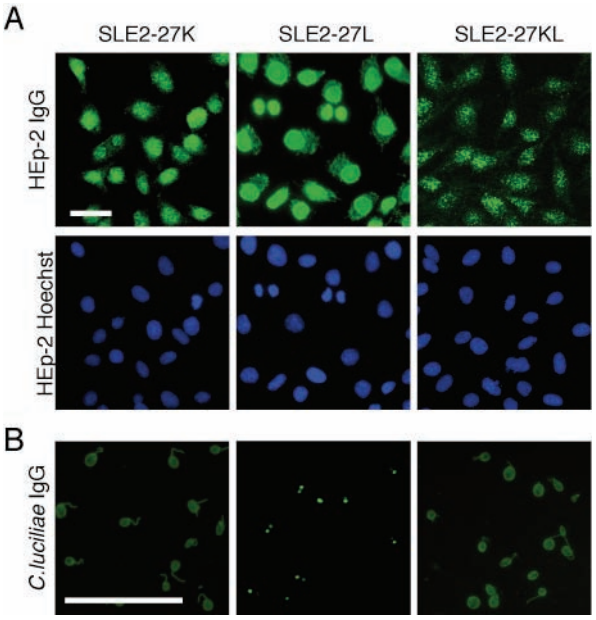



**Figure 12. Nuclear Reactive Antibody SLE2-27.**
HEp-2 cell (A) and *Crithidia luciliae* (B) IFA staining patterns of SLE2-27K, SLE2-27L and SLE2-27KL. Scale bar represents 50 µm.

### 4.5 Reversion of Somatic Hypermutations Abolishes Reactivity in Poly- and Self-Reactive IgG+ Bone Marrow Plasma Cells from SLE Patients

Self- and polyreactive antibodies in the antigen-experienced memory B cell compartment frequently arise from non-self-reactive precursors by somatic hypermutation (Hervé et al., 2005; Tsuiji et al., 2006; Tiller et al., 2007). Moreover, somatic mutations play a major role in the generation of SLE-associated autoantibodies (Wellmann et al., 2005; Mietzner et al., 2008). To determine if self- and polyreactivity in the IgG+ bone marrow plasma cell compartment is mediated by somatic hypermutations, six poly- and self-reactive antibodies from patient SLE2 were reverted to their germline sequence by using a PCR-based reversion strategy (Figure S6; Table S6). The reverted recombinant monoclonal antibodies were tested for polyreactivity in ELISAs and for HEp-2 self-reactivity in IFAs, and compared to their mutated counterparts (Figure 13). The germline configuration diminished binding in both assays for all tested antibodies. These data thus show that autoreactive IgG+ bone marrow plasma cells can develop from naïve precursors lacking detectable levels of poly- and self-reactivity by the introduction of somatic hypermutations.

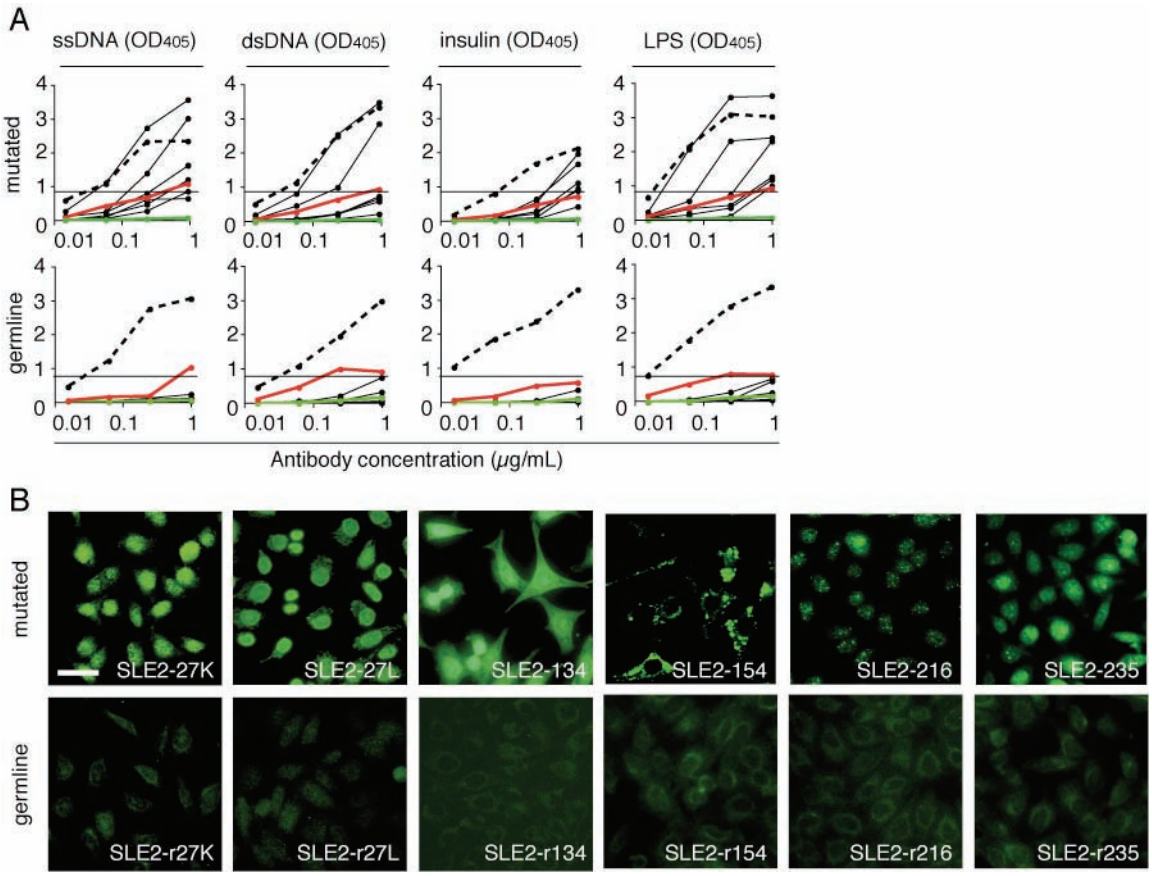



**Figure 13. Somatic Hypermutation Contributes to Poly- and Self-Reactivity in IgG Plasma Cell Antibodies in SLE.**

IgH and IgL chains from six IgG+ plasma cells of one SLE patient were reverted into their germline counterparts by PCR (SLE2-27K, SLE2-27L, SLE2-134, SLE2-154, SLE2-216, SLE2-235). Recombinant mutated IgG plasma cell antibodies (upper panel) and their germline counterparts (lower panel) were tested for polyreactivity (A) and self-reactivity by HEp-2 cell IFA (B). (A) IgG plasma cell antibodies (black lines) were tested for polyreactivity by ELISA with ss/dsDNA, insulin, and LPS. Dashed lines represent the high positive control antibody ED38, red lines indicate the low positive control eiJB40, green lines display the negative control antibody mGO53. (B) HEp-2 IFA of the same antibodies as tested in (A). Scale bar represents 50 μm.

# 5 DISCUSSION

## 5.1 Unbiased Analysis of the Plasma Cell Antibody Repertoire by Single Cell Ig Gene Cloning Implies a Rigid Selection Process in Healthy Humans

Immunoglobulin (Ig) gene sequence analysis of distinct B cell populations allowed a basic understanding of the expressed human antibody repertoire at different stages during B cell development (Huang and Stollar, 1991; Huang et al., 1992; Brezinschek et al., 1995; Wang and Stollar, 2000). However, sequence analysis alone does not permit predictions on antibody reactivities. Human monoclonal antibodies can be produced by different methods such as immortalization of B cells with Ebstein-Barr virus (Steinitz et al., 1977; Lanzavecchia et al., 2007), the production of B-cell hybridomas (Kohler and Milstein, 1975), using phage display libraries (McCafferty et al., 1990) or the humanization of antibodies from other mammalian species (Jones et al., 1986). Still, immortalization and fusion efficiencies are low, largely depend on the maturation status of the B cell and frequently require additional B cell stimulation. Phage display technology on the other hand relies on random pairing of human Ig heavy and light chains *in vitro*, thus not providing information on the epitope specificities that humans generate *in vivo*.

This study takes advantage of an unbiased and efficient approach that combines Ig gene repertoire analysis and Ig reactivity profiling at the single cell level (Wardemann et al., 2003; Tiller et al., 2008). The applied single-cell strategy facilitates the isolation of single B cells at any stage of their development and requires only small cell numbers. Using this methodology it was demonstrated that selection into the human bone marrow plasma cell compartment requires a positive selection step after the germinal center reaction under normal immune conditions (Scheid et al., 2011), as was elegantly demonstrated in mouse models (Smith et al., 1997; Takahashi et al., 1998; Phan et al., 2006; Paus et al., 2006). This counterselection against poly- and self-reactivity in the bone marrow plasma cell compartment may be essential to avoid the production of poly- and self-reactive serum IgG antibodies that might lead to the induction of inflammatory autoimmune reactions and the development of autoimmune disease.

### 5.2 Serum Autoantibodies are a Hallmark of Disease in SLE Patients

B cell lymphopenia and B cell overactivity are among the most striking abnormalities encountered in systemic lupus erythematosus (Rahman and Isenberg, 2008; Crispín et al., 2010) and were a characteristic of the analyzed SLE patients. Patient selection was based on elevated serum ANA IgG levels indicating the presence of autoantibody secreting cells. Enhanced B cell activity results in the production of characteristic patterns of autoantibodies and has been postulated to be the defining pathogenic event in human SLE (Lipsky, 2001; Rahman and Isenberg, 2008). Serum IgG autoantibodies against dsDNA and nucleosomes, as detected in the analyzed lupus patients with SLEDAI scores above 4, indicate disease activity and vanish during remission (Grammer and Lipsky, 2003; Vallerskog et al., 2007; Hiepe et al., 2011). However, a recent study analyzing serum autoantibodies at low dilutions demonstrated a persisting SLE antibody profile independently of disease activity (Fattal et al., 2010). This specific antibody profile revealed increased anti-dsDNA IgG antibody reactivities in all tested patients. Thus, baseline anti-dsDNA serum IgG reactivity may be sustained by terminally differentiated plasma cells during remission and plasmablasts may contribute to elevated anti-dsDNA serum IgG levels during flares.

### 5.3 The Ig Gene Repertoire in IgG+ Bone Marrow Plasma Cells from SLE Patients

The present study demonstrates that SLE is associated with variable abnormalities in subclass distribution, Vλ gene usage and Ig light chain isotype expression in IgG+ bone marrow plasma cells, whereas somatic mutation levels and IgH and Igκ gene usage appear normal. Previous studies have reported molecular abnormalities in the Ig gene repertoire of peripheral B cells from SLE patients, including altered IgH and IgL V gene usage, abnormal IgH CDR3 features, altered somatic mutation frequencies and differences in IgG subclass distribution (Demaison et al, 1994; Manheimer-Lory et al., 1997; Dorner et al., 1998; Dorner et al., 1999; Jacobi et al., 2000; Odendahl et al., 2000; Yurasov et al., 2005; Yurasov et al., 2006; Mietzner et al., 2008). The present study demonstrates that some of these SLE-associated Ig gene features, such as underrepresentation of IgG2 and Vλ gene biases extend to the bone marrow plasma cell compartment. The data moreover confirms previous findings on a high variability in the Ig gene repertoire among individual SLE patients (Yurasov et al., 2005;

Yurasov et al., 2006; Foreman et al., 2007; Mietzner et al., 2008) and this diversity of the Ig gene repertoire likely reflects the high degree of disease diversity in SLE.

B cell receptor editing has been proposed to be the main mechanism in establishing self-tolerance and is mainly conferred by secondary Ig gene rearrangements that generate a new antigen receptor with an innocuous specificity (Gay et al., 1993; Radic et al., 1993; Tiegs et al., 1993). Abnormalities in Igκ gene usage indicating secondary rearrangement were not detected in the IgG+ bone marrow plasma cell compartment and alterations in Vλ gene usage were not linked to chromosomal location, as was also reported in previous studies on peripheral B cells (Bensimon et al., 1994; Dorner et al., 1999; Mietzner et al., 2008). However, other studies have demonstrated that SLE is associated with abnormalities in Ig light chain editing in peripheral B cells (Suzuki et al., 1996; Dorner et al., 1998; Dorner et al., 1999; Yurasov et al., 2006).

High frequencies of Igκ and Igλ double positive B cells were detected in the IgG+ bone marrow plasma cell compartment of two SLE patients, as previously reported for IgG+ memory B cells from one SLE patient (Mietzner et al., 2008). Evidence from experimental mouse models has proven that editing of self-reactive B cell antigen receptors may be associated with Ig light chain inclusion (Nemazee and Weigert, 2000; Li et al., 2001; Li et al., 2002; Liu et al., 2005; Doyle et al., 2006; Casellas et al., 2007; Zouali, 2008). Moreover, the two patients with an increased frequency of allelically included light chains also displayed elevated ratios of *VH* to *VL* gene somatic mutations, suggesting that secondary Ig gene recombination in these individuals occurred after antigen-mediated activation in the periphery and after initial affinity maturation.

### 5.4 The Bone Marrow Contributes to Serum ANA Production in SLE Patients

Exclusion of polyreactive IgG+ plasma cells from the bone marrow compartment is impaired in some SLE patients. In agreement with this observation, previous studies demonstrated that pathogenic lupus autoantibodies are frequently polyreactive (Sabbaga et al., 1990; Spatz, 1997) and naïve B cells of SLE patients are enriched for polyreactive antibodies both during relapse and remission (Yurasov et al., 2005; Yurasov et al., 2006). In healthy donors, polyreactivity is a common feature of IgG+ memory B cells (Tiller et al., 2007) but rare

in the long-lived bone marrow plasma cell compartment (Scheid et al., 2011). As observed in two SLE patients, polyreactive antibodies can accumulate in the plasma cell compartment to a higher extent than detected in healthy donor plasma cells. This increase in polyreactivity was not associated with an increase in self-reactivity in one patient, which indicates that chronic inflammation provides a constant supply of antigen to stimulate lymphocytes in this patient (Goodnow, 2007). In summary, the data suggest that an accumulation of polyreactive antibodies in the plasma cell compartment is not a universal feature of the disease, but likely reflects differences in genetic and environmental factors that both are important in the development of SLE (Rahman and Isenberg, 2008).

The bone marrow in SLE patients harbors IgG+ plasma cells expressing disease-associated ANAs including anti-chromatin IgGs. Anti-nuclear IgG antibodies with chromatin reactivity represent a hallmark of SLE (Kotzin, 1996) and may arise from autoreactive B cells that fail to be excluded from germinal center reactions (Cappione et al., 2005). One of the identified chromatin-reactive antibodies in this study could be further characterized to be specific for native dsDNA. Both anti-dsDNA and anti-nucleosome antibodies have been demonstrated to mediate lupus nephritis (Rahman and Isenberg, 2008; van Bavel et al., 2008). However, chromatin-specific antibodies detected in immunofluorescence assays frequently show no or very low reactivity against individual histones or native dsDNA (van Bavel et al., 2008). Nevertheless, the present study clearly demonstrates that the bone marrow plasma cell compartment contributes to serum ANA production in SLE patients as previously reported for an autoimmune lupus mouse model (Hoyer et al., 2004).

## 5.5 The Role of Somatic Hypermutations in the Generation of Self-Reactive Antibodies

In agreement with previously published data on the impact of somatic hypermutation in the generation of disease-associated SLE autoantibodies (Shlomchik et al., 1987; van Es et al., 1991; Radic et al., 1993; Manheimer-Lory et al., 1997; Wellmann et al., 2005; Mietzner et al., 2008; Guo et al., 2010), the germline forms of all tested SLE plasma cell antibodies lacked detectable levels of self- and polyreactivity. This finding implies that these antibody specificities developed by somatic mutations introduced during affinity maturation in germinal center reactions. The data thus confirm previous findings demonstrating the importance of somatic mutations for the development of SLE autoantibodies.

### 5.6 Genetic and Environmental Factors Generate Disease Diversity in SLE Patients

All patients differed in clinical features as well as serology and hematology. Further, differences among SLE patients were detected at both the Ig molecular level and the antibody reactivity level in the bone marrow plasma cell compartment. This high degree of disease diversity is one of the main obstacles in lupus treatment. Many different pathways, processes and cell types of the immune system are involved in the generation of the SLE phenotype (Harley et al., 2009).

All SLE patients with active disease had low complement serum levels. These low complement levels indicate poor waste disposal due to deficiency in complement components or an increase in cell death rates that may be one of the main factors triggering the onset of the disease (Rahman and Isenberg, 2008; Muñoz et al., 2010). Further, the IgG+ bone marrow plasma cell compartment is enriched for self-reactive antibodies with nuclear specificity. Due to defective clearance mechanisms, cellular debris containing nuclear antigens is exposed and can activate immune cells. Nuclear antigens like chromatin contain both B cell and T cell epitopes and additionally activate immune cells through TLR9 specific for DNA (Goodnow, 2007; Harley et al., 2009). This simultaneous activation of different pathways subsequently enhances inflammatory cascades and provides stimuli for chronic inflammation (Isenberg et al., 1990; Rahman et al., 2002).

The presence of autoantibody titers like those detected in the analyzed patients correlates with elevated BAFF serum levels (Cambridge et al., 2008; Tieng and Peeva, 2008). Alterations in the expression of components involved in BCR signaling can affect thresholds for B cell survival and activation. The threshold for naïve and memory B cell activation, proliferation and antibody secretion is lowered by the presence of both IL-17 and BAFF (Doreau et al., 2009) and may also influence the bone marrow plasma cell compartment. In situations of B cell hyperresponsiveness, affinity maturation would be censored less stringently (Tarlinton and Smith, 2000) and selection into the long-lived plasma cell compartment may be impaired.

Elevated levels of poly- and self-reactive plasma cells are probably a result of B cell hyperresponsiveness. The IgG inhibitory Fc gamma receptor IIB (FcγRIIB) is the only inhibitory Fc receptor on B cells (Nimmerjahn and Ravetch, 2008) and has been shown to be involved in SLE pathogenesis of mice and man at both genetic and functional levels (Morel,

2010; Tiller et al., 2010). The frequency of FcγRIIB on circulating memory B cells is significantly decreased in SLE patients (Mackay et al., 2006), thus may also be impaired on terminally differentiated plasma cells. A decrease of IgG inhibitory (FcγRIIB) is suggested to promote B cell hyperresponsiveness and thus nourishes an unregulated expansion of activated autoreactive B cells and the production of pathogenic autoantibodies (Morel, 2010; Mackay et al., 2006).

### 5.7 Therapeutic Options to Eliminate Autoreactive Plasma Cells

SLE is associated with high frequencies of IgG ANA-secreting bone marrow plasma cells that may represent an important therapeutic target in patients refractory to conventional therapies. Self-reactive high-affinity germinal center B cells are difficult to target once they have been selected into the bone marrow plasma pool. As soon as plasma cells have reached a survival niche in the bone marrow, they lose the ability to respond to antigenic changes in their environment (Amanna and Slifka, 2010) and are thus resistant to glucocorticoids, immunosuppressive and cytotoxic treatments (Hoyer et al., 2004; Hiepe et al., 2011). However, targeting plasma cells is possible but universal depletion of plasma cells also eliminates the main source of protective antimicrobial antibodies and is accompanied with severe side effects (Neubert et al., 2008; Hoyer et al., 2008; Sanz and Lee, 2010).

Therapeutic drugs abolishing autoantibodies and preventing flares in SLE patients are still missing in disease treatment. Approaches of the last decades focused on specific immunomodulation to alter B cell populations and several strategies targeting B cells are currently available (Sanz and Lee, 2010). Current approaches comprise direct B cell death using depleting antibodies, inhibition of factors involved in B cell differentiation or survival and interruption of signaling through the B cell receptor or co-stimulatory receptors (Sanz and Lee, 2010). For example, CD20-mediated depletion eliminates autoreactive mature B cells but does not target plasmablasts and plasma cells and thus, protective as well as autoreactive antibodies secreted by plasma cells are still detected in the patients' serum (Traggiai et al., 2003; Vallerskog et al., 2007; Cambridge et al., 2008; Lipsky and Dorner, 2010). On the other hand, using a tolerogen to selectively target DNA-reactive B cells has not proven to be clinically beneficial (Rahman and Isenberg, 2008). An expansion of regulatory B cells, a recently discovered B cell subpopulation, might provide an alternative treatment strategy

(Yanaba et al., 2008), as regulatory B cells are capable of inhibiting proinflammatory cascades by secretion of the regulatory cytokines IL-10 and TGF-β.

The formation of a stable pool of autoreactive plasma cells that contribute to serum IgG production may represent an important therapeutic target in patients refractory to conventional therapies (Hiepe et al., 2011). A clinical long-term benefit for patients with refractory SLE has been demonstrated for autologous stem cell transplantation along with non-specific immunosuppressive agents. This approach does not specifically target plasma cells, but resets adaptive immunity and thereby eliminates autoreactive lymphocytes (Alexander et al., 2009). However, this therapeutic approach goes along with a high mortality (Jayne et al., 2004; Alexander et al., 2009) and thus requires further optimization.

## 5.8 Conclusion and Outlook

Long-lived plasma cells do not divide after selection into the bone marrow compartment (Manz et al., 1997; Slifka et al., 1998; Amanna and Slifka, 2010) and long-term antibody production after infection or vaccination is often maintained by bone marrow plasma cells (Benner et al., 1981). Physical space in survival niches is limited (Radbruch et al., 2006) and once detached from this microenvironment, plasma cells do not relocate (Hoyer et al., 2004). However, specific antibody titers are stable for decades (Amanna et al., 2007) and are shown to sustain for more than 70 years even without antigenic challenge (Hammarlund et al., 2003).

The checkpoint omitting poly- and self-reactive specificities in human bone marrow plasma cells appears to be disturbed in SLE patients with active disease, as reflected by elevated levels of poly- and self-reactivity. As the circulating memory B cell compartment in the majority of SLE patients with active disease is not enriched for poly- or selfreactive antibodies, the bone marrow might be the major source for high-affinity serum IgG antibodies in SLE patients.

Differences in the frequency and specificity of autoreactive bone marrow plasma cells among individual SLE patients likely reflects the high degree of disease diversity. The identification of patient subgroups by B cell profiling could thus provide additional information that might affect the choice of therapeutic treatment. CD20-mediated B cell depletion trials

have already shown that patients with distinct B cell characteristics are more likely to benefit from such regimens (Lipsky and Dorner, 2010).

Inflamed tissues provide additional survival niches for plasma cells (Cassese et al., 2001; Scheel et al., 2011). The analysis of plasma cell frequencies of affected organs during immunosuppressive treatment should elucidate the impact of these plasma cells on disease pathogenesis. Further, experiments analyzing plasma cell frequencies and properties in the blood and affected organs in the course of different immunomodulatory treatments might foster further understanding of the disease. However, the latter experiments are not feasible in humans but have to be conducted in an autoimmune lupus mouse model.

The analysis of B cells participating in germinal center reactions should provide further insights into the proposed checkpoint that controls selection into the long-lived plasma cell compartment in healthy individuals but fails to exclude self-reactive specificities in SLE patients with active disease. Mouse studies have demonstrated that affinity maturation in a primary immune response occurs by early selective differentiation of high-affinity variants into plasma cells that subsequently persist in the bone marrow (Smith et al., 1997; Smith et al, 2000). Further, analysis of plasma cells in an autoimmune lupus mouse model suggests that recruitment of self-reactive plasma cells into the bone marrow compartment occurs early in life (Hoyer et al., 2004). Thus, optimizing the treatment of refractory SLE will require thorough research investigating the critical components of plasma cell biology during development, proliferation and maturation, and elucidating the integral parts of the plasma cell survival niche (Tarlinton and Hodgkin, 2004). Future work should moreover focus on safer strategies for resetting adaptive immunity or targeting autoreactive effector and memory cells specifically while compromising protective immunity as little as possible.

# 6 REFERENCES

Alberts, B, Johnson, A, Walter, P, Lewis, J, and Raff, M (2002). Molecular Biology of the Cell. 5th edition. Taylor & Francis Ltd., London, ISBN 0-8153-4072-9

Alexander, T, Thiel, A, Rosen, O, Massenkeil, G, Sattler, A, Kohler, S, Mei, H, Radtke, H, Gromnica-Ihle, E, Burmester, GR, Arnold, R, Radbruch, A, and Hiepe, F (2009). Depletion of autoreactive immunologic memory followed by autologous hematopoietic stem cell transplantation in patients with refractory SLE induces long-term remission through de novo generation of a juvenile and tolerant immune system. Blood *113*, 214-23.

Alt, FW, Yancopoulos, GD, Blackwell, TK, Wood, C, Thomas, E, Boss, M, Coffman, R, Rosenberg, N, Tonegawa, S, and Baltimore, D (1984). Ordered rearrangement of immunoglobulin heavy chain variable region segments. EMBO J *3*, 1209-1219.

Amanna, IJ, Carlson, NE, and Slifka, MK (2007). Duration of humoral immunity to common viral and vaccine antigens. N Engl J Med *357*,1903-1915.

Amanna, IJ and Slifka, MK (2010). Mechanisms that determine plasma cell lifespan and the duration of humoral immunity. Immunol Rev *236*, 125-138.

Arbuckle, MR, McClain, MT, Rubertone, MV, Scofield, RH, Dennis, GJ, James, JA, and Harley, JB (2003). Development of autoantibodies before the clinical onset of systemic lupus erythematosus, N Engl J Med 349, 1526-33.

Barbas, SM, Ditzel, HJ, Salonen, EM, Yang, WP, Silverman, GJ, and Burton, DR (1995). Human autoantibody recognition of DNA. Proc Natl Acad Sci U S A *92*, 2529-2533.

Benner, R, Hijmans, W, and Haaijman, JJ (1981). The bone marrow: the major source of serum immunoglobulins, but still a neglected site of antibody formation. Clin Exp Immunol *46*, 1-8.

Bensimon, C, Chastagner, P, and Zouali, M (1994). Human lupus anti-DNA autoantibodies undergo essentially primary V kappa gene rearrangements. EMBO J *13*, 2951–2962.

Berek, C, Berger, A, and Apel, M (1991). Maturation of the immune response in germinal centers. Cell *67*, 1121-1129.

Berek, C and Milstein, C (1988). The dynamic nature of the antibody repertoire. Immunol Rev *105*, 5-26.

Boussif, O, Lezoualc'h, F, Zanta, MA, Mergny, MD, Scherman, D, Demeneix, B, and Behr, JP (1995). A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proceedings of the National Academy of Sciences of the United States of America *92*, 7297-7301.

Brezinschek, HP, Foster, SJ, Dorner, T, Brezinschek, RI, and Lipsky, PE (1998). Pairing of variable heavy and variable kappa chains in individual naive and memory B cells. J Immunol *160*, 4762-4767.

Burnet FM (1972). Auto-immunity and Auto-immune Disease. Medical and Technical Publishing Co Ltd, Lancaster, UK.

Cambridge, G, Isenberg, DA, Edwards, JC, Leandro, MJ, Migone, TS, Teodorescu, M, and Stohl, W (2008). B cell depletion therapy in systemic lupus erythematosus: relationships among serum B lymphocyte stimulator levels, autoantibody profile and clinical response. Ann Rheum Dis *67*, 1011-1016.

Cappione, A 3rd, Anolik, JH, Pugh-Bernard, A., Barnard, J., Dutcher, P., Silverman, G., and Sanz, I. (2005). Germinal center exclusion of autoreactive B cells is defective in human systemic lupus erythematosus. J Clin Invest *115*, 3205-3216.

Casellas, R, Shih, TA, Kleinewietfeld, M, Rakonjac, J, Nemazee, D, Rajewsky, K, and Nussenzweig, MC (2001). Contribution of receptor editing to the antibody repertoire. Science *291*, 1541-1544.

Casellas, R, Zhang, Q, Zheng, NY, Mathias, MD, Smith, K, and Wilson, PC (2007). Igk allelic inclusion is a consequence of receptor editing. J Exp Med *204*, 153–160.

Cassese, G, Lindenau, S, de Boer, B, Arce, S, Hauser, A, Riemekasten, G, Berek, C, Hiepe, F, Krenn, V, Radbruch, A, and Manz, RA (2001). Inflamed kidneys of NZB / W mice are a major site for the homeostasis of plasma cells. Eur J Immunol 31, 2726-2732.

Cornall, RJ, Goodnow, CC, and Cyster, JG (1995). The regulation of self-reactive B cells. Curr Opin Immunol *7*, 804-811.

Crispín, JC, Liossis, SN, Kis-Toth, K, Lieberman, LA, Kyttaris, VC, Juang, YT, and Tsokos, GC (2010). Pathogenesis of human systemic lupus erythematosus: recent advances. Trends Mol Med *16*, 47-57.

Crouzier, R, Martin, T, and Pasquali, JL (1995). Heavy chain variable region, light chain variable region, and heavy chain CDR3 influences on the mono- and polyreactivity and on the affinity of human monoclonal rheumatoid factors. J Immunol *154*, 4526-35.

Davidson, A and Diamond, B (2001). Autoimmune Diseases. N Engl J Med *345*, 340-350.

de Wildt, RM, Hoet, RM, van Venrooij, WJ, Tomlinson, IM, and Winter, G (1999). Analysis of heavy and light chain pairings indicates that receptor editing shapes the human antibody repertoire. J Mol Biol *285*, 895-901.

de Wildt, RM, Tomlinson, IM, van Venrooij, WJ, Winter, G, and Hoet, RM (2000). Comparable heavy and light chain pairings in normal and systemic lupus erythematosus IgG(+) B cells. Eur J Immunol *30*, 254-261.

Demaison, C, Chastagner, P, Thèze, J, and Zouali, M (1994). Somatic diversification in the heavy chain variable region genes expressed by human autoantibodies bearing a lupus-associated nephritogenic anti-DNA idiotype. Proc Natl Acad Sci U S A. *91*, 514-518.

Demaison, C, David, D, Fautrel, B, and Theze, J (1996). V-H genefamily representation in peripheral activated B cells from systemic lupus erythematosus (SLE) patients. Clin Exp Immunol *104*, 439–445.

Dighiero, G and Rose, NR (1999). Critical self-epitopes are key to the understanding of self-tolerance and autoimmunity. Immunol Today *20*, 423-428.

Doreau, A, Belot, A, Bastid, J, Riche, B, Trescol-Biemont, MC, Ranchin, B, Fabien, N, Cochat, P, Pouteil-Noble, C, Trolliet, P, Durieu, I, Tebib, J, Kassai, B, Ansieau, S, Puisieux, A, Eliaou, JF, and Bonnefoy-Berard, N (2009). Interleukin 17 acts in synergy with B cell-activating factor to influence B cell biology and the pathophysiology of systemic lupus erythematosus. Nat Immunol *10*, 778-785.

Dorner, T, Foster, SJ, Farner, NL, and Lipsky, PE (1998). Immunoglobulin kappa chain receptor editing in systemic lupus erythematosus. J Clin Invest *102*, 688–694.

Dorner, T, Farner, NL, and Lipsky, PE (1999). Ig lambda and heavy chain gene usage in early untreated systemic lupus erythematosus suggests intensive B cell stimulation. J Immunol *163*, 1027-1036.

Dorner, T, Heimbacher, C, Farner, NL, and Lipsky, PE (1999). Enhanced mutational activity of Vkappa gene rearrangements in systemic lupus erythematosus. Clin Immunol *92*, 188-196.

Dorner, T and Lipsky, PE (2001). Immunoglobulin variable-region gene usage in systemic autoimmune diseases, Arthritis Rheum *44*, 2715-2727.

Doyle, CM, Han, J, Weigert, MG, and Prak, ET (2006). Consequences of receptor editing at the lambda locus: multireactivity and light chain secretion. Proc Natl Acad Sci U S A *103*, 11264-11269.

Egner, W (2000). The use of laboratory tests in the diagnosis of SLE. J Clin Pathol *53*, 424-432.

Ellyard, JI, Avery, DT, Phan, TG, Hare, NJ, Hodgkin, PD, and Tangye, SG (2004). Antigen-selected, immunoglobulin-secreting cells persist in human spleen and bone marrow. Blood *103*, 3805-3812.

Fattal, I, Shental, N, Mevorach, D, Anaya, JM, Livneh, A, Langevitz, P, Zandman-Goddard, G, Pauzner, R, Lerner, M, Blank, M, Hincapie, ME, Gafter, U, Naparstek, Y, Shoenfeld, Y, Domany, E, and Cohen, IR (2010). An antibody profile of systemic lupus erythematosus detected by antigen microarray. Immunology *130*, 337-343.

Feuillard, J, Taylor, D, Casamayor-Palleja, M, Johnson, GD, and MacLennan, IC (1995). Isolation and characteristics of tonsil centroblasts with reference to Ig class switching. Int Immunol *7*, 121-130.

Foreman, AL, Van de Water, J, Gougeon, ML, and Gershwin, ME (2007). B cells in autoimmune diseases: insights from analyses of immunoglobulin variable (Ig V) gene usage. Autoimmun Rev *6*, 387-401.

Gaipl, US, Munoz, LE, Grossmayer, G, Lauber, K, Franz, S, Sarter, K, Voll, RE, Winkler, T, Kuhn, A, Kalden, J, Kern, P, and Herrmann, M (2007). Clearance deficiency and systemic lupus erythematosus (SLE). J Autoimmun *28*, 114-121.

Gay, D, Saunders, T, Camper, S, and Weigert, M (1993). Receptor editing: an approach by autoreactive B cells to escape tolerance. J Exp Med *177*, 999-1008.

Goodnow, CC, Crosbie, J, Adelstein, S, Lavoie, TB, Smith-Gill, SJ. Brink, RA, Pritchard-Briscoe, H, Wotherspoon, JS, Loblay, RH, Raphael, K, Trent, RJ, and Basten, A (1988). Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature *334*, 676-682.

Goodnow, CC, Cyster JG, Hartley SB, Bell SE, Cooke MP, Healy JI, Akkaraju S, Rathmell JC, Pogue SL, and Shokat KP (1995). Self-Tolerance Checkpoints in B Lymphocyte Development. Adv Immunol *59*, 279-368.

Goodnow, CC (2007), Multistep pathogenesis of autoimmune disease, Cell *130*, 25-35.

Goodnow, CC, Vinuesa, CG, Randall, KL, Mackay, F, and Brink, R (2010). Control systems and decision making for antibody production. Nat Immunol *11*, 681-688.

Graham, FL, and van der Eb, AJ (1973). A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology *52*, 456-467.

Grammer, AC and Lipsky, PE (2003). Arthritis Res Ther *5* (Suppl 4), S22-S27.

Guo, W, Smith, D, Aviszus, K, Detanico, T, Heiser, RA, and Wysocki, LJ (2010). Somatic hypermutation as a generator of antinuclear antibodies in a murine model of systemic autoimmunity. J Exp Med *207*, 2225-2237.

Hahn, BH (1998). Antibodies to DNA. N Engl J Med *338*, 1359-1368.

Halverson, R, Torres, RM, and Pelanda, R (2004): Receptor editing is the main mechanism of B cell tolerance toward membrane antigens. Nat Immunol *5*, 645-650.

Hammarlund, E, Lewis, MW, Hansen, SG, Strelow, LI, Nelson, JA, Sexton, GJ, Hanifin, JM, and Slifka, MK (2003). Duration of antiviral immunity after smallpox vaccination. Nat Med *9*, 1131-1137.

Harley, ITW, Kaufman, KM , Langefeld, CD, Harley, JB, and Kelly, JA (2009). Genetic susceptibility to SLE: new insights from fine mapping and genome-wide association studies. Nature Reviews Genetics *10*, 285-290.

Herve, M, Xu, K, Ng, YS, Wardemann, H, Albesiano, E, Messmer, BT, Chiorazzi, N, and Meffre, E (2005). Unmutated and mutated chronic lymphocytic leukemias derive from self-reactive B cell precursors despite expressing different antibody reactivity. J Clin Invest *115*, 1636-1643.

Hibi, T and Dosch, HM (1986). Limiting dilution analysis of the B cell compartment in human bone marrow. Eur J Immunol *16*, 139-145.

Hiepe, F, Dorner, T, Hauser, AE, Hoyer, BF, Mei, H, and Radbruch, A (2011). Long-lived autoreactive plasma cells drive persistent autoimmune inflammation. Nat Rev Rheumatol *Epub,* DOI 10.1038/nrrheum.2011.1.

Hieter, PA, Max, EE, Seidman, JG, Maizel, JV Jr, and Leder, P (1980). Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments. Cell *22*, 197-207.

Hieter, PA, Korsmeyer, SJ,Waldmann, TA, and Leder, P (1981a). Human immunoglobulin kappa light-chain genes are deleted or rearranged in lambda-producing B cells. Nature *290*, 368-372.

Hieter, PA, Hollis, GF, Korsmeyer, SJ, Waldmann, TA, and Leder, P (1981b). Clustered arrangement of immunoglobulin lambda constant region genes in man. Nature *294*, 536-540.

Hochberg, MC (1997). Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum *40*, 1725.

Hoyer, BF, Moser, K, Hauser, AE, Peddinghaus, A, Voigt, C, Eilat, D, Radbruch, A, Hiepe, F, Manz, RA (2004). Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. J Exp Med 199, 1577-1584

Hoyer, BF, Mumtaz, IM, Yoshida, T, Hiepe, F, and Radbruch, A (2008). How to cope with pathogenic long-lived plasma cells in autoimmune diseases. Ann Rheum Dis *67 Suppl 3*, iii87-9.

Huang, C and Stollar, BD (1991). Construction of representative immunoglobulin variable region cDNA libraries from human peripheral blood lymphocytes without in vitro stimulation. J Immunol Methods 141, 227-236.

Huang, C, Stewart, AK, Schwartz, RS and Stollar, BD (1992). Immunoglobulin heavy chain gene expression in peripheral blood B lymphocytes. J Clin Invest 89, 1331-1343.

Ichiyoshi, Y and Casali, P (1994). Analysis of the structural correlates for antibody polyreactivity by multiple reassortments of chimeric human immunoglobulin heavy and light chain V segments. J Exp Med *180*, 885-895.

Isenberg, D, Williams, W, Axford, J, Bakimer, R, Bell, D, Casaseca-Grayson, T, Diamond, B, Ebling, F, Hahn, B, Harkiss, G, Mackworth-Young, C, Le Page, S, Massicotte, H, Rauch, J, Ravirajan, C, Schwartz, R, Shoenfeld, Y, Staines, NA, Todd-Pokropek, A, Tucker, L, Watts, R and Zouali, M (1990). Comparison of DNA antibody idiotypes in human sera: an international collaborative study of 19 idiotypes from 11 different laboratories. J Autoimmun 3, 393-414.

Jacob J, Kelsoe G, Rajewsky K, and Weiss U (1991). Intraclonal generation of antibody mutants in germinal centres. Nature 354, 389-392.

Jacobi, AM, Hansen, A, Burmester, GR, Dorner, T, and Lipsky, PE (2000). Enhanced mutational activity and disturbed selection of mutations in V(H) gene rearrangements in a patient with systemic lupus erythematosus. Autoimmunity 33 , 61-76.

Janeway, CA, Murphy, K, Travers, P, and Walport, M (2008). Immunobiology, 7th edition, Garland Science, New York and London, ISBN: 0-8153-4123-7.

Jayne, D, Passweg, J, Marmont, A, Farge, D, Zhao, X, Arnold, R, Hiepe, F, Lisukov, I, Musso, M, Ou-Yang, J, Marsh, J, Wulffraat, N, Besalduch, J, Bingham, SJ, Emery, P, Brune, M, Fassas, A, Faulkner, L, Ferster, A, Fiehn, C, Fouillard, L, Geromin, A, Greinix, H, Rabusin, M, Saccardi, R, Schneider, P, Zintl, F, Gratwohl, A, and Tyndall, A (2004). Autologous stem cell transplantation for systemic lupus erythematosus, Lupus *13*, 168-76.

Jones, PT, Dear, PH, Foote, J, Neuberger, MS, and Winter, G (1986). Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature 321, 522-525.

Kabat, EA, and Wu, TT (1991). Identical V region amino acid sequences and segments of sequences in antibodies of different specificities. Relative contributions of VH and VL genes, minigenes, and complementarity-determining regions to binding of antibody-combining sites. J Immunol *147*, 1709-1719.

Klein, U and Dalla-Favera, R (2008). Germinal centres: role in B-cell physiology and malignancy. Nat Rev Immunol *8*, 22-33.

Klein U, Kuppers R, and Rajewsky K (1993). Human IgM+IgD+ B cells, the major B cell subset in the peripheral blood, express V kappa genes with no or little somatic mutation throughout life. Eur J Immunol *23*, 3272-3277.

Kohler, G and Milstein, C (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256, 495-497.

Korsmeyer, SJ, Hieter, PA, Ravetch, JV, Poplack, DG, Waldmann, TA, and Leder, P (1981). Developmental hierarchy of immunoglobulin gene rearrangements in human leukemic pre-B-cells. Proc Natl Acad Sci U S A 78, 7096-7100.

Kotzin, BL (1996). Systemic lupus erythematosus, Cell 85, 303-306.

Kuppers R, Zhao M, Hansmann ML, & Rajewsky K (1993). Tracing B cell development in human germinal centres by molecular analysis of single cells picked from histological sections. EMBO J *12*, 4955-4967.

Lanzavecchia, A, Corti, D, and Sallusto, F (2007). Human monoclonal antibodies by immortalization of memory B cells. Current opinion in Biotechnology 18, 523-528.

Lanzavecchia A and Sallusto F (2009). Human B cell memory. Curr Opin Immunol 21, 298-304.

Lefranc, MP and Lefranc G (2001). The Immunoglobulin FactsBook. Academic Press, ISBN: 012441351X

Li, H, Jiang, Y, Cao, H, Radic, M, Prak, EL, and Weigert, M (2001). Editors and editing of anti-DNA receptors. Immunity 15, 947–957.

Li, Y, Louzoun, Y, and Weigert, M (2002). Autoreactive B cells in the marginal zone that express dual receptors. J Exp Med 195, 181–188.

Liu, S, Velez, MG, Humann, J, Rowland, S, Conrad, FJ, Halverson, R, Torres, RM, and Pelanda, R (2005). Receptor editing can lead to allelic inclusion and development of B cells that retain antibodies reacting with high avidity autoantigens. J Immunol 175, 5067–5076.

Lipsky, PE (2001): Systemic lupus erythematosus: an autoimmune disease of B cell hyperactivity, Nat Immunol 2, 764-766.

Lipsky, P E, and Dorner, T (2010). The red wolf remains a wily foe. Nat Rev Rheumatol *6*, 307-308.

Loder, F, Mutschler, B, Ray, RJ, Paige, CJ, Sideras, P, Torres, R, Lamers, MC, and Carsetti, R (1999). B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals. J Exp Med *190*, 75-89.

Mackay, M, Stanevsky, A, Wang, T, Aranow, C, Li, M, Koenig, S, Ravetch, JV, and Diamond, B (2006). Selective dysregulation of the FcgammaIIB receptor on memory B cells in SLE. J Exp Med *203*, 2157-2164.

Manheimer-Lory, AJ, Zandman-Goddard, G, Davidson, A, Aranow, C, and Diamond, B (1997). Lupus-specific antibodies reveal an altered pattern of somatic mutation. J Clin Invest *100*, 2538-2546.

Manz, RA, Hauser, AE, Hiepe, F, and Radbruch, A (2005). Maintenance of serum antibody levels. Annu Rev Immunol *23*, 367-386.

Manz, RA, Thiel, A and Radbruch, A (1997). Lifetime of plasma cells in the bone marrow. Nature *388*, 133-134.

McCafferty, J, Griffiths, AD, Winter, G, and Chiswell, DJ (1990). Phage antibodies: filamentous phage displaying antibody variable domains. Nature *348*, 552-554.

McClain, MT, Heinlen, LD, Dennis, GJ, Roebuck, J, Harley, JB, and James, JA (2005). Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. Nat Med *11*, 85-89.

McGaha, TL, Sorrentino, B and Ravetch, JV (2005). Restoration of tolerance in lupus by targeted inhibitory receptor expression. Science *307*, 590-593.

McMillan, R, Longmire, RL, Yelenosky, R, Lang, JE, Heath, V, and Craddock, CG (1972). Immunoglobulin synthesis by human lymphoid tissues: normal bone marrow as a major site of IgG production. J Immunol *109*, 1386-1394.

Meffre, E, Schaefer, A, Wardemann, H, Wilson, P, Davis, E, and Nussenzweig, MC (2004). Surrogate light chain expressing human peripheral B cells produce self-reactive antibodies. J Exp Med 199, 145-150.

Meffre, E and Wardemann, H (2008). B-cell tolerance checkpoints in health and autoimmunity. Curr Opin Immunol *20*, 632-638.

Melchers, F, Rolink, A, Grawunder, U, Winkler, TH, Karasuyama, H, Ghia, P, and Andersson, J (1995). Positive and negative selection events during B lymphopoiesis. Curr Opin Immunol 7, 214-227.

Mietzner, B, Tsuiji, M, Scheid, J, Velinzon, K, Tiller, T, Abraham, K, Gonzalez, JB, Pascual, V, Stichweh, D, Wardemann, H, and Nussenzweig, MC (2008). Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors. Proc Natl Acad Sci U S A 105, 9727-9732.

Morel, L (2010). Genetics of SLE: evidence from mouse models. Nat Rev Rheumatol 6, 348-357.

Munakata, Y, Saito, S, Hoshino, A, Muryoi, T, Hirabayashi, Y, Shibata, S, Miura, T, Ishii, T, Funato, T and Sasaki, T (1998). Somatic mutation in autoantibody-associated VH genes of circulating IgM+IgD+ B cells. Eur J Immunol 28, 1435–1444.

Muñoz, LE, Lauber, K, Schiller, M, Manfredi, AA, and Herrmann, M (2010). The role of defective clearance of apoptotic cells in systemic autoimmunity. Nat Rev Rheumatol *6*, 280-289.

Muramatsu, M, Kinoshita, K, Fagarasan, S, Yamada, S, Shinkai, Y, and Honjo, T (2000). Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell *102*, 553-563.

Nemazee, D and Burki, K (1989). Clonal deletion of autoreactive B lymphocytes in bone marrow chimeras. Proc Natl Acad Sci U S A *86*, 8039-8043.

Nemazee, DA and Sato, VL (1983). Induction of rheumatoid antibodies in the mouse. Regulated production of autoantibody in the secondary humoral response. J Exp Med *158*, 529-545.

Nemazee, D and Weigert, M (2000). Revising B cell receptors. J Exp Med *191*, 1813-1817.

Neubert, K, Meister, S, Moser, K, Weisel, F, Maseda, D, Amann, K, Wiethe, C, Winkler, TH, Kalden, JR, Manz, RA, and Voll, RE (2008). The proteasome inhibitor bortezomib depletes plasma cells and protects mice with lupus-like disease from nephritis. Nat Med *14*, 748-755.

Nimmerjahn, F, and Ravetch, JV (2008). Fcgamma receptors as regulators of immune responses. Nat Rev Immunol *8*, 34-47.

Nossal, GJV, Austin, CM, and Ada, GL (1965). Antigens in immunity, VII Analysis of immunological memory. Immunology *9*. 333-348.

Nossal, GJ and Lederberg, J (1958). Antibody production by single cells. Nature *181*, 1419-1420.

Nossal, GJ and Pike, BL (1980). Clonal anergy: persistence in tolerant mice of antigen-binding B lymphocytes incapable of responding to antigen or mitogen. Proc Natl Acad Sci U S A *77*, 1602-1606.

Odendahl, M, Jacobi, A, Hansen, A, Feist, E, Hiepe, F, Burmester, GR, Lipsky, PE, Radbruch, A, and Dorner, T (2000). Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. J Immunol *165*, 5970-5979.

Oettinger, MA, Schatz, DG, Gorka, C, and Baltimore, D (1990). RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. Science 248, 1517-1523.

Parhami-Seren, B and Margolies, MN (1996). Contribution of heavy chain junctional amino acid diversity to antibody affinity among p-azophenylarsonate-specific antibodies. J Immunol *157*, 2066-2072.

Paus, D, Phan, TG, Chan, TD, Gardam, S, Basten, A, and Brink, R. (2006) Antigen recognition strength regulates the choice between extrafollicular plasma cell and germinal center B cell differentiation. J Exp Med *203*,1081-1091.

Phan, TG, Paus, D, Chan, TD, Turner, ML, Nutt, SL, Basten, A, and Brink, R (2006). High affinity germinal center B cells are actively selected into the plasma cell compartment. J Exp Med *203*, 2419-2424.

Pisetsky, DS and Ronnblom, L (2009). Systemic lupus erythematosus: a matter of life and death. Arthritis Rheum *60*, 1567-1570.

Plebani, A, Ugazio, AG, Avanzini, MA, Massimi, P, Zonta, L, Monafo, V, and Burgio, GR (1989). Serum IgG subclass concentrations in healthy subjects at different age: age normal percentile charts. Eur J Pediatr *149*, 164-167.

Prak, EL and Weigert, M (1995). Light chain replacement: a new model for antibody gene rearrangement. J Exp Med *182*, 541-548.

Radbruch, A, Muehlinghaus, G, Luger, EO, Inamine, A, Smith, KG, Dorner, T, and Hiepe, F (2006). Competence and competition: the challenge of becoming a long-lived plasma cell. Nat Rev Immunol *6*, 741-750.

Radic, MZ, Mackle, J, Erikson, J, Mol, C, Anderson, WF, and Weigert, M (1993). Residues that mediate DNA binding of autoimmune antibodies. J Immunol *150*, 4966-4977.

Radic, MZ, and Weigert, M (1994). Genetic and structural evidence for antigen selection of anti-DNA antibodies. Annu Rev Immunol *12*, 487-520.

Radic, MZ and Zouali, M (1996). Receptor editing, immune diversification, and self-tolerance. Immunity *5*, 505–511.

Rahman, A, Giles, I, Haley, J, and Isenberg, D (2002). Systematic analysis of sequences of anti-DNA antibodies--relevance to theories of origin and pathogenicity. Lupus *11*, 807-823.

Rahman, A and Isenberg, DA (2008). Systemic lupus erythematosus. N Engl J Med *358*, 929-939.

Rajewsky K (1996). Clonal selection and learning in the antibody system. Nature *381*, 751-758.

Reth (1995). The B-cell antigen receptor complex and co-receptors. Immunol Today *16*, 310-313.

Retter, MW and Nemazee, D (1998). Receptor editing occurs frequently during normal B cell development. J Exp Med *188*, 1231-1238.

Sabbaga, J, Pankewycz, OG, Lufft, V, Schwartz, RS, and Madaio, MP (1990). Cross-reactivity distinguishes serum and nephritogenic anti-DNA antibodies in human lupus from their natural counterparts in normal serum. J Autoimmun *3*, 215-235.

Sallusto, F, Lanzavecchia, A, Araki, K, and Ahmed, R (2010). From vaccines to memory and back. Immunity *33*, 451-463.

Sanz, I and Lee, FE (2010). B cells as therapeutic targets in SLE. Nat Rev Rheumatol *6*, 326-337.

Scheel, T, Gursche, A, Zacher, J, Haupl, T, and Berek, C (2011). V-region gene analysis of locally defined synovial B and plasma cells reveals selected B cell expansion and accumulation of plasma cell clones in rheumatoid arthritis. Arthritis Rheum *63*, 63-72.

Scheid, J, Mouquet, H, Kofer, J, Yurasov, S, Nussenzweig, MC, and Wardemann, H (2011). A checkpoint for autoreactivity between memory B cells and bone marrow plasma cells in humans. manuscript submitted

Sherer, Y, Gorstein, A, Fritzler, MJ, and Shoenfeld, Y (2004). Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients. Semin Arthritis Rheum *34*, 501-537.

Shapiro-Shelef, M and Calame, K (2005). Regulation of plasma-cell development. Nat Rev Immunol *5*, 230-242.

Shimizu, A, and Honjo, T (1984). Immunoglobulin class switching. Cell *36*, 801-803.

Shlomchik, MJ, Marshak-Rothstein, A, Wolfowicz, CB, Rothstein, TL, and Weigert, MG (1987). The role of clonal selection and somatic mutation in autoimmunity. Nature *328*, 805-811.

Shlomchik, MJ, Zharhary, D, Saunders, T, Camper, SA, and Weigert, MG (1993). A rheumatoid factor transgenic mouse model of autoantibody regulation. Int Immunol *5*, 1329-1341.

Shlomchik, MJ, Madaio, MP, Ni, D, Trounstein, M, and Huszar, D (1994). The role of B cells in lpr/lpr-induced autoimmunity. J Exp Med *180*, 1295-1306.

Slifka MK, Antia R, Whitmire JK, and Ahmed R (1998). Humoral immunity due to long-lived plasma cells. Immunity *8*, 363-372

Smith, KGC, Light, A, Nossal, GJV, and Tarlinton, DM (1997). The extent of affinity maturation differs between the memory and antibody-forming cell compartments in the primary immune response. EMBO J *16*, 2996–3006.

Smith, KG, Light, A, O′Reilly, LA, Ang, SM, Strasser, A, and Tarlinton, D (2000). bcl-2 transgene expression inhibits apoptosis in the germinal center and reveals differences in the selection of memory B cells and bone marrow antibody-forming cells. J Exp Med *191*, 475-484.

Souroujon, M, White-Scharf, ME, Andreschwartz, J, Gefter, ML, and Schwartz, RS (1988). Preferential autoantibody reactivity of the preimmune B cell repertoire in normal mice. J Immunol *140*, 4173-4179.

Spatz, L, Iliev, A, Saenko, V, Jones, L, Irigoyen, M, Manheimer-Lory, A, Gaynor, B, Putterman, C, Bynoe, M, Kowal, C, Kuo, P, Newman, J, and Diamond, B (1997). Studies on the structure, regulation, and pathogenic potential of anti-dsDNA antibodies. Methods *11*, 70-78.

Steinitz, M, Klein, G, Koskimies, S, and Makel, O (1977). EB virus-induced B lymphocyte cell lines producing specific antibody. Nature *269*, 420-422.

Suzuki, N, Harada, T, Mihara, S, and Sakane, T (1996). Characterization of a germline Vk gene encoding cationic anti-DNA antibody and role of receptor editing for development of the autoantibody in patients with systemic lupus erythematosus. J Clin Invest *98*, 1843-1850.

Takahashi Y, Dutta PR, Cerasoli DM, and Kelsoe G (1998). In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. V. Affinity maturation develops in two stages of clonal selection. J Exp Med *187*, 885-895.

Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller, JG, Talal, N, and Winchester, RJ (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum *25*, 1271-1277.

Tarasenko, T, Dean, JA, and Bolland, S (2007). FcγRIIB as a modulator of autoimmune disease susceptibility. Autoimmunity *40*, 409-417.

Tarlinton, DM (2008). Evolution in miniature: selection, survival and distribution of antigen reactive cells in the germinal centre. Immunol Cell Biol *86*, 133-138

Tarlinton, DM and Hodgkin, PD (2004). Targeting Plasma Cells in Autoimmune Diseases. J Exp Med *199*, 1451-1454.

Tarlinton, DM and Smith, KG (2000). Dissecting affinity maturation: a model explaining selection of antibody-forming cells and memory B cells in the germinal centre. Immunol Today *21*, 436-441.

Terstappen LW, Johnsen S, Segers-Nolten IM, and Loken MR (1990). Identification and characterization of plasma cells in normal human bone marrow by high-resolution flow cytometry. Blood 76, 1739-1747.

Tiegs, SL, Russell, DM and Nemazee, D (1993). Receptor editing in self-reactive bone marrow B cells. J Exp Med *177*, 1009-1020.

Tieng, AT and Peeva, E (2008). B-cell-directed therapies in systemic lupus erythematosus. Semin Arthritis Rheum *38*, 218-227.

Tiller, T (2009). The Frequency of Self-Reactive Human and Mouse IgG+ B Lineage Cells in Health and Autoimmunity. Thesis. Humboldt Universität zu Berlin.

Tiller, T, Kofer, J, Kreschel, C, Busse, CE, Riebel, S, Wickert, S, Oden, F, Mertes, MM, Ehlers, M, and Wardemann, H (2010). Development of self-reactive germinal center B cells and plasma cells in autoimmune Fc gammaRIIB-deficient mice. J Exp Med *207*, 2767-2778.

Tiller, T, Meffre, E, Yurasov, S, Tsuiji, M, Nussenzweig, MC, and Wardemann, H (2008). Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. J Immunol Methods *329*, 112-124.

Tiller, T, Tsuiji, M, Yurasov, S, Velinzon, K, Nussenzweig, MC, and Wardemann, H (2007). Autoreactivity in human IgG+ memory B cells. Immunity *26*, 205-213.

Tonegawa, S (1983). Somatic generation of antibody diversity. Nature *302*, 575-581.

Traggiai, E., Puzone, R., and Lanzavecchia, A. (2003). Antigen dependent and independent mechanisms that sustain serum antibody levels. Vaccine *21*, Suppl 2:S35-37.

Tsuiji, M, Yurasov, S, Velinzon, K.; Thomas, S, Nussenzweig, MC, and Wardemann, H (2006). A checkpoint for autoreactivity in human IgM+ memory B cell development. J Exp Med *203*, 393-400.

Vallerskog, T, Gunnarsson, I, Widhe, M, Risselada, A, Klareskog, L, van Vollenhoven, R, Malmstrom, V, and Trollmo, C (2007). Treatment with rituximab affects both the cellular and the humoral arm of the immune system in patients with SLE. Clin Immunol *122*, 62-74.

van Bavel, CC, Fenton, KA, Rekvig, OP, van der Vlag, J, and Berden, JH (2008). Glomerular targets of nephritogenic autoantibodies in systemic lupus erythematosus. Arthritis Rheum *58*, 1892-1899

van der Burg, M, Tumkaya, T, Boerma, M, de Bruin-Versteeg, S, Langerak, AW, and van Dongen, JJ (2001). Ordered recombination of immunoglobulin light chain genes occurs at the IGK locus but seems less strict at the IGL locus. Blood *97*, 1001-1008.

van Es, JH, Gmelig Meyling, FH, van de Akker, WR, Aanstoot, H, Derksen, RH, and Logtenberg, T (1991). Somatic mutations in the variable regions of a human IgG anti-double-stranded DNA autoantibody suggest a role for antigen in the induction of systemic lupus erythematosus. J Exp Med *173*, 461-470.

Wang, X and Stollar, BD (2000). Human immunoglobulin variable region gene analysis by single cell RT-PCR. J Immunol Methods *244*, 217-225.

Wardemann, H, Yurasov, S, Schaefer, A, Young, J, Meffre, E, and Nussenzweig, MC (2003). Predominant autoantibody production by early human B cell precursors. Science *301*, 1374-1377.

Wellmann, U, Letz, M, Herrmann, M, Angermuller, S, Kalden, JR, and Winkler, TH (2005). The evolution of human anti-double-stranded DNA autoantibodies. Proc Natl Acad Sci U S A *102*, 9258-9263.

Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, and Tedder TF (2008). A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. Immunity *28*, 639-50.

Yurasov, S, Tiller, T, Tsuiji, M, Velinzon, K, Pascual, V, Wardemann, H, and Nussenzweig, MC (2006). Persistent expression of autoantibodies in SLE patients in remission. J Exp Med *203*, 2255-2261.

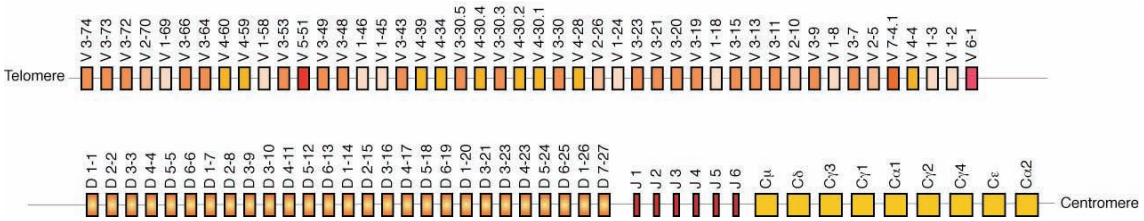
Yurasov, S, Wardemann, H, Hammersen, J, Tsuiji, M, Meffre, E, Pascual, V, and Nussenzweig, MC (2005). Defective B cell tolerance checkpoints in systemic lupus erythematosus. J Exp Med *201*, 703-711.

Zouali, M (1997). The structure of human lupus anti-DNA antibodies. Methods *11*, 27–35.

Zouali, M (2008). Receptor editing and receptor revision in rheumatic autoimmune diseases. Trends Immunol *29*, 103-109.

# 7 SUPPLEMENTAL INFORMATION

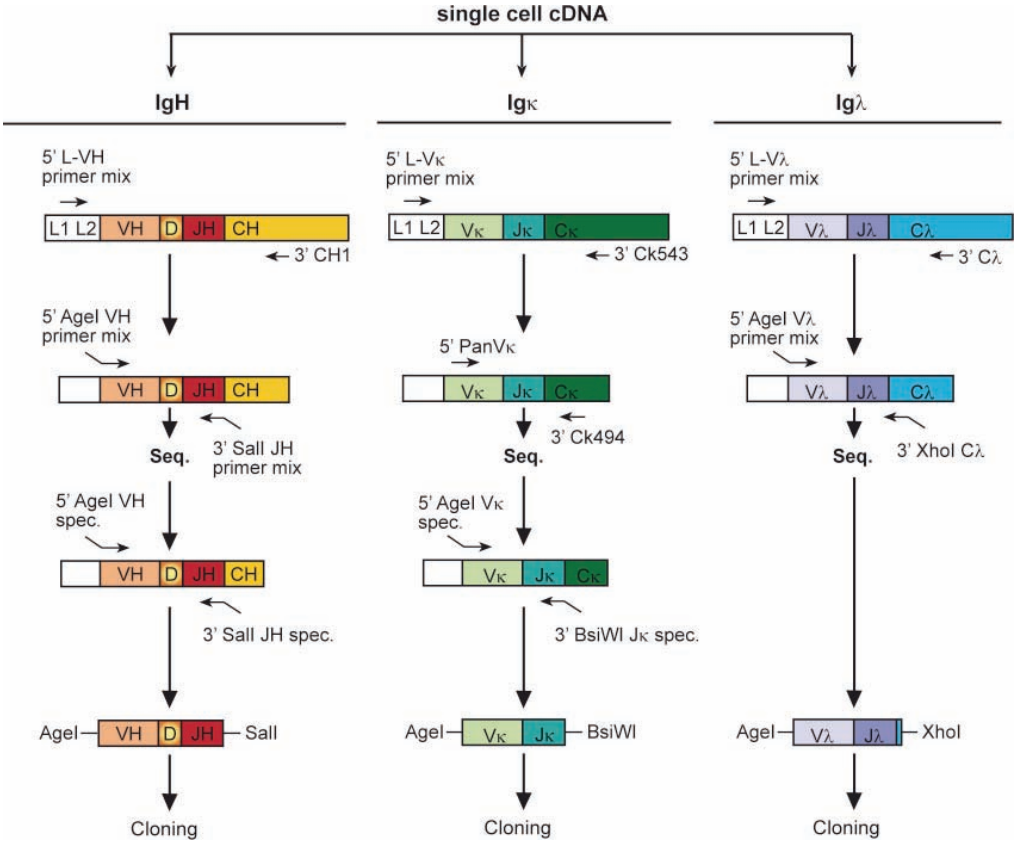
## 7.1 Supplemental Figures




**Supplementary Figure S1. The Human Ig Heavy Chain Locus.**
The VH, D and JH gene elements of the human Ig heavy chain variable region and the CH gene segments that determine the Ig constant region. These gene elements are located on chromosome 14, the relative order of functional gene elements is shown schematically (modified; Tiller, 2009).

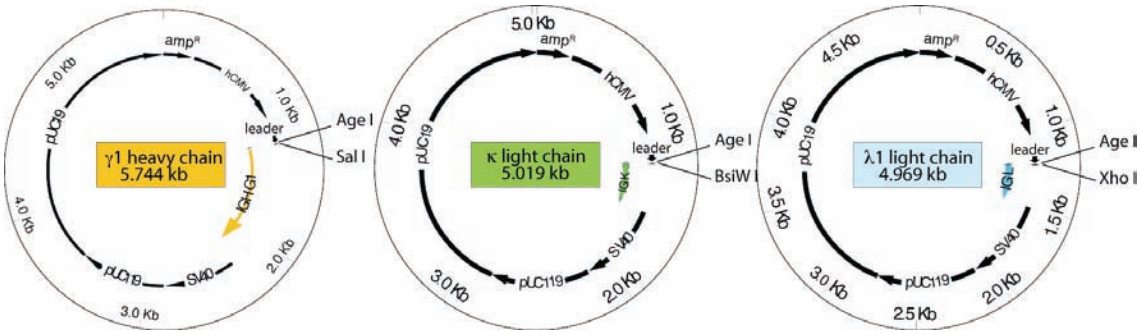

**Supplementary Figure S2. The Human Ig Kappa Light Chain Locus.**
The Vκ and Jκ gene elements of the human Ig kappa light chain variable region and the Cκ gene segment that determines the Ig kappa constant region. These gene elements are located on chromosome 2, the relative order of functional gene elements is shown schematically (modified; Tiller, 2009).


**Supplementary Figure S3. The Human Ig Lambda Light Chain Locus.**
The Vλ and Jλ gene elements of the human Ig lambda light chain variable region and the Cλ gene segments that determine the Ig lambda constant region. These gene elements are located on chromosome 22, the relative order of functional gene elements is shown schematically (modified; Tiller, 2009).



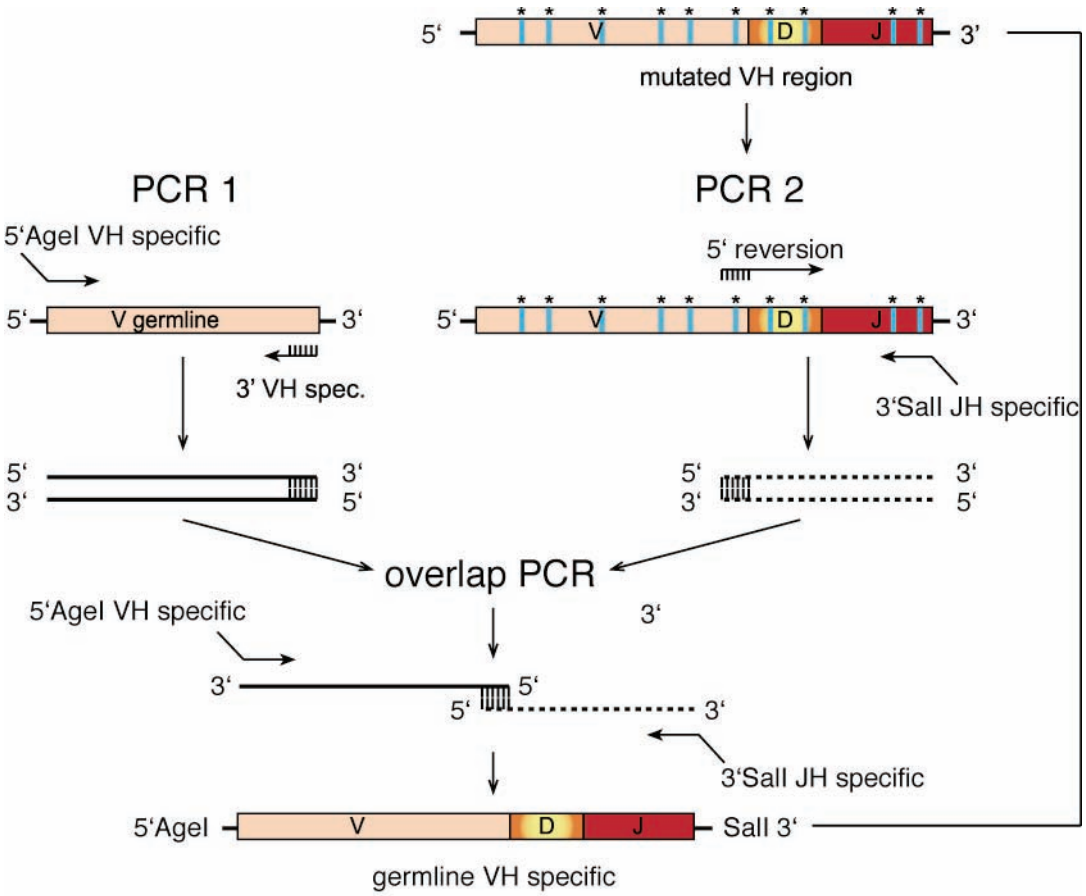

**Supplementary Figure S4. Single Cell RT-PCR Strategy to Amplify Expressed Human Ig Gene Transcripts.**

First PCRs were performed with forward primer mixes specific for the leader region and reverse primers specific for the respective Igγ, Igκ or Igλ constant region. Second PCRs were performed with forward primer mixes specific for FWR1 and respective nested reverse primers specific for the Igγ, Igκ or Igλ J genes or constant regions. Second PCR products were sequenced to determine the respective V and J gene segement. Before cloning, V- and J gene element specific primers containing restriction sites were used in another round of amplification using the first PCR product as template (modified, Tiller et al., 2008).

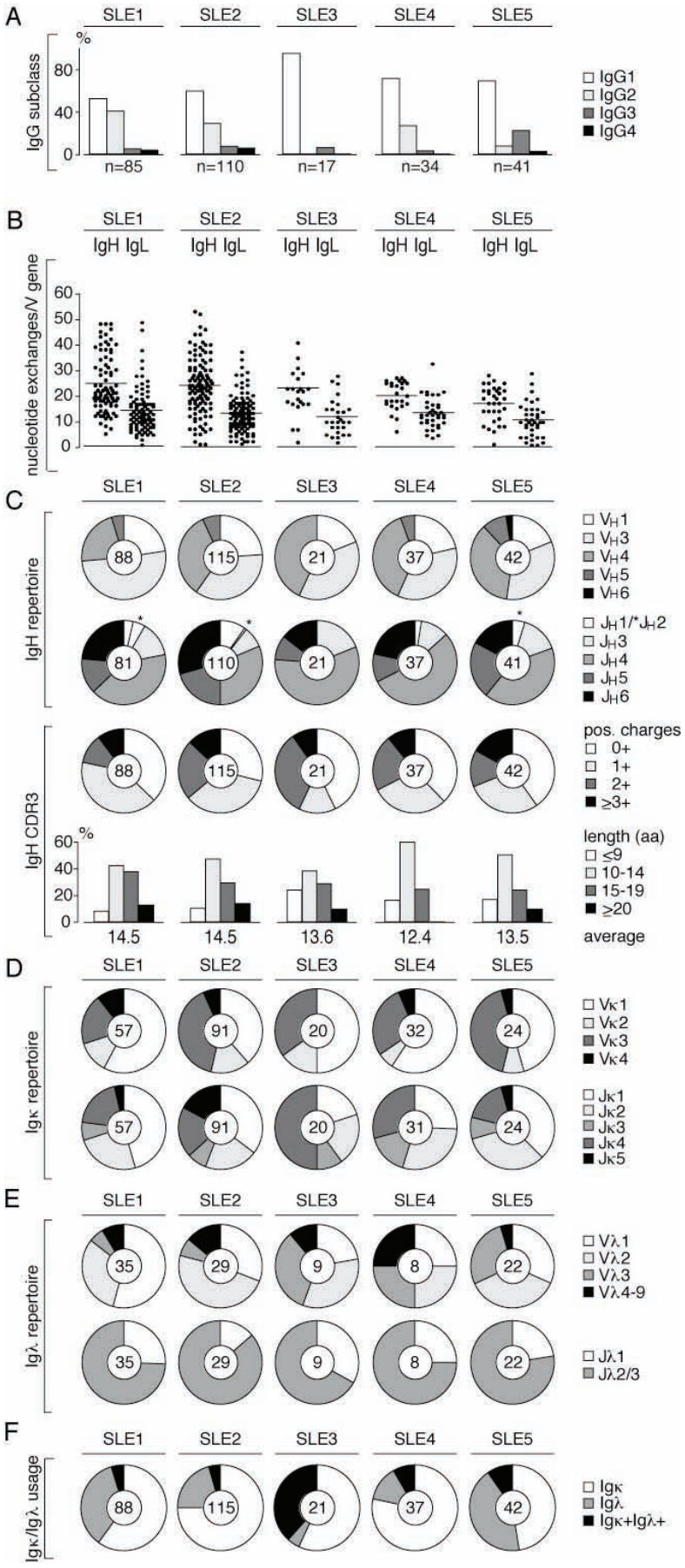



**Supplementary Figure S5. Igγ, Igκ and Igλ Expression Vector Maps.**

PCR products are ligated into human Igg1, Igk or Igl eukaryotic expression vectors containing a murine Ig gene leader peptide (GenBank accession number DQ407610) and a multiple cloning site upstream of the human Igg1, Igk or Igl_constant regions. Transcription is under the influence of the human cytomegalovirus (HCMV) promoter and clones can be selected based on resistance to ampicillin (modified; Tiller, 2009).




**Supplementary Figure S6. Reversion of Somatic Mutations.**
PCR strategy to revert mutated Ig genes into their germline counterparts exemplified on an IgH chain gene. Asterisks and blue lines indicate somatic mutations. PCR 1 amplifies a germline VH gene corresponding to the VH in the mutated clone with gene specific primers. Primers used in PCR 2 revert somatic mutations in the mutated clone. Homology of the PCR 2 forward primer to the reverse primer used in PCR 1 is indicated. The PCR 2 reverse primer is JH-specific and contains the SalI restriction site. PCR products 1 and 2 are fused via the homologous region (indicated) in a subsequent overlap PCR using the same 5′AgeI VH specific forward primer as in PCR 1 and the 3′SalI JH specific reverse primer used in PCR 2 to generate the complete germline VDJ sequence. IgL chain somatic mutations are reverted following the same principle. Overlap PCR products are cloned into the respective expression vectors (modified; Tiller, 2009).




**Supplementary Figure S7. Ig Gene Features of IgG+ Plasma Cells from Individual SLE Patients.**

Ig gene features of IgG+ plasma cell antibodies from five SLE patients (SLE1-5; age 31-69) are shown in comparison to pooled IgG+ plasma cell antibodies from four healthy donors. (A) IgG subclass distribution for individual patients and pooled data (SLE1-5) for comparison with healthy donors; n indicates the number sequences analyzed. (B) Dots represent the absolute number of somatic mutations (nucleotide exchanges compared to the nearest germline gene segment) in individual *Igh* and *Igl Vκ* and *Vλ* genes (FWR1-FWR3) in IgG+ plasma cell antibodies from individual patients and pooled data (SLE1-5) for comparison with healthy donors. Horizontal lines indicate averages. VH/JH gene family usage and IgH CDR3 positive charges and length (C), Vκ/Jκ (D), Vλ/Jλ (E) gene family usage, and Ig light chain isotype (F) are shown. Pie charts represent the data for individual patients and pooled data (SLE1-5) for comparison with healthy donors. The number of sequences analyzed is indicated in the pie chart centers.

## 7.2 Supplemental Tables

**Table S1.** Sequence data and reactivity of IgG antibodies from bone marrow plasma cells of SLE patient SLE1.

| Ig | HEAVY | | | | | | LIGHT | | | | REACTIVITY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SLE1 | VH | D | JH | CDR3 (aa) | IgG isotype | V-mut number | Vκ | Jκ | CDR3 (aa) | V-mut number | ssDNA | dsDNA | insulin | LPS | HEp-2 IFA |
| p-SLE1-2 | 5-51 | 3-9/2-21 | 4 | VTAYYLFDY | 1 | 9 | 1-39 | 4 | QQSYSTPLT | 15 | / | / | / | / | c |
| p-SLE1-5 | 4-59 | 6-19 | 4 | RGEGAVTGTGPFDY | 1 | 16 | 3-20 | 2 | QQYGRSPRT | 16 | / | / | / | / | / |
| p-SLE1-9 | 1-69 | / | 4 | QRDRGHYYFDY | 2 | 18 | 3-20 | 1 | QQYAGSPWT | 10 | + | + | + | + | / |
| p-SLE1-19 | 3-23 | 5-12 | 3 | VGRGYEGAFDV | 1 | 28 | 1-5 | 1 | QQFNSYPWT | 19 | / | / | / | / | / |
| p-SLE1-45 | 1-18 | 1-1 | 6 | ETIVDGLDV | 1 | 62 | 1-5 | 1 | EQHTVDPRV | 45 | + | + | + | + | / |
| p-SLE1-55# | 4-39 | 1-14 | 6 | PVENGGAANRALFYGLDV | 1 | 37 | 1-9 | 4 | QQLTDYPRSIT | 33 | | | | | |
| p-SLE1-56 | 3-23 | 3-16 | 4 | YVSGDYKPPFDY | 2 | 48 | 1-16 | 1 | QQYDHYPWT | 22 | / | / | / | / | / |
| p-SLE1-67 | 1-3 | 3-22 | 3 | PPLPTYDTSGPDAFDT | 1 | 22 | 4-1 | 4 | QQYYNSAALT | 17 | / | / | / | / | / |
| p-SLE1-68# | 1-69 | 1-20/1-1 | 4 | PRLGTTKTAFDY | 1 | 35 | 3-15 | 1 | QHYNNWLSWT | 11 | | | | | |
| p-SLE1-69κ# | 1-18 | 3-9 | 4/5 | DRDDYDVLAGYAPYDF | 1 | 44 | 4-1 | 1 | QHYFSGPT | 21 | | | | | |
| p-SLE1-83# | 4-31 | 4-23 | 3 | SSMSLRDYDGSDAFDI | 2 | 13 | 1-39 | 1 | QQSFSIPRT | 9 | | | | | |
| p-SLE1-91# | 4-49 | 2-15 | 4 | CPRGAKDSSGFFDS | 2 | 20 | 1-39 | 1 | QQSFNVPRT | 16 | | | | | |
| p-SLE1-93 | 3-15 | 2-2 | 6 | AEDIVVVPAVAKYYYYGMEV | 2 | 16 | 1-39 | 1 | QQSYSTPWT | 13 | / | / | / | + | c |
| p-SLE1-103 | 3-33 | 4-17 | 6 | SARPYGDYSYFYYGMDV | 1 | 18 | 1-39 | 2 | QQSYSSPFT | 11 | / | / | / | / | / |
| p-SLE1-108 | 3-48 | 6-19 | 6 | DPTGGGQWPVRYYYYGMDV | 4 | 23 | 2-28 | 1 | MQALETPQT | 11 | / | / | / | / | / |
| p-SLE1-109κ# | 4-31 | 4-17/3-10/4-23 | 1/4 | DSGYGAPEY | 1 | 8 | 1-17 | 1 | LQHNNYPRT | 3 | | | | | |
| p-SLE1-110# | 1-69 | 5-12 | 1 | GGDGGYDGEH | 1 | 48 | 1-27 | 3 | QNYKTVPRD | 27 | | | | | |
| p-SLE1-111 | 4-59 | 3-10 | 6 | FDMFREALGPTYYYGMDV | 1 | 19 | 1-9 | 4 | QQFALGGLT | 10 | + | + | + | + | c |
| p-SLE1-112κ | 4-39 | 3-9 | 5 | RNDLLSTYYRTRPFFDS | ND | 38 | 3-20 | 2 | QHYGDSQYT | 17 | + | + | / | + | n |
| p-SLE1-115 | 3-15 | 1-26 | 5 | DRIEGVFFADNNWLGR | 1 | 40 | 3-20 | 1 | QQCGPSPPWT | 7 | + | + | + | + | / |
| p-SLE1-118 | 3-23 | 2-15 | 4 | SMPLIDYCSGGSCHWTDDY | 2 | 21 | 1-5 | 1 | QQYYRLRA | 10 | / | / | / | / | / |
| p-SLE1-124# | 3-23 | / | 4/5 | GQGSWVDSIFFQS | 2 | | 1-5 | 4 | QQYRGYLKLT | 13 | | | | | |
| p-SLE1-129 | 3-23 | 5-5/5-18 | 4 | SSGGYSYGALGY | 1 | 20 | 4-1 | 2 | QQYSTTPCS | 13 | / | / | / | / | / |
| p-SLE1-136 | 1-24 | 6-19 | 3 | ASSGWPDAFDI | 4 | 18 | 1-13 | 4 | QHFDNSPLT | 15 | / | / | / | / | / |
| p-SLE1-142# | 3-11 | 2-2 | 6 | DRPCNSNSCDIFYGMDV | 1 | 19 | 1-9 | 1 | QLNSYPRT | 7 | | | | | |
| p-SLE1-151 | 4-39 | 5-5/5-18 | 5 | QSWMIPEENS | 2 | 22 | 3-15 | 5 | QHYNNWPIA | 23 | + | + | + | + | / |
| p-SLE1-154# | 3-7 | 5-24 | 3 | DANPGDNKDAFDV | 2 | 21 | 1-6 | 1 | LQDYSYPRT | 12 | | | | | |
| p-SLE1-160κ | 3-30 | 1-26 | 5 | DLGVGATGIDS | 1 | 5 | 3-20 | 1 | QQYGTAPGT | 27 | / | / | / | / | / |
| p-SLE1-164 | 1-8 | 1-20/1-7 | 6 | SRNDWNPRFEGYDYHGFDV | 3 | 48 | 1-5 | 2 | QQCNSFSCS | 37 | + | + | + | + | / |
| p-SLE1-166# | 1-2 | 1-1 | 5 | ADTWNNDWFDP | 1 | 38 | 1-39 | 2 | QQTYRPPQT | 8 | | | | | |
| p-SLE1-169 | 4-28 | 3-22 | 4 | GQIYHYDSSGPFDF | 2 | 42 | 1-5 | 1 | QQYHYPPWT | 30 | / | / | / | / | / |
| p-SLE1-173 | 3-9 | 7-27 | 4 | DNTAGLSNGIWGLDY | 1 | 12 | 1-39 | 2 | QQSYSDPST | 8 | / | / | / | / | / |
| p-SLE1-176 | 3-7 | 3-10 | 6 | TRAGKDYYGSGSYRQDGMDV | 1 | 13 | 1-39 | 3 | QQTYNTRFFT | 10 | / | / | / | / | / |
| p-SLE1-177# | 3-9 | 6-19 | 3 | DRIAVAGRDALDF | 1 | 18 | 1-39 | 5 | QQGFATPVT | 16 | | | | | |
| p-SLE1-178# | 5-51 | 6-19 | 3 | QAGDDGIDL | 1 | 32 | 3-20 | 1 | HQHGNSPQT | 16 | | | | | |
| p-SLE1-181 | 4-31 | 3-16 | 3 | ITQGRYYDGDGAFDI | 2 | 28 | 1-5 | 1 | QQYRSYSWT | 11 | / | / | / | / | / |
| p-SLE1-189 | 3-23 | 2-15 | 4 | SMPLINFCFGGGCYWTDDY | 1 | 40 | 1-5 | 1 | QQYNRLRT | 12 | / | / | / | / | / |
| p-SLE1-194# | 1-18 | 3-10 | 4 | SGLFRLGELPFDY | 1 | 12 | 1-9 | 4 | QQLNSKPLT | 7 | | | | | |
| p-SLE1-201# | 3-23 | 3-9 | 5 | TPATSYDVLNDYYWSASIDS | 2 | 34 | 1-17 | 1 | LQHRSYPWT | 15 | | | | | |
| p-SLE1-208# | 4-7 | / | 2 | HSFDDYRHWYFDL | ND | 19 | 3-20 | 3 | QQYTGPPPT | 4 | | | | | |
| p-SLE1-209# | 4-31 | 4-11/4-4 | 5 | DYSNYGWFDP | 2 | 14 | 1-39 | 1 | QQSYNIPPWT | 10 | | | | | |
| p-SLE1-214 | 3-30 | 3-16 | 4/5 | DLSADYDFIWGTPHF | 1 | 12 | 2-28 | 4 | MQALQAPLS | 4 | / | / | / | / | / |
| p-SLE1-219 | 3-7 | 6-19 | 4 | SDSGWTDFDS | 2 | 19 | 2-30 | 1 | MQGTQWPWT | 4 | / | / | / | / | / |
| p-SLE1-226# | 3-23 | 3-22+6-13 | 4 | NSEAALLYDAAGVDALDY | 2 | 29 | 1-39 | 2 | QQSYSIPYT | 24 | | | | | |
| p-SLE1-229 | 4-39 | 4-17 | 5 | HVVPYDYGDYVPGWFDP | 2 | 18 | 2-28 | 1 | MQGLQTLWT | 5 | / | / | / | / | / |
| p-SLE1-230 | 5-51 | 6-6 | 4 | HPADWAARPTYFDF | 1 | 29 | 3-20 | 2 | LQYGHSPNS | 19 | + | / | / | / | / |
| p-SLE1-232 | 4-39 | 6-19/1-26 | 6 | PAAVGGATNRANFYGSDV | 1 | 45 | 1-9 | 4 | QGLHDYPRAMT | 48 | / | / | / | / | / |
| p-SLE1-240# | 4-31 | 2-15 | 6 | EGWYGVYTYYYYGMDV | 1 | 30 | 1-17 | 2 | LQHNRYPRT | 20 | | | | | |
| p-SLE1-250# | 4-39 | 6-19 | 4 | LASPWVAGPDF | 1 | 39 | 3-20 | 3 | QHYGHSIFT | 15 | | | | | |
| p-SLE1-261# | 3-9 | 3-3 | 4 | GPFGVGYYFAS | 1 | 12 | 4-1 | 2 | QQYFAIPPCS | 8 | | | | | |
| p-SLE1-274 | 3-23 | 5-18/5-5 | 4 | NGKRLWSLHY | 2 | 22 | 2-28 | 4 | MQTLQTSLT | 12 | / | / | / | / | / |
| p-SLE1-281 | 3-53 | 1-1 | 4 | GQLERPLDY | 4 | 22 | 4-1 | 1 | QQYYSSPRT | 5 | + | / | / | / | c |
| p-SLE1-284 | 1-8 | / | 1 | GLIRFDDNAYYYVH | 2 | 18 | 1-12 | 1 | QQANAFRT | 21 | / | / | / | / | / |
| p-SLE1-288# | 1-3 | 6-13 | 4 | SGTAGGTLYFDN | 2 | 38 | 1-5 | 4 | QQYNKYPLT | 12 | | | | | |
| p-SLE1-289# | 3-11 | 3-3 | 4 | RIAFDY | 2 | 19 | 2-29 | 2 | MQAIRLYT | 10 | | | | | |
| p-SLE1-290# | 3-74 | 2-2 | 6 | GRDCGTRTPCYGHPYYGMDV | 2 | 24 | 2-28 | 2 | MQGQEPPYT | 8 | | | | | |
| p-SLE1-295 | 3-23 | 6-25 | 2 | TYWGYSTRGYFDL | 3 | 16 | 4-1 | 2 | QQYYTNPCN | 9 | / | / | / | / | / |

**Table S1 (cont).** Sequence data and reactivity of IgG antibodies from bone marrow plasma cells of SLE patient SLE1.

| SLE1 | VH | D | JH | CDR3 (aa) | IgG isotype | V-mut number | Vλ | Jλ | CDR3 (aa) | V-mut number | ssDNA | dsDNA | insulin | LPS | HEp-2 IFA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| p-SLE1-18 | 1-18 | 3-16/2-15/1-26 | 4 | YTFGGVEASGQDHIDY | 2 | 19 | 1-47 | 3 | AAWDDSLSGWV | 10 | / | + | / | + | / |
| p-SLE1-27# | 3-7 | 1-7/1-20 | 2 | TARIAGTGGGYFDV | 1 | 23 | 2-14 | 2 | SSYTTDSTPL | 9 | | | | | |
| p-SLE1-39 | 3-74 | 5-12/1-26 | 6 | GDVYSGFDKESYVMDV | 3 | 43 | 1-51 | 2 | GTWDSSLSTGV | 6 | / | / | / | / | / |
| p-SLE1-47 | 3-11 | 3-22 | 4 | DLHYTDSSGYSFDY | 2 | 12 | 1-44 | 3 | ATWDDSLKGWV | 14 | + | + | + | + | c |
| p-SLE1-69λ | | | | | | | 2-14 | 1 | SSYTSSSTRYV | 4 | + | / | + | + | / |
| p-SLE1-72 | 3-15 | 5-12 | 4 | VRNDGETFDN | 2 | 21 | 1-40 | 3 | QSYDTSLTAV | 12 | + | + | + | + | c |
| p-SLE1-73# | 1-46 | 1-1/1-20 | 4 | PRLGTTKTAFDY | ND | | 1-44 | 3 | AAWDDSLNGRV | 0 | | | | | |
| p-SLE1-78# | 1-18 | 3-9 | 6 | DCGVDCLDSYGLDV | 2 | 31 | 2-11 | 2 | CSYAGNYRII | 10 | | | | | |
| p-SLE1-84 | 3-23 | 3-10 | 1/5 | DDYGSGNYYNVPDR | 1 | 24 | 1-47 | 3 | AGWDDSLGRPV | 18 | / | / | / | / | c |
| p-SLE1-102# | 1-18 | 6-13 | 4 | DQGKRAAAGNFDY | 1 | 14 | 1-40 | 3 | QSYDTSLTAV | 11 | | | | | |
| p-SLE1-104 | 1-2 | 1-1 | 6 | DLEHNGRPLYYGMDV | 2 | 21 | 1-44 | 3 | ATWDDSLKGWV | 16 | / | / | / | / | / |
| p-SLE1-109λ# | | | | | | | 7-43 | 1 | LLFYGGTYV | 10 | | | | | |
| p-SLE1-112λ | | | | | | | 2-14 | 1 | SSYTSSSTLYV | 2 | + | + | + | + | c |
| p-SLE1-117 | 3-64 | 1-26 | 6 | SGSAWYNMDV | 2 | 30 | 1-51 | 3 | ETWENSLNSGV | 9 | / | / | / | / | / |
| p-SLE1-123# | 4-39 | 6-19 | 5 | HVLRGWHFDS | 1 | 26 | 1-47 | 2 | AAWDDSVSGPV | 14 | | | | | |
| p-SLE1-125 | 5-51 | 3-9 | 4 | HSGPLSLNFDY | 3 | 20 | 2-8 | 2 | TSYAGRDNFVV | 7 | + | + | + | + | c |
| p-SLE1-128# | 3-30 | 3-10 | 5 | SARGRVSVVQGVINWFDP | 1 | 17 | 3-25 | 1 | QSTDSSGSYV | 5 | | | | | |
| p-SLE1-131 | 3-33 | 3-10 | 6 | QLQYYGAGSMDV | 2 | 21 | 1-51 | 2 | GSWDSSLNVVL | 6 | / | / | / | / | / |
| p-SLE1-137# | 1-46 | 3-22 | 4 | EGYYDTTGYKNFDY | 2 | 31 | 2-14 | 1 | CSNFHSISYV | 16 | | | | | |
| p-SLE1-138# | 3-21 | 2-15 | 4 | GAGYCSGVGCYGLGDYFDY | 1 | 20 | 1-47 | 3 | ATWDDSLSTWV | 8 | | | | | |
| p-SLE1-158 | 3-23 | / | 6 | MKGPLRREDYGMDV | 2 | 19 | 1-40 | 3 | QSYDSSLRGWP | 13 | / | + | + | + | / |
| p-SLE1-160λ# | | | | | | | 4-69 | 2/3 | QTWGTGVQV | 11 | | | | | |
| p-SLE1-163# | 3-74 | 7-27 | 1/4 | AWGNIQGEFDH | 1 | 41 | 1-40 | 3 | QSYDSSLSGREV | 23 | | | | | |
| p-SLE1-188# | 4-39 | 3-3 | 1 | GVDDDQSFLEEHFQY | 2 | 46 | 1-51 | 3 | ATWDTSLIITWV | 16 | | | | | |
| p-SLE1-191 | 3-11 | 4-23/4-17 | 6 | DQIPQPDYEGVPPYYYYYGMDV | 1 | 11 | 2-18 | 3 | SSYTSTSTLV | 6 | / | / | / | / | / |
| p-SLE1-206 | 3-23 | 3-22/3-10 | 4/5 | DITV | 1 | 7 | 1-36 | 2 | ATSEDIMNGPV | 9 | + | + | + | + | c |
| p-SLE1-210# | 3-23 | 3-10 | 3 | DLLSGSGSRGNGFDV | 1 | 13 | 1-40 | 3 | QSYDSSLNCVV | 7 | | | | | |
| p-SLE1-223# | 1-2 | 6-13 | 5 | ADTWNDDWFDP | 1 | 38 | 2-14 | 1 | SSYRSGDTLYV | 18 | | | | | |
| p-SLE1-225# | 3-15 | 2-8 | 4 | EYWYAFDY | 2 | 34 | 1-44 | 1 | AAWDARLNAYV | 25 | | | | | |
| p-SLE1-227 | 3-48 | 1-26 | 4 | SLDSYSESYYEWFDH | 1 | 16 | 2-11 | 2/3 | CSYEGGYI | 5 | / | / | / | / | / |
| p-SLE1-231# | 3-64 | / | 2 | DFGL | 1 | 11 | 6-57 | 3 | QSYDSTSHWV | 9 | | | | | |
| p-SLE1-234# | 4-59 | 3-10 | 3 | EPVESVVWGHAFEI | 2 | 24 | 2-23 | 2 | CSYAGSTSIVL | 14 | | | | | |
| p-SLE1-249 | 1-2 | 3-16 | 3 | STLTSGGLFIFAFDI | 1 | 40 | 2-14 | 1 | SSYTSSATHV | 16 | / | / | / | / | / |
| p-SLE1-256 | 3-11 | 5-18/5-5 | 4 | EWWGDAALAD | 2 | 17 | 1-51 | 2 | GTWDSSLSAVV | 6 | / | / | / | / | / |
| p-SLE1-275# | 3-15 | 3-16/1-26 | 4 | DTLLGGNGSQLDY | 1 | 13 | 3-1 | 1 | QAWDSITARV | 12 | | | | | |

p, plasma cell; SLE, SLE patient; CDR3, complementarity determining region 3; aa, amino acid; IFA, indirect immunofluorescence assay; ND, not determined; c, cytoplasmic; n, nuclear; +,reactive; /, non-reactive; #, antibody not expressed

**Table S2.** Sequence data and reactivity of IgG antibodies from bone marrow plasma cells of SLE patient SLE2.

| Ig | HEAVY | | | | | | LIGHT | | | | REACTIVITY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SLE2 | VH | D | JH | CDR3 (aa) | IgG isotype | V-mut number | Vκ | Jκ | CDR3 (aa) | V-mut number | ssDNA | dsDNA | insulin | LPS | HEp-2 IFA |
| p-SLE2-3# | 3-23 | 3-10 | 4 | SYYGEDIDY | 1 | 18 | 1-33 | 1 | QQYDDLPPWT | 10 | | | | | |
| p-SLE2-4# | 3-23 | 5-18 | 4 | QATGYYYGR | 1 | 11 | 3-20 | 2 | QQYGSSPYT | 1 | | | | | |
| p-SLE2-5# | 5-51 | 6-25 | 4 | QADYFDH | 1 | 40 | 3-15 | 2 | QQYISWPPMHT | 22 | | | | | |
| p-SLE2-7 | 3-21 | 4-17 | 6 | RHHDYGDSVYYYYAMDL | 1 | 14 | 2-28 | 4 | MQTLQIPR | 1 | + | + | / | + | c |
| p-SLE2-10 | 5-51 | 1-1 | 3 | HHDNPRVHAFDI | 1 | 36 | 1-33 | 2 | QQYDDLPYT | 11 | / | / | / | / | / |
| p-SLE2-14 | 1-3 | 3-3 | 5 | DRIMEWLFDS | 1 | 27 | 1-33 | 1 | QQYDELPT | 7 | + | + | / | + | n |
| p-SLE2-17# | 1-3 | 2-8 | 4 | DRCTVAPCLDLDN | 1 | 23 | 1-39 | 4 | QQSYSTPLT | 14 | | | | | |
| p-SLE2-18 | 3-33 | 2-21 | 6 | AQLVVVTAQTYYYYYDMDV | 1 | 14 | 2-28 | 1 | MQALQTPPWT | 5 | / | / | / | / | / |
| p-SLE2-21# | 3-30 | 6-19 | 4 | GYSTDWYGFFIY | 3 | 13 | 3-20 | 4 | HQYGGAPLT | 9 | | | | | |
| p-SLE2-22 | 4-59 | 2-21 | 6 | GEVVVTGTLEDHYGMDV | 1 | 11 | 1-33 | 1 | QQYDTFPWT | 8 | / | / | / | / | / |
| p-SLE2-24# | 3-23 | 3-10 | 4 | GRRGSGSYGAYYFDY | 2 | 0 | 1-39 | 2 | QQSYSTPVT | 0 | | | | | |
| p-SLE2-27κ | 3-23 | 2-15 | 6 | DRRVCSGGSCYPLGSYYGMDV | 3 | 6 | 3-20 | 1 | QQYETSPPT | 10 | / | / | / | / | n |
| p-SLE2-28# | 3-21 | 5-18 | 5 | DPHTAMGRGSDF | 1 | 39 | 3-11 | 4 | QQRSNRPPFS | 15 | | | | | |
| p-SLE2-32κ# | 4-61 | 2-15 | 6 | YCTGVNCYVARGGVDV | 2 | 37 | 1-12 | 1 | QQATSFPLT | 13 | | | | | |
| p-SLE2-33# | 3-21 | 3-9 | 3 | DVVVLRNFDHFPYDAFDM | 1 | 43 | 4-1 | 4 | QQYYSSLSVT | 27 | | | | | |
| p-SLE2-35# | 4-34 | / | 6 | DQDGAYGMDV | 1 | 19 | 3-20 | 2 | QQYDSSPYT | 10 | | | | | |
| p-SLE2-36# | 4-31 | 3-9 | 4 | TPAYWDTETGFHPAHFDY | 1 | 29 | 3-11 | 1 | QQRDNWA | 10 | | | | | |
| p-SLE2-37 | 3-74 | 5-5/5-18 | 4 | APSYIYGRYYFDY | 1 | 6 | 3-20 | 5 | QQYGSSPIT | 4 | / | + | / | / | / |
| p-SLE2-43 | 3-74 | 4-17/4-23 | 6 | DDYGDHFYYGMDV | 1 | 4 | 3-11 | 1 | QQRINWPPT | 2 | / | / | / | / | / |
| p-SLE2-49# | 4-59 | / | 5 | FRRGPPDIWFDP | 1 | 27 | 1-39 | 4 | QQSYRTPLT | 12 | | | | | |
| p-SLE2-50# | 1-69 | 7-27 | 6 | LPGERRHDYYGLDV | 1 | 18 | 2-28 | 5 | MQALQTPIT | 3 | | | | | |
| p-SLE2-51# | 5-51 | 4-17 | 3 | HQGNTVTSADAFDI | 1 | 24 | 1-39 | 1 | QQGFTALWT | 10 | | | | | |
| p-SLE2-54# | 4-31 | 3-10 | 5 | GHFYYGSGSYSDR | 1 | 21 | 3-20 | 5 | QQYGRFPGT | 16 | | | | | |
| p-SLE2-60κ | 3-30 | 3-22 | 3 | GGYYLDNSGYDGPFDV | ND | 31 | 1-39 | 5 | QQSYGTFLT | 27 | / | / | / | / | / |
| p-SLE2-61# | 4-30 | 1-1 | 5 | DPRQQLEHWFDP | 1 | 21 | 1-39 | 3 | QQGYSTPLT | 8 | | | | | |
| p-SLE2-63# | 1-24 | 1-7 | 1/5 | DLAITGTTD | 2 | 8 | 3-11 | 4 | QHRANWPLT | 8 | | | | | |
| p-SLE2-64# | 5-51 | 3-22 | 6 | AELYYENLYTSFGV | 1 | 25 | 2-28 | 4 | MQALQTPLT | 4 | | | | | |
| p-SLE2-66# | 4-4 | 4-17 | 5 | GRHGDGLDS | 1 | 19 | 1-16 | 4 | QQYDTYPIT | 15 | | | | | |
| p-SLE2-67# | 1-69 | 4-17 | 6 | AGETTVTNYYGMDV | 4 | 14 | 2-29 | 3 | MQSSDLPPT | 13 | | | | | |
| p-SLE2-71 | 4-31 | 3-10 | 6 | SLFYYGSGHYANA | 1 | 16 | 3-15 | 1 | QHYNNWT | 9 | / | / | / | / | / |
| p-SLE2-72# | 3-33 | 3-22 | 6 | DVDYDSSGYYYGMDV | 1 | 4 | 2-28 | 1 | MQALQTPRT | 4 | | | | | |
| p-SLE2-77# | 1-18 | 2-8 | 4 | DGYCDKGICLAY | 1 | 39 | 3-11 | 4 | QQRLNWP | 12 | | | | | |
| p-SLE2-78# | 3-74 | / | 6 | GTDAWRGMDV | 2 | 51 | 2-24 | 2 | MQSTQFPHT | 14 | | | | | |
| p-SLE2-83# | 3-53 | 3-22 | 1 | LTHYYDKNTYYPGYMRH | 1 | 23 | 1-33 | 2 | QQYDNLPYT | 17 | | | | | |
| p-SLE2-84 | 3-53 | / | 5 | MGRG | 2 | 6 | 1-27 | 1 | QKYNSAPET | 5 | / | / | / | / | / |
| p-SLE2-92# | 4-39 | 6-13 | 4 | HWPTSSSYGAIDY | 1 | 7 | 3-15 | 5 | QQANSFPLT | 7 | | | | | |
| p-SLE2-103# | 4-59 | / | 5 | FRRGPPDIWFDP | 1 | 26 | 1-39 | 4 | QQSYRTPLT | 13 | | | | | |
| p-SLE2-105κ# | 3-74 | 1-20 | 5 | DVAHNYNRGWFDP | 2 | 22 | 3-11 | 1 | QQRAIWPPT | 7 | | | | | |
| p-SLE2-106# | 4-28 | 3-22 | 3 | RSSGYYIDAFDI | 2 | 29 | 3-15 | 1 | QQYNNWPT | 7 | | | | | |
| p-SLE2-109 | 1-69 | 3-10 | 6 | TYDSGNQLNLGDQPNYYYGMDV | 1 | 10 | 4-1 | 1 | QQYYSTSWT | 3 | / | / | / | + | / |
| p-SLE2-114# | 4-31 | 4-11 | 4 | ATGAANYFDY | 2 | 28 | 1-33 | 1 | QQYDELPRT | 18 | | | | | |
| p-SLE2-117 | 1-69 | 3-3 | 6 | ETSYYDFSSGPQSHVNNGMDV | 1 | 21 | 3-20 | 1 | QHFGGLMWT | 12 | / | / | / | / | / |
| p-SLE2-119# | 3-15 | | 1 | GSLVLHTAAEDH | 2 | 30 | 2-24 | 5 | LQSAQLPFT | 14 | | | | | |
| p-SLE2-120 | 3-23 | 6-25 | 4 | VTAAGSAGPCDY | 1 | 26 | 3-20 | 1 | QQYVSSVWT | 7 | / | / | / | / | / |
| p-SLE2-121# | 1-69 | 3-22 | 1 | VSQVSFSDSSGHYRAGYFHP | 1 | | 1-39 | 4 | QQSDSAPVT | 25 | | | | | |
| p-SLE2-122 | 1-69 | 6-13 | 5 | ASTGYGRTWDNWFEP | ND | 36 | 1-39 | 2 | QQTYNSPYT | 24 | / | / | / | / | / |
| p-SLE2-123 | 4-31 | 2-15 | 4/2 | LRRVGGDDF | ND | 33 | 3-20 | 3 | HQYGALPLT | 16 | / | / | / | / | / |
| p-SLE2-124 | 1-2 | 3-10 | 6 | DPLYYGSGSSLEVPAALNAFYGMDV | ND | 25 | 3-20 | 5 | QYYGRSPPIT | 7 | / | / | / | / | / |
| p-SLE2-134 | 3-30 | 6-13/2-2 | 4 | GASAAAVFDH | 3 | 30 | 4-1 | 1 | QQYYRPPWT | 16 | + | + | + | + | c/n |
| p-SLE2-135 | 3-30 | 3-3 | 4 | DRGWRGHWYGGDY | 2 | 21 | 1-33 | 2 | HQYDNLPPYT | 11 | / | / | / | / | / |
| p-SLE2-136 | 4-31 | 3-10 | 6 | GLYYHGSGHYANV | 1 | 10 | 3-15 | 1 | QPYNNWT | 8 | / | / | / | / | n |
| p-SLE2-139# | 4-39 | 6-13 | 6 | SRSSWSNMDV | 2 | 22 | 2-28 | 5 | MQAVQTPVT | 8 | | | | | |
| p-SLE2-140 | 3-23 | 5-12 | 4 | DCGYSGSFES | 2 | 22 | 3-15 | 2 | HQYDKWPPGGAYT | 18 | / | / | / | / | / |
| p-SLE2-144# | 3-74 | 6-6 | 4 | DRNPTVLDN | 2 | 33 | 4-1 | 5 | QEYYSTPAIT | 11 | | | | | |
| p-SLE2-145# | 4-31 | 2-2 | 4 | GRGSQHQLLHAFDY | 4 | 18 | 1-33 | 5 | QQYDDFLIT | 6 | | | | | |
| p-SLE2-147 | 4-39 | 4-17 | 4 | DFGDHRTDY | 2 | 16 | 3-11 | 5 | QQQSS | 10 | / | / | / | / | / |
| p-SLE2-148 | 3-23 | 3-9 | 5 | TPATSYDVLNDYYWSASIDS | 2 | 33 | 3-20 | 2 | QQYANSQT | 28 | / | / | / | / | / |
| p-SLE2-152# | 4-39 | 6-6 | 5 | NSSSSPWFDP | 4 | 23 | 3-15 | 2 | QQYNNWPPYT | 9 | | | | | |
| p-SLE2-154 | 4-31 | 3-10 | 4 | GLYYYGSGSYWDH | 1 | 21 | 3-20 | 5 | QQYGTSPVT | 10 | / | / | / | / | c |
| p-SLE2-155# | 1-69 | 3-3 | 4 | SPRGGEIWSGHQFHFDH | 1 | 35 | 3-15 | 1 | QQYETWQT | 8 | | | | | |
| p-SLE2-158 | 3-72 | 6-6 | 4 | DSMAGGGFDY | 2 | 27 | 2-28 | 1 | MQSLQTPRT | 13 | / | / | / | / | / |
| p-SLE2-159# | 3-21 | 7-27 | 2 | SQTGLYWYFDL | 1 | 9 | 1-13 | 3 | QQLIGYPFT | 6 | | | | | |
| p-SLE2-160 | 1-18 | 3-16 | 4 | YTFGGVEASGQDHIDY | 2 | 20 | 1-13 | 3 | QQLIGYPFT | 8 | / | / | / | + | / |
| p-SLE2-168# | 4-39 | 6-19 | 4 | PDFLASPWVAG | 1 | 22 | 2-30 | 1 | MQGTHWPPA | 15 | | | | | |
| p-SLE2-180 | 1-69 | 6-13 | 4 | EGGPASGTAGYDN | 1 | 24 | 4-1 | 1 | QQYHSPPPT | 7 | / | / | / | / | / |
| p-SLE2-187 | 1-69 | 6-19 | 5 | ERVAVAGGWFDP | 4 | 36 | 3-15 | 3 | QQYNNWPPL | 6 | / | / | / | / | / |
| p-SLE2-188κ# | 1-3 | 3-16 | 6 | LRLGEISQNRFYHYDMDV | 1 | 24 | 3-11 | 1 | QQRTDWPT | 14 | | | | | |

**Table S2 (cont).** Sequence data and reactivity of IgG antibodies from bone marrow plasma cells of SLE patient SLE2.

| SLE2 | VH | D | JH | CDR3 (aa) | IgG isotype | V-mut number | Vκ | Jκ | CDR3 (aa) | V-mut number | ssDNA | dsDNA | insulin | LPS | HEp-2 IFA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| p-SLE2-192# | 4-39 | 6-19 | 4 | LASPWVAGPDF | 1 | 16 | 3-11 | 5 | QQRANWPPIT | 23 | | | | | |
| p-SLE2-204 | 3-23 | 3-10 | 1 | ELQISMQGILIRQYFQH | 1 | 23 | 3-11 | 2 | QQRSNWPPYI | 6 | / | / | / | / | / |
| p-SLE2-205 | 3-48 | 1-26 | 5 | SLDSYSESYYEWFDH | 1 | 29 | 1-39 | 3 | QQSYSTPLT | 11 | / | / | / | / | / |
| p-SLE2-207 | 3-74 | 1-20 | 5 | DVAHNYNRGWFDP | 2 | 17 | 3-11 | 1 | QQRAIWPPT | 3 | / | / | / | / | / |
| p-SLE2-208 | 3-23 | 1-20 | 1 | QLQQ | 2 | 27 | 3-11 | 4 | QQRYNWPPLT | 15 | + | + | + | + | / |
| p-SLE2-216 | 3-48 | 3-3 | 5 | EPRSAWAHWFDP | 1 | 22 | 3-15 | 2 | QQYNNWPPYT | 16 | / | / | / | / | n |
| p-SLE2-224 | 4-59 | 1-1 | 3 | EGPGTSGFDALDV | 1 | 22 | 1-39 | 1 | QQSYSTPWT | 12 | / | / | / | / | / |
| p-SLE2-226 | 1-69 | 3-3 | 6 | DPSYYDFWSGRRSHVKSGMDV | 2 | 17 | 3-20 | 1 | QQFDISPWT | 10 | / | / | / | / | / |
| p-SLE2-229# | 4-39 | 3-3 | 5 | QSRMIPEENS | 2 | 45 | 1-5 | 1 | QQYNNYPWT | 30 | | | | | |
| p-SLE2-238 | 1-8 | 1-7/1-20 | 6 | SRNDWNPRFEGYDYHGFDV | 3 | 17 | 2-30 | 2 | MQGKLWPHT | 8 | / | / | / | / | / |
| p-SLE2-239# | 4-34 | 4-4 | 6 | EEFDSGSHYGPTYYYGMDV | 2 | 18 | 1-9 | 1 | QQLNNYPWT | 23 | | | | | |
| p-SLE2-240 | 1-8 | 1-7/1-20 | 6 | SRNDWNPRFEGYDYHGFDV | 3 | 52 | 1-39 | 2 | QQSFSTPYT | 20 | / | / | / | + | / |
| p-SLE2-248 | 1-18 | 1-26 | 3 | KGGGTHYDALDM | 4 | 16 | 1-5 | 1 | QQYHYPPWT | 5 | / | / | / | / | / |
| p-SLE2-253# | 3-7 | 1-26 | 4 | EGSGRYLPPRDFDL | 2 | 43 | 2-30 | 5 | MQGSHWVT | 33 | | | | | |
| p-SLE2-256 | 1-46 | 1-26 | 5 | DRLWAVEEVGANTYSFFDP | 1 | 26 | 2-30 | 4 | MDGTYWPT | 11 | / | / | / | / | / |
| p-SLE2-257# | 5-51 | 5-12/3-9 | 6 | HPIPSPFDLVMSGLKGAFNYYTMDA | 1 | 13 | 1-5 | 2 | QQYNYFYS | 9 | | | | | |
| p-SLE2-265 | 4-61 | 3-9 | 3 | SHNDILTEMVFDI | ND | 39 | 3-11 | 4 | QQRSNWPLT | 36 | / | / | / | / | c |
| p-SLE2-267# | 1-69 | 3-22 | 1 | VSQLSFSDSTGHYRGGHLHP | 1 | 0 | 1-39 | 1 | QQSDSAPVT | 1 | | | | | |
| p-SLE2-271# | 4-59 | 6-13 | 5 | GSTNWYDP | 2 | 20 | 4-1 | 4 | QQYYSTPST | 11 | | | | | |
| p-SLE2-274# | 4-34 | 2-2 | 3 | VVDIEVQPTGSDGFDI | 3 | 25 | 1-16 | 5 | QQYNSYPIT | 15 | | | | | |
| p-SLE2-277# | 1-24 | 3-9 | 4 | PGDYKGYYFAF | 1 | 11 | 1-12 | 2 | QQADSLPLS | 12 | | | | | |
| p-SLE2-282# | 3-21 | 1-14 | 4 | VEPFWAVDY | 3 | 12 | 1-39 | 1 | QQSYSTLKT | 12 | | | | | |
| p-SLE2-283# | 4-34 | / | 6 | ENRVLDV | 3 | | 3-20 | 2 | QQCAVSPYT | | | | | | |
| p-SLE2-286# | 4-39 | 3-10 | 5 | VTSYYGSGSYFFGT | 1 | | 1-39 | 5 | QQSFITPIT | | | | | | |

| SLE2 | VH | D | JH | CDR3 (aa) | IgG isotype | V-mut number | Vλ | Jλ | CDR3 (aa) | V-mut number | ssDNA | dsDNA | insulin | LPS | HEp-2 IFA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| p-SLE2-6# | 4-61 | 7-27 | 6 | QLGRGGYYYYGMDV | 2 | 1 | 2-11 | 2 | CSYAGSYTFMV | 1 | | | | | |
| p-SLE2-8# | 4-59 | / | 4/5 | DFAEGEFSSSLGD | 1 | 20 | 1-44 | 2 | AAWDDSLSSRGVV | 8 | | | | | |
| p-SLE2-26 | 3-7 | 7-27 | 1 | DLNWESH | 2 | 25 | 2-14 | 3 | SSYTTRHTWV | 16 | / | / | / | / | / |
| p-SLE2-27λ | | | | | | | 8-61 | 3 | VLYMGSGIWV | 3 | + | + | / | + | n |
| p-SLE2-32# | | | | | | | 2-11 | 3 | YSHAGSYT | 1 | | | | | |
| p-SLE2-60λ | | | | | | | 3-21 | 2 | QVWDNDIDHVV | 12 | / | / | / | / | / |
| p-SLE2-62# | 5-51 | 3-16 | 4 | EGDYIWGTYNY | 1 | 6 | 4-69 | 3 | QTWDTDIRV | 13 | | | | | |
| p-SLE2-69 | 3-53 | 3-10 | 5 | DPGYGSGTLSFDP | 2 | 23 | 7-46 | 3 | LLFYSGARV | 14 | / | / | / | / | / |
| p-SLE2-74# | 4-59 | 6-19/5-18/3-3 | 4 | GPIAVDPVEATFLSGFFDY | 1 | 29 | 2-11 | 3 | CSYAGSYTWI | 17 | | | | | |
| p-SLE2-80 | 3-23 | 5-24 | 4/5 | NSVTMTTFTRWAFDS | 2 | 21 | 2-14 | 2 | SSYRSTNNLV | 16 | / | / | / | / | / |
| p-SLE2-95 | 3-64 | 4-23/4-17 | 1 | GESLVTTGAQ | 1 | 30 | 1-36 | 2 | ATWDDSLNGPV | 10 | / | / | / | / | / |
| p-SLE2-101 | 3-33 | 6-13 | 4 | GGWGQQLVRDVGGY | 1 | 16 | 2-14 | 1 | SSYASGNTYV | 12 | + | + | / | + | / |
| p-SLE2-102# | 1-2 | 5-12 | 6 | DLYPRQYNGYRDSLDL | 1 | 26 | 2-14 | 2 | SSYTSASTYVV | 18 | | | | | |
| p-SLE2-105λ# | | | | | | | 1-44 | 3 | ATWDDSLKGWV | 14 | | | | | |
| p-SLE2-107 | 4-39 | 1-26 | 1 | SIVGVTVYFQL | 1 | 32 | 2-8 | 2 | TSYAGRDNFVV | 7 | / | / | / | + | / |
| p-SLE2-170# | 4-31 | 1-1/1-20/2-26 | 1 | GVWNDVLHWGGGVTPFWH | 1 | 20 | 2-14 | 3 | TSYIGINTVV | 11 | | | | | |
| p-SLE2-188λ | | | | | | | 1-47 | 2 | AAWDDILSGVV | 14 | / | / | / | / | / |
| p-SLE2-206 | 4-31 | 3-16/3-10 | 4 | EGVSAVRGYFDY | 2 | 35 | 2-14 | 1 | CSNFHSISYV | 17 | / | / | / | / | / |
| p-SLE2-215# | 4-39 | 1-20 | 6 | HFAITGSLFDMDV | 2 | 25 | 3-25 | 2 | QSADTSGPSVV | 20 | | | | | |
| p-SLE2-217 | 3-30 | 6-19 | 6 | DRGHSSGWSSGYGMDV | 1 | 15 | 2-14 | 1 | SSYSSSNTYV | 11 | / | / | / | / | / |
| p-SLE2-219# | 1-69 | 3-3 | 6 | GHNDFWRGYSKTPLDYKDAMDA | 1 | 46 | 1-40 | 3 | QSYDSSLSGSV | 13 | | | | | |
| p-SLE2-228 | 3-21 | 6-19 | 4/2 | GPSGGEWQSGVDY | 1 | 33 | 1-44 | 3 | AAWDDSLNNWV | 15 | / | / | / | / | / |
| p-SLE2-235 | 5-51 | / | 4 | HYDYPDY | 1 | 5 | 6-57 | 3 | HSYDSNNHWV | 4 | / | / | / | / | n |
| p-SLE2-254 | 5-51 | 2-15 | 4 | RATSRGTFDY | 1 | 14 | 2-11 | 3 | CSYAGDFTVM | 16 | / | / | / | / | / |
| p-SLE2-269# | 4-61 | 6-19 | 4 | HPGGNGWYKYYFDQ | 2 | 22 | 2-14 | 2 | SSFTTSSTLVI | 12 | | | | | |
| p-SLE2-270 | 4-39 | 3-9 | 5 | RNDLLSTYYRTRPFFD | 4 | 38 | 2-11 | 1 | QSYDSSLSGFYV | 11 | | | | | |
| p-SLE2-273 | 3-73 | 4-23 | 6 | QRETELRAEVADYGMDV | 1 | 18 | 1-40 | 3 | QSYGTSLTAV | 12 | | | | | |
| p-SLE2-278 | 1-69 | 5-24 | 5 | GRDDYNNYGIDP | 2 | 40 | 1-44 | 2/3 | SAWDDSLNGII | 19 | | | | | |
| p-SLE2-281 | 3-30 | 3-22 | 1 | GVGGYYSSPFEH | 1 | 37 | 1-44 | 2 | ATWDVSLNGVV | 19 | | | | | |

p, plasma cell; SLE, SLE patient; CDR3, complementarity determining region 3; aa, amino acid; IFA, indirect immunofluorescence assay; ND, not determined; c, cytoplasmic; n, nuclear; +,reactive; /, non-reactive; #, antibody not expressed

**Table S3.** Sequence data and reactivity of IgG antibodies from bone marrow plasma cells of SLE patient SLE3.

| Ig | HEAVY | | | | | | LIGHT | | | | REACTIVITY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SLE3 | VH | D | JH | CDR3 (aa) | IgG isotype | V-mut number | Vκ | Jκ | CDR3 (aa) | V-mut number | ssDNA | dsDNA | insulin | LPS | HEp-2 IFA |
| p-SLE3-6 | 3-30 | 3-10 | 5 | EGEEVQGRISNPGPLHS | 1 | 22 | 3-20 | 4 | QQYGTLPLT | 13 | / | / | / | / | / |
| p-SLE3-8κ# | 3-23 | 1-26 | 4 | TRRALSYFDY | 1 | 18 | 1-39 | 4 | QQSYSNPPT | 14 | | | | | |
| p-SLE3-11 | 3-30 | 3-16 | 3 | DRTDSDEGVFDI | 1 | 17 | 3-11 | 4 | QQRYNWPLT | 7 | / | / | / | / | n |
| p-SLE3-14 | 4-59 | 2-2 | 4 | YIVVIPAYYFDS | 1 | 22 | 2-28 | 2 | MQALQTPFT | 9 | / | / | / | / | / |
| p-SLE3-15 | 1-69 | 6-6 | 6 | DPRIAARPLYYYYGMDV | 1 | 1 | 1-16 | 1 | QQYNTYPPT | 9 | + | + | + | + | c |
| p-SLE3-23κ# | 4-39 | 6-13 | 4 | EVYGNSWSLYYFDH | 1 | 21 | 1-9 | 4 | QQLNSYPRALT | | | | | | |
| p-SLE3-24κ | 3-30 | 4-17 | 4 | DDPWDGDSILDS | 1 | 16 | 1-39 | 2 | QQSYTIPFT | 9 | / | / | / | / | c/n |
| p-SLE3-30κ | 4-59 | 3-9 | 6 | GGEILTGFVQNNYYFYMDV | 1 | 21 | 1-39 | 3 | QQSYSAPFT | 27 | + | / | + | + | / |
| p-SLE3-33 | 4-39 | 3-9 | 4 | RKVDSAFDF | 1 | 26 | 1-17 | 3 | QKYNSAPFT | 24 | / | / | / | + | c |
| p-SLE3-39 | 3-33 | 6-19 | 4 | TTGKIAVAGSLAY | 3 | 22 | 1-27 | 1 | QQYNSAPQT | 14 | / | / | / | / | / |
| p-SLE3-42κ | 4-39 | 3-9 | 5 | RRVDSAFDS | ND | 16 | 1-9 | 4 | QQLNSYPRALT | 1 | / | / | / | / | c/n |
| p-SLE3-44 | 1-69 | 3-22 | 3 | GDSTGYDITAFYAFDI | 1 | 16 | 2-28 | 4 | MQALQTPLT | 4 | / | / | / | / | / |
| p-SLE3-50κ | 1-18 | 3-22 | 4 | GYDNSGPDY | 1 | 15 | 1-5 | 2 | QDYNSYT | 9 | / | / | / | / | c |
| p-SLE3-55κ | 3-21 | 6-6 | 6 | ESSSSLYYYAMDV | 1 | 28 | 3-11 | 2 | QHRVNWPPGYT | 9 | / | / | / | / | / |
| p-SLE3-57 | 4-59 | 7-27 | 4 | ANWDVFAY | ND | 69 | 3-20 | 4 | QQYTEIPLT | 20 | + | + | + | + | c |
| p-SLE3-61# | 1-46 | 5-5 | 4 | DQVGRYSFGFATGQQRVSAISD | 1 | 40 | 3-11 | 4 | QQRSNCLS | 4 | | | | | |
| p-SLE3-63 | 4-30 | 3-22 | 3 | NRKSYDSYGHPLFGVFEI | 1 | 30 | 3-20 | 1 | QQYASSPWT | 16 | / | / | / | / | / |
| p-SLE3-65 | 3-73 | 6-6 | 4 | PFGSSSY | ND | 28 | 2-29 | 4 | MQSVQLLT | 13 | + | / | / | / | c/n |
| p-SLE3-68 | 3-23 | 3-3 | 3 | YHRNNYWTARDAFDL | ND | 22 | 1-5 | 1 | QQYYNYRT | 5 | / | / | / | / | / |
| p-SLE3-69# | 4-59 | 2-2 | 4 | AWPYVLNEPYCSSSSCPYFFDY | 1 | 17 | 3-20 | 4 | QQYGSSPLT | 3 | | | | | |
| SLE3 | VH | D | JH | CDR3 (aa) | IgG isotype | V-mut number | Vλ | Jλ | CDR3 (aa) | V-mut number | ssDNA | dsDNA | insulin | LPS | HEp-2 IFA |
| p-SLE3-8λ | | | | | | | 4-69 | 2/3 | QTWGTGVQV | 10 | / | / | / | / | c |
| p-SLE3-23λ | | | | | | | 2-14 | 1 | SSYTSSTTRL | 7 | / | / | / | / | c |
| p-SLE3-24λ | | | | | | | 1-47 | 2/3 | AAWDDTLSGLI | 17 | / | / | / | / | / |
| p-SLE3-30λ | | | | | | | 2-11 | 2/3 | CSYAGSYHVL | 3 | / | / | + | + | c |
| p-SLE3-42λ | | | | | | | 3-21 | 1 | QVWDNFRDHYV | 14 | / | / | / | / | / |
| p-SLE3-49 | 4-4 | 3-10 | 4 | LLGDYGSGRFDH | 1 | 34 | 3-21 | 3 | QVWDNPSEHRL | 25 | / | / | / | / | / |
| p-SLE3-50# | | | | | | | 3-1 | 1 | QAWDSSTSYV | 4 | | | | | |
| p-SLE3-55λ | | | | | | | 1-44 | 3 | AAWDDSRNGVV | 7 | / | / | / | / | / |
| p-SLE3-69λ | | | | | | | 2-8 | 3 | SSYAGDYSFVV | 11 | / | / | / | / | c |

p, plasma cell; SLE, SLE patient; CDR3, complementarity determining region 3; aa, amino acid; IFA, indirect immunofluorescence assay; ND, not determined; c, cytoplasmic; n, nuclear; +,reactive; /, non-reactive; #, antibody not expressed

**Table S4.** Sequence data and reactivity of IgG antibodies from bone marrow plasma cells of SLE patient SLE4.

| Ig | HEAVY | | | | | | LIGHT | | | | REACTIVITY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SLE4 | VH | D | JH | CDR3 (aa) | IgG isotype | V-mut number | Vκ | Jκ | CDR3 (aa) | V-mut number | ssDNA | dsDNA | insulin | LPS | HEp-2 IFA |
| p-SLE4-5 | 5-51 | 7-27 | 4 | QEEVGMSFGS | 2 | 27 | 1-5 | 1 | LQYRAMLWT | 18 | / | / | / | / | / |
| p-SLE4-11 | 1-69 | 3-3 | 4 | AQAPGFWSGYYHVH | 1 | 18 | 1-13 | 4 | QQFNGFPQT | 16 | / | / | / | / | / |
| p-SLE4-16 | 3-15 | 2-2 | 4 | VTFRYCSSTSCFIDH | 1 | 18 | 3-20 | 3 | QRYGISIFT | 14 | / | / | / | / | / |
| p-SLE4-17 | 3-30 | 6-13/2-2 | 4 | DYSHRSSWFTPTFNDY | 2 | 11 | 3-20 | 3 | QHYGTSRIT | 7 | / | / | / | / | / |
| p-SLE4-21 | 3-15 | 3-22/6-19/6-25 | 4 | DVFVSLDSSGYPVLLY | 1 | 27 | 2-29 | 2 | MQAIELPRT | 22 | / | / | / | / | / |
| p-SLE4-27 | 4-39 | 3-22 | 3 | SPISEISDPLWAFDI | 1 | 16 | 3-20 | 2 | QQYSISTGYT | 13 | / | / | / | + | c/n |
| p-SLE4-30 | 5-a | 2-15/6-25 | 4 | RGGGSSWIDY | 2 | 17 | 3-15 | 3 | QQYNTWPPFT | 14 | / | / | / | / | / |
| p-SLE4-32 | 1-46 | 2-21 | 4 | ECSADCYNTFDF | ND | 25 | 1-5 | 2 | QQYNSYYT | 7 | / | / | / | / | / |
| p-SLE4-34 | 4-59 | 3-3/3-9 | 3 | VRINRSVFGTDILGPFDL | 1 | 29 | 1-27 | 3 | QNYNSAPLT | 17 | / | / | / | / | / |
| p-SLE4-41 | 4-39 | 6-19 | 4 | IYMHTSGWIPFDY | 2 | 25 | 1-5 | 1 | QQYHTYPWA | 21 | / | / | / | / | / |
| p-SLE4-49κ# | 1-18 | 1-26 | 4 | RSGSYHDH | 1 | 26 | 1-5 | 2 | QQYNSYPYT | | | | | | |
| p-SLE4-52 | 3-7 | 3-3 | 5 | ELATKHSWFDS | ND | 23 | 3-15 | 2 | QQHDNWPPYT | 11 | / | / | / | / | / |
| p-SLE4-53 | 3-15 | 5-12 | 6 | LEATDDGRGYYYFSGMDV | 1 | 20 | 4-1 | 4 | HQYYSLPLT | 12 | / | / | / | / | c/n |
| p-SLE4-55 | 1-46 | 5-12 | 6 | DLYRSDKYGMDV | 1 | 12 | 1-39 | 4 | QQSYSSPPT | 9 | / | / | / | / | / |
| p-SLE4-56# | 4-59 | / | 5 | GRDLPIDNWFDH | 2 | 18 | 3-15 | 1 | QQYSNWPPWT | 22 | | | | | |
| p-SLE4-64 | 4-b | 6-13 | 5 | VSEQQLVTP | 1 | 35 | 1-5 | 4 | QQYNTSPLT | 22 | / | / | / | / | / |
| p-SLE4-66 | 4-30 | 5-5/5-18 | 4 | YSTALEYFDN | 1 | 21 | 1-13 | 4 | QQFNGFPQT | 16 | / | / | / | / | c |
| p-SLE4-68 | 1-69 | 1-1/1-20 | 6 | TQNDYLVL | 2 | 20 | 3-20 | 2 | QQYGRPPLS | 17 | / | / | / | / | c |
| p-SLE4-70 | 4-30 | 5-12 | 5 | GTRDSGYDLVWFDP | 1 | 24 | 4-1 | 4 | QQYDSSPLT | 10 | / | / | / | / | / |
| p-SLE4-74# | 4-61 | / | 6 | AAARVYYYGMDV | 1 | | 1-5 | 1 | LQYNSYLA | | | | | | |
| p-SLE4-77κ# | 4-34 | 6-6 | 4 | ASSTYTSSPLDY | 1 | 6 | 1-13 | 1/4 | QQYNSFPQT | | | | | | |
| p-SLE4-78 | 1-24 | 5-5 | 4 | ETRGWLQAFEY | 1 | 26 | 1-33 | 4 | QQFEDLPSGT | 10 | / | / | / | / | / |
| p-SLE4-82κ | 3-21 | / | 4 | DLAPGYYFDY | 1 | 16 | 1-17 | 1 | LQHNNYPRT | 4 | / | / | / | / | / |
| p-SLE4-84 | 1-3 | / | 3 | REYAFDI | 2 | 15 | 1-5 | 1 | QQYINYPWT | 10 | / | / | / | / | / |
| p-SLE4-86 | 3-33 | 3-3/3-9 | 4 | APYYDVWNSIEVPYYLDY | ND | 32 | 1-5 | 2 | QQSWLHPYT | 33 | / | / | + | / | c |
| p-SLE4-87 | 3-33 | 5-5 | 3 | TKWIKDEYGDVIDL | 1 | 25 | 3-11 | 4 | QQRSNWPLT | 13 | / | / | / | / | / |
| p-SLE4-91 | 4-34 | 3-22 | 4 | RSGYYWGLGY | 1 | 26 | 3-15 | 3 | QQYDNWPLT | 12 | / | / | / | / | c/n |
| p-SLE4-92 | 3-23 | / | 4 | GAAGNYDH | 1 | 29 | 2-29 | 1 | MQGIHLPWT | 5 | / | / | / | / | / |
| p-SLE4-103 | 3-21 | 3-16 | 6 | DKGRLGESEDKHYGMDV | 2 | 28 | 1-39 | 2 | QQTFTTPGT | 11 | / | / | / | / | / |
| p-SLE4-107 | 4-61 | 1-1 | 6 | DRKTTGTTIRGMDV | 1 | 25 | 1-5 | 4 | QQYNGFPIT | 13 | / | / | / | / | / |
| p-SLE4-112 | 4-31 | / | 6 | DKRVAGRQIYYYYGLDV | 2 | 24 | 1-9 | 2 | QQLNSYPS | 9 | / | / | / | / | / |
| p-SLE4-118 | 4-59 | / | 6 | FGISASHYYYAMDV | 1 | 44 | 1-5 | 1 | QQYHIYPWT | 8 | + | / | + | + | c/n |
| SLE4 | VH | D | JH | CDR3 (aa) | IgG isotype | V-mut number | Vλ | Jλ | CDR3 (aa) | V-mut number | ssDNA | dsDNA | insulin | LPS | HEp-2 IFA |
| p-SLE4-24 | 3-30 | 3-10 | 4 | EDYYNSSPDY | 1 | 27 | 2-14 | 1 | CSYTSISTYV | 13 | / | / | / | / | / |
| p-SLE4-37 | 1-2 | 5-12 | 4 | AWGAYDSSGDF | 3 | 29 | 1-40 | 3 | QSYDTRLSGWV | 15 | / | / | / | / | c/n |
| p-SLE4-40 | 3-33 | 2-21 | 4 | DGCGGECYHPIDY | 1 | 17 | 3-10 | 3 | YSVDSSGDHGV | 14 | / | / | / | / | / |
| p-SLE4-49λ | | | | | | | 1-51 | 1 | ATWDTSLSVVYV | 12 | / | / | / | / | / |
| p-SLE4-77λ | | | | | | | 9-49 | 3 | ATDPGTGSNFVWV | 13 | / | / | / | / | c |
| p-SLE4-82λ | | | | | | | 2-23 | 2/3 | CSYAGRSIVV | 5 | / | / | + | / | / |
| p-SLE4-93 | 3-30 | 3-9 | 4 | DEEETAFDY | 1 | 26 | 4-69 | 3 | QTWATGMPWV | 21 | / | / | / | / | c |
| p-SLE4-101 | 4-59 | 4-23 | 1 | TYGGNGPIQQ | 1 | 33 | 3-21 | 3 | HVWDSTTEPSDWA | 21 | / | / | / | / | / |

p, plasma cell; SLE, SLE patient; CDR3, complementarity determining region 3; aa, amino acid; IFA, indirect immunofluorescence assay; ND, not determined; c, cytoplasmic; n, nuclear; +,reactive; /, non-reactive; #, antibody not expressed

**Table S5.** Sequence data and reactivity of IgG antibodies from bone marrow plasma cells of SLE patient SLE5.

| Ig | HEAVY | | | | | | LIGHT | | | | REACTIVITY | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SLE5 | VH | D | JH | CDR3 (aa) | IgG isotype | V-mut number | Vκ | Jκ | CDR3 (aa) | V-mut number | ssDNA | dsDNA | insulin | LPS | HEp-2 IFA |
| p-SLE5-2 | 3-30 | 6-6 | 2 | GDYTTSFWFFDL | 1 | 16 | 3-20 | 4 | QQYGSSLT | 7 | / | / | / | / | c/n |
| p-SLE5-11 | 5-a | 5-5/5-18 | 5 | RGTAMALDP | 1 | 21 | 3-15 | 2 | HQYSNWPPYT | 9 | / | / | / | / | / |
| p-SLE5-13 | 1-2 | / | 4 | GRAYRSRFPGGY | 1 | 9 | 1-5 | 2 | QQYNSYPYT | 1 | / | / | / | / | n |
| p-SLE5-16# | 3-23 | 6-13 | 4 o 5 | GGYGSSWF | 1 | | 1-27 | 1 | QKYNSAPWT | | | | | | |
| p-SLE5-17# | 4-59 | 3-10 | 6 | DGRHLIYGSVYYYMDF | 1 | | 1-39 | 1 | QQSQTTPRT | 24 | | | | | |
| p-SLE5-19κ# | 3-23 | 6-19 | 5 | HWGAAGWYPMDS | 1 | 20 | 1-9 | 2 | QQLNSYPST | | | | | | |
| p-SLE5-25 | 4-31 | 6-19 | 4 | RQWFERADYFDY | 1 | 10 | 2-28 | 1 | MQALQTPWT | 0 | / | / | / | / | / |
| p-SLE5-32κ# | 3-21 | 6-13 | 4 | VGFTSSWHFRFFDS | 3 | | 1-9 | 2 | QQLNSYPST | | | | | | |
| p-SLE5-41κ | 4-59 | 3-3 | 4 | WLRSGFDY | 1 | 5 | 1-33 | 2 | QQYADLPCT | 14 | / | / | / | / | c |
| p-SLE5-47 | 4-30 | / | 5 | MAS | 1 | 33 | 2-28 | 4 | MQALQTPLT | 18 | + | + | + | + | n |
| p-SLE5-50 | 3-30 | 6-19 | 4 | VTSGWNVGSFLD | 3 | 22 | 3-15 | 3 | QQYNSWTS | 17 | / | + | / | + | / |
| p-SLE5-52# | 1-2 | 3-22 | 4 | KNYDTGLDS | 1 | 21 | 1-5 | 1 | QQYSSYSWT | | | | | | |
| p-SLE5-62# | 4-4 | 5-5/5-18 | 4 | VGRGSAMDGIDY | 1 | | 3-11 | 5 | QQRSNWIT | 4 | | | | | |
| p-SLE5-63 | 3-21 | 3-3 | 4 | LGCSWTSCYLYDFWSGYYSHYFDF | nd | 13 | 4-1 | 3 | QQYYGSPPGT | 8 | + | + | + | + | / |
| p-SLE5-64 | 4-30 | 1-14 | 3 | EVDRPAPDSDAFDI | 2 | 29 | 3-11 | 4 | QQRANWPLT | 7 | / | / | / | / | / |
| p-SLE5-74κ# | 3-30 | 6-13 | 6 | DILVRWRQQLVRGMRYYYGDV | 1 | 0 | 1-39 | 2 | QQTHSTPCT | | | | | | |
| p-SLE5-75 | 3-21 | 4-4/4-11 | 4 | STSVDYYFDY | 3 | 18 | 1-5 | 1 | QQYKKSPWT | 13 | / | / | / | / | / |
| p-SLE5-78 | 1-69 | 3-3 | 5 | DGGWVDDDLWSGYDS | 1 | 18 | 3-11 | 2 | QQRSNWPCS | 3 | / | / | / | / | c/n |
| p-SLE5-81 | 3-30 | / | 3 | DRTHSDEGVFDI | 1 | 20 | 3-11 | 4 | QQRSNWPLT | 9 | / | / | / | / | / |
| p-SLE5-84# | 4-61 | 6-19 | 4 | IHSSLWYGFDY | 1 | 18 | 1-39 | 1 | HQSYIVPWT | 15 | | | | | |
| p-SLE5-92 | 4-39 | / | 6 | PVASDYYMDV | 1 | 24 | 1-5 | 2 | QQYNTYCS | 12 | / | / | / | / | / |
| p-SLE5-94 | 5-a | / | 4 | LLLLGGLGSYYNDH | 3 | 24 | 3-20 | 1 | QQYKT | 14 | + | + | + | + | c |
| p-SLE5-95 | 5-51 | 1-26 | 4 | QVGANWLDY | 3 | 22 | 3-15 | 1 | QQYNFSPTWT | 14 | / | / | / | / | n |
| p-SLE5-96 | 1-24 | 3-9 | 3 | ERNDDILTGYYNRNGALDI | 2 | 6 | 3-11 | 1 | QQGSYWPPWT | 2 | / | / | / | / | c/n |
| SLE5 | VH | D | JH | CDR3 (aa) | IgG isotype | V-mut number | Vλ | Jλ | CDR3 (aa) | V-mut number | ssDNA | dsDNA | insulin | LPS | HEp-2 IFA |
| p-SLE5-3# | 1-2 | 2-15 | 6 | YSPYFYGSSWDWFFDV | 1 | | 3-21 | 3 | QVWDTSSDHMV | | | | | | |
| p-SLE5-4# | 4-61 | 6-19 | 6 | DEAALAGSLGDGYFYGMDV | 1 | 13 | 3-1 | 1 | QAWDSSTSYV | | | | | | |
| p-SLE5-12 | 3-33 | / | 4 | DNYYFSGSFDY | 3 | 7 | 2-8 | 1 | STYGGSDNYV | 9 | / | / | / | / | c/n |
| p-SLE5-18# | 4-b | 2-2 | 5 | DGGYCSSTTCYRWFDP | 1 | 21 | 3-1 | 3 | QAWDSSTGVV | | | | | | |
| p-SLE5-19λ | | | | | | | 1-51 | 2/3 | GTWISLSAVV | 9 | / | / | / | + | / |
| p-SLE5-20 | 3-30 | 4-17 | 6 | GPTTLITSPYYFYYYYYMDV | 1 | 13 | 2-14 | 2/3 | NSYTSGTTPVF | 12 | / | / | / | / | / |
| p-SLE5-30 | 3-53 | 2-8 | 4 | GRWLRHCTEINCLDYFDF | 1 | 19 | 3-21 | 2/3 | QVWHSSAHLVV | 18 | / | / | / | / | c/n |
| p-SLE5-31 | 1-69 | 6-19 | 4 | DSVRYSRDWYVRQIRPNFDF | 3 | 46 | 2-14 | 2/3 | SSYYTSNTGPIAAVI | 28 | + | + | + | + | c |
| p-SLE5-32λ# | | | | | | | 1-44 | 1 | AAWDDSLNGYV | 8 | | | | | |
| p-SLE5-41λ# | | | | | | | 2-14 | 2/3 | SSYTRSSTLVV | 3 | | | | | |
| p-SLE5-44 | 4-59 | 6-13 | 3 | NSTSSSPLDI | 4 | 24 | 3-21 | 2/3 | QVWDSSSDHPV | 0 | / | / | / | / | / |
| p-SLE5-45 | 5-a | 3-16 | 2 | RGHQTSPRLWYFDV | 1 | 13 | 1-40 | 3 | QSYDFSLNASWV | 11 | + | / | + | / | / |
| p-SLE5-49 | 4-39 | 5-5/5-18 | 5 | DDRIYTHGYFRFDS | 1 | 16 | 2-14 | 2/3 | SSYTSSSTLVV | 3 | / | / | / | / | / |
| p-SLE5-51 | 3-30 | 6-6 | 6 | IALPDLDWYYYYMDV | 1 | 21 | 3-21 | 3 | QLWDSGSEYSWV | 12 | / | / | / | / | / |
| p-SLE5-67 | 4-59 | / | 5 | EENGYSSSWFYY | 3 | 6 | 2-14 | 2/3 | SSYTSSSSHVV | 10 | / | / | / | / | / |
| p-SLE5-69 | 3-21 | 3-10 | 5 | DRLQTYYYGLGSPHTTFDP | 1 | 16 | 1-51 | 3 | GTWDNSLSAGV | 9 | / | / | / | / | / |
| p-SLE5-73 | 6-1 | 6-19 | 3 | EGDGSGWYDDAFDI | 1 | 7 | 2-23 | 1 | CSFADRSPSFYV | 21 | / | / | / | / | / |
| p-SLE5-74λ | | | | | | | 7-46 | 3 | LLSYNGVRWV | 10 | + | + | + | + | c/n |
| p-SLE5-77 | 1-69 | 4-17 | 3 | RHDYADYRFAFDI | 1 | 7 | 1-44 | 2/3 | AAWDDSLNGVV | 6 | / | / | / | / | n |
| p-SLE5-79# | 4-b | 6-19 | 4 | DEQWLFDY | 3 | 27 | 2-8 | 1 | SSYAGSNNYL | 3 | | | | | |
| p-SLE5-90 | 1-18 | 6-19 | 4 | DPLGRQWLTNFDY | 2 | 14 | 1-51 | 3 | GTWDSSLNTGV | 8 | / | / | / | / | n |
| p-SLE5-93 | 4-30 | 3-16 | 5 | EQAMLAIGGVLVRAWFDP | 1 | 25 | 1-44 | 3 | AAWDDSLSGVV | 1 | / | / | / | / | / |

p, plasma cell; SLE, SLE patient; CDR3, complementarity determining region 3; aa, amino acid; IFA, indirect immunofluorescence assay; ND, not determined; c, cytoplasmic; n, nuclear; +,reactive; /, non-reactive; #, antibody not expressed

**Table S6.** Ig gene usage, CDR3 amino acid sequence, and reactivity of mutated and respective unmutated germline IgG+ plasma cells from patient SLE2 after reversion of somatic mutations.

| Ig | HEAVY | | | | LIGHT | | | | REACTIVITY | |
|---|---|---|---|---|---|---|---|---|---|---|
| | VH | JH | V-Mut | CDR3 (aa) | Vκ/λ | Jκ/λ | V-Mut. | CDR3 (aa) | poly | HEp2 |
| p-SLE2-27 | 3-23 | 6 | 6 | DRRVCSGGSCYPLGSYYGMDV | κ 3-20 | 1 | 10 | QQYETSPPT | / | n |
| | | | | | λ 8-61 | 3 | 3 | VLYMGSGIWV | + | n |
| p-SLE2-r27 | 3-23 | 6 | 0 | DRRVCSGGSCYPLGSYYGMDV | κ 3-20 | 1 | 0 | QQY**GS**SPPT | / | / |
| | | | | | λ 8-61 | 3 | 0 | VLYMGSGIWV | / | / |
| p-SLE2-134 | 3-30 | 4 | 30 | GASAAAVFDH | κ 4-1 | 1 | 16 | QQYYRPPWT | + | c/n |
| p-SLE2-r134 | 3-30 | 4 | 0 | G**GI**AAAVFDH | κ 4-1 | 1 | 0 | QQYY**ST**PWT | / | / |
| p-SLE2-154 | 4-31 | 3-10 | 4 | GLYYYGSGSYWDH | κ 3-20 | 5 | 10 | QQYGTSPVT | / | c |
| p-SLE2-r154 | 4-31 | 3-10 | 0 | GLYYYGSGSYWD**Y** | κ 3-20 | 5 | 0 | QQYG**S**SPI**T** | / | / |
| p-SLE2-216 | 3-48 | 5 | 22 | EPRSAWAHWFDP | κ 3-15 | 2 | 16 | QQYNNWPPYT | / | n |
| p-SLE2-r216 | 3-48 | 5 | 0 | EPRSAWAHWFDP | κ 3-15 | 2 | 0 | QQYNNWPPYT | / | / |
| p-SLE2-235 | 5-51 | 5 | 22 | HYDYPDY | λ 6-57 | 3 | 4 | HSYDSNNHWV | / | n |
| p-SLE2-r235 | 5-51 | 5 | 0 | HYDYPDY | λ 6-57 | 3 | 0 | **Q**SYDS**S**NHWV | / | / |

reverted amino acids in CDR3s are in bold; RF, reading frame; V-Mut, number of V gene mutations from FWR1 to FWR3, inclusively; mut, mutated antibody; rev, reverted antibody; '+, reactive in ELISA/IFA; /, non-reactive in ELISA/IFA; poly, polyreactive to ss/dsDNA, LPS, and insulin; HEp2, reactive in HEp2 IFA

## 7.3 Supplemental Material

### Antibodies

| Specificity | Clone/Source | Conjugate | Application | Supplier |
|---|---|---|---|---|
| Human CD19 | SJ25C1 | PECy7 | FACS | BD Biosciences |
| Human CD27 | M-T271 | FITC | FACS | BD Biosciences |
| Human CD38 | HIT2 | APC | FACS | BD Biosciences |
| Human CD138 | MI15 | PE | FACS | BD Biosciences |
| Human IgG, Fcγ | Goat | - | ELISA | Jackson ImmunoResearch |
| Human IgG, Fcγ | Goat | HRP | ELISA | Jackson ImmunoResearch |
| Human IgG, Fcγ | Goat | Cy3 | IFA | Jackson ImmunoResearch |

| Name | | Application | Reference |
|---|---|---|---|
| ED38 | High polyreactive control | ELISA | Meffre, et al., 2004 |
| eiJB40 | Low polyreactive control | ELISA | Wardemann et al., 2003 |
| mGO53 | Non-polyreactive control | ELISA | Wardemann et al., 2003 |

### Antigens

| Name | Application | Supplier |
|---|---|---|
| DNA sodium salt from salmon testes | ELISA | Sigma-Aldrich |
| Human recombinant insulin | ELISA | Sigma-Aldrich |
| LPS, E.coli 055:B5 | ELISA | Sigma-Aldrich |
| Human IgG1, kappa (as standard) | ELISA | Sigma-Aldrich |

### Bacteria

| Name | Supplier |
|---|---|
| *E.coli* DH10B | Invitrogen |

## Bacterial Culture Media

| Name | Compounds | | |
|---|---|---|---|
| Luria Bertani (LB) | 1 % (w/v) | Bacto-Tryptone | |
| medium | 0.5 % (w/v) | yeast extract | |
| | 85.5 mM | NaCl | were dissolved in water under stirring, |
| | the pH was adjusted to 7.5 and the medium was autoclaved (15 min at 121 °C), | | |
| | if required, ampicillin Na salt (Roche) was used at a concentration of 100 µg/ml, | | |
| | 1.5 % (w/v) Agar-agar was added prior to autoclaving (15 min at 121 °C) for LB- plates | | |
| Terrific Broth (TB) | 47.6 % (w/v) | TB powder | were dissolved in water under stirring, |
| medium | 4 ml | Glycerol | was added prior to autoclaving (15 min at 121 °C) |

## Buffers and Solutions

| Name | Compounds | |
|---|---|---|
| 2x HEPES-buffered saline, pH 7.05 | 50 mM | HEPES |
| | 10 mM | KCl |
| | 12 mM | Dextrose |
| | 280 mM | NaCl |
| | 1.5 mM | $Na_2HPO_4$-$7H_2O$ |
| 5x loading dye for DNA gels | 60 % (w/v) | Sucrose |
| | 1 mM | Cresol Red |
| 50x Tris Acetate EDTA (TAE) buffer | 2 M | Tris Base |
| | 0.05 % (v/v) | Acetic Acid |
| | 0.05 M | EDTA (pH 8.0) |
| ABTS buffer, pH 4.2 | 29.4 mM | $Na_2HPO_4$-$7H_2O$ |
| | 41.2 mM | Citric Acid, anhydrous |
| ELISA blocking buffer | 0.05 % | Tween® 20 |
| | 1 mM | EDTA |
| | 1x | PBS |
| 1x Phosphate buffered Saline (PBS) | 137 mM | NaCl |

| | | |
|---|---|---|
| | 2.7 mM | KCl |
| | 10 mM | $Na_2HPO_4$ |
| | 1.76 mM | $KH_2PO_4$ |
| | the pH was adjusted to 7.4 prior to was autoclaving | |
| 10x Phosphate buffered Saline (PBS) | 1.37 M | NaCl |
| | 27 mM | KCl |
| | 100 mM | $Na_2HPO_4$ |
| | 17.6 mM | $KH_2PO_4$ |

## Cell Line

| **Name** | | **Supplier** |
|---|---|---|
| HEK293T | ATCC® No. CRL-11268™ | Invitrogen |

## Cell Culture Media

| **Name** | **Supplier** |
|---|---|
| Antibiotic-Antimycotic (100x) | Gibco BRL |
| Dulbecco's Modified Eagle Medium (D-MEM) - GlutaMAX™ | Gibco BRL |
| Ultra Low IgG FCS | Biochrom |
| Nutridoma-SP | Roche |

## Chemicals

Standard laboratory chemicals were purchased from Sigma and were all in per analysis quality.

| Name | Formula |
|---|---|
| Ammonium Chloride | $NH_4Cl$ |
| Calcium Chloride | $CaCl_2 \cdot 2H_2O$ |
| Citric Acid, anhydrous | $C_6H_8O_7$ |
| Cresol Red | $C_{21}H_{17}O_5SNa$ |
| D-(+)-Glucose | $C_6H_{12}O_6$ |
| Dithiothreitol (DTT) | $C_4H_{10}O_2S_2$ |
| Ethidium Bromide | $C_{21}H_{20}BrN_3$ |
| EDTA Disodium Salt | $C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$ |
| Glycerol | $C_3H_8O_3$ |
| Glycine | $C_2H_5NO_2$ |
| Glacial Acetic Acid | $C_2H_4O_2$ |
| HEPES | $C_8H_{18}N_2O_4S$ |

| Name | Formula |
|---|---|
| Igepal CA-630 | $C_{16}H_{26}O_2$ |
| Polyethylenimine | $H(NHCH_2CH_2)_nNH_2$ |
| Potassium Hydrogen Carbonate | $KHCO_3$ |
| Potassium Chloride | KCl |
| Potassium Phosphate | $KH_2PO_4$ |
| Sodium Azide | $NaN_3$ |
| Sodium Chloride | NaCl |
| Sodium Phosphate | $NaH_2PO_4 \cdot 7H_20$ |
| Sucrose | $C_{12}H_{22}O_{11}$ |
| Trizma® Base | $C_4H_{11}NO_3$ |
| Tween®20 | $C_{58}H_{114}O_2$ |

## Commercial Kits

| Name | Supplier |
|---|---|
| ANA HEp-2 microscopic slides | Bios GmbH Labordiagnostik |
| NucleoBond Xtra Maxi Kit | Macherey-Nagel |
| NucleoSpin Extract II Kit | Macherey-Nagel |
| NucleoSpin® Plasmid Kit | Macherey-Nagel |

## Enzymes

| Name | Application | Supplier |
|---|---|---|
| SuperScript III Reverse Transcriptase | Reverse Transcription | Invitrogen |
| HotStar Taq DNA Polymerase | PCR | Qiagen |
| T4 DNA Ligase | Ligation | New England Biolabs (NEB) |
| Age I | Restriction enzyme digest | New England Biolabs (NEB) |
| Sal I | Restriction enzyme digest | New England Biolabs (NEB) |
| BsiW I | Restriction enzyme digest | New England Biolabs (NEB) |
| Xho I | Restriction enzyme digest | New England Biolabs (NEB) |

## Expression Vectors

Eukaryotic expression vectors with human γ1, κ or λ constant regions were a kind gift of Prof. Dr. Michel Nussenzweig, The Rockefeller University, New York, USA.

## Human Samples

Bone marrow samples from SLE patients were collected after patients signed informed consent in accordance with protocols reviewed by the Institutional Review Board of the Charité University Medical Center.

## Nucleotides and Nucleic Acids

| Name | Supplier |
|---|---|
| 100 bp DNA Ladder | New England Biolabs |
| 1 kb DNA Ladder | Invitrogen |
| dATP | Invitrogen |
| dCTP | Invitrogen |
| dGTP | Invitrogen |
| dTTP | Invitrogen |
| Random Hexamer Primer | Roche Applied Science |

## Primer Sequences for Amplification of Human Ig Genes

Primer Sequences for Amplification of Human Ig Genes.

1st γHeavy Chain PCR

| Forward Primer | 5'-3' Sequence |
|---|---|
| 5' L-VH 1 | ACAGGTGCCCACTCCCAGGTGCAG |
| 5' L-VH 3 | AAGGTGTCCAGTGTGARGTGCAG |
| 5' L-VH 4/6 | CCCAGATGGGTCCTGTCCCAGGTGCAG |
| 5' L-VH 5 | CAAGGAGTCTGTTCCGAGGTGCAG |

| Reverse Primer | 5'-3' Sequence |
|---|---|
| 3' Cγ CH1 | GGAAGGTGTGCACGCCGCTGGTC |

2nd γHeavy Chain PCR

| Forward Primer | 5'-3' Sequence |
|---|---|
| 5' AgeI VH1 | CTGCAACCGGTGTACATTCCCAGGTGCAGCTGGTGCAG |
| 5' AgeI VH1/5 | CTGCAACCGGTGTACATTCCGAGGTGCAGCTGGTGCAG |
| 5' AgeI VH 1-18 | CTGCAACCGGTGTACATTCCCAGGTTCAGCTGGTGCAG |
| 5' AgeI VH 1-24 | CTGCAACCGGTGTACATTCCCAGGTCCAGCTGGTACAG |
| 5' AgeI VH3 | CTGCAACCGGTGTACATTCTGAGGTGCAGCTGGTGGAG |
| 5' AgeI VH3-23 | CTGCAACCGGTGTACATTCTGAGGTGCAGCTGTTGGAG |
| 5'AgeI VH3-33 | CTGCAACCGGTGTACATTCTCAGGTGCAGCTGGTGGAG |
| 5' AgeI VH 3-9 | CTGCAACCGGTGTACATTCTGAAGTGCAGCTGGTGGAG |
| 5' AgeI VH4 | CTGCAACCGGTGTACATTCCCAGGTGCAGCTGCAGGAG |
| 5' AgeI VH 4-34 | CTGCAACCGGTGTACATTCCCAGGTGCAGCTACAGCAGTG |
| 5'AgeI VH4-39 | CTGCAACCGGTGTACATTCCCAGCTGCAGCTGCAGGAG |
| 5' AgeI VH 6-1 | CTGCAACCGGTGTACATTCCCAGGTACAGCTGCAGCAG |

| Reverse Primer | 5'-3' Sequence |
|---|---|
| 3' IgG (internal) | GTTCGGGGAAGTAGTCCTTGAC |
| 3' SalI JH 1/2/4/5 | TGCGAAGTCGACGCTGAGGAGACGGTGACCAG |
| 3' SalI JH 3 | TGCGAAGTCGACGCTGAAGAGACGGTGACCATTG |
| 3' SalI JH 6 | TGCGAAGTCGACGCTGAGGAGACGGTGACCGTG |

1st κLight Chain PCR

| Forward Primer | 5'-3' Sequence |
|---|---|
| 5' L Vκ 1/2 | ATGAGGSTCCCYGCTCAGCTGCTGG |
| 5' L Vκ 3 | CTCTTCCTCCTGCTACTCTGGCTCCCAG |
| 5' L Vκ 4 | ATTTCTCTGTTGCTCTGGATCTCTG |
| **Reverse Primer** | **5'-3' Sequence** |

| | |
|---|---|
| 3' Cκ 543 | GTTTCTCGTAGTCTGCTTTGCTCA |

### 2nd κLight Chain PCR

| **Forward Primer** | **5'-3' Sequence** |
|---|---|
| 5' Pan Vκ | ATGACCCAGWCTCCABYCWCCCTG |

| **Reverse Primer** | **5'-3' Sequence** |
|---|---|
| 3' Cκ 494 | GTGCTGTCCTTGCTGTCCTGCT |

### Gene-specific κLight Chain PCR

| **Forward Primer** | **5'-3' Sequence** |
|---|---|
| 5' AgeI Vκ 1-5 | CTGCAACCGGTGTACATTCTGACATCCAGATGACCCAGTC |
| 5' AgeI Vκ 1-9 | TTGTGCTGCAACCGGTGTACATTCAGACATCCAGTTGACCCAGTCT |
| 5' AgeI Vκ 1D-43 | CTGCAACCGGTGTACATTGTGCCATCCGGATGACCCAGTC |
| 5' AgeI Vκ 2-24 | CTGCAACCGGTGTACATGGGGATATTGTGATGACCCAGAC |
| 5' AgeI Vκ 2-28 | CTGCAACCGGTGTACATGGGGATATTGTGATGACTCAGTC |
| 5' AgeI Vκ 2-30 | CTGCAACCGGTGTACATGGGGATGTTGTGATGACTCAGTC |
| 5' Age Vκ 3-11 | TTGTGCTGCAACCGGTGTACATTCAGAAATTGTGTTGACACAGTC |
| 5' Age Vκ 3-15 | CTGCAACCGGTGTACATTCAGAAATAGTGATGACGCAGTC |
| 5' Age Vκ 3-20 | TTGTGCTGCAACCGGTGTACATTCAGAAATTGTGTTGACGCAGTCT |
| 5' Age Vκ 4-1 | CTGCAACCGGTGTACATTCGGACATCGTGATGACCCAGTC |

| **Reverse Primer** | **5'-3' Sequence** |
|---|---|
| 3' BsiWI Jκ 1/4 | GCCACCGTACGTTTGATYTCCACCTTGGTC |
| 3' BsiWI Jκ 2 | GCCACCGTACGTTTGATCTCCAGCTTGGTC |
| 3' BsiWI Jκ 3 | GCCACCGTACGTTTGATATCCACTTTGGTC |
| 3' BsiWI Jκ 5 | GCCACCGTACGTTTAATCTCCAGTCGTGTC |

### 1st λLight Chain PCR

| **Forward Primer** | **5'-3' Sequence** |
|---|---|
| 5' L Vλ 1 | GGTCCTGGGCCCAGTCTGTGCTG |
| 5' L Vλ 2 | GGTCCTGGGCCCAGTCTGCCCTG |
| 5' L Vλ 3 | GCTCTGTGACCTCCTATGAGCTG |
| 5' L Vλ 4/5 | GGTCTCTCTCSCAGCYTGTGCTG |
| 5' L Vλ 6 | GTTCTTGGGCCAATTTTATGCTG |
| 5' L Vλ 7 | GGTCCAATTCYCAGGCTGTGGTG |
| 5' L Vλ 8 | GAGTGGATTCTCAGACTGTGGTG |

| **Reverse Primer** | **5'-3' Sequence** |
|---|---|

| | |
|---|---|
| 3' Cλ | CACCAGTGTGGCCTTGTTGGCTTG |

2nd λLight Chain PCR

| **Forward Primer** | **5'-3' Sequence** |
|---|---|
| 5'AgeI Vλ 1 | CTGCTACCGGTTCCTGGGCCCAGTCTGTGCTGACKCAG |
| 5'AgeI Vλ 2 | CTGCTACCGGTTCCTGGGCCCAGTCTGCCCTGACTCAG |
| 5'AgeI Vλ 3 | CTGCTACCGGTTCTGTGACCTCCTATGAGCTGACWCAG |
| 5'AgeI Vλ 4/5 | CTGCTACCGGTTCTCTCTCSCAGCYTGTGCTGACTCA |
| 5'AgeI Vλ 6 | CTGCTACCGGTTCTTGGGCCAATTTTATGCTGACTCAG |
| 5'AgeI Vλ 7/8 | CTGCTACCGGTTCCAATTCYCAGRCTGTGGTGACYCAG |

| **Reverse Primer** | **5'-3' Sequence** |
|---|---|
| 3' XhoI Cλ | CTCCTCACTCGAGGGYGGGAACAGAGTG |

## Primer for Sequencing and Bacterial Colony Insert Screening

Primer Sequences for Sequencing and Bacterial Colony Insert Screening.

| **Forward Primer** | **5'-3' Sequence** |
|---|---|
| 5' Ab sense | GCTTCGTTAGAACGCGGCTAC |

| **Reverse Primer** | **5'-3' Sequence** |
|---|---|
| 3' IgG (internal) | GTTCGGGGAAGTAGTCCTTGAC |
| 3' Cκ 494 | GTGCTGTCCTTGCTGTCCTGCT |
| 3' Cλ | CACCAGTGTGGCCTTGTTGGCTTG |

## Reagents

| Name | Supplier |
|---|---|
| ABTS tablets, 50 mg | Roche Applied Science |
| Nuclease-free water | Qiagen |
| Protein G sepharose beads | GE Healthcare |
| RNAsin | Promega |
| SeaKem® LE Agarose | Cambrex Bioscience |

## Software

| Name | Producer |
|---|---|
| Adobe Illustrator CS3 | Adobe |
| EditSeq™ | DNASTAR |
| FlowJow7 | Tree Star |
| GraphPad Prism 4 | GraphPad Software Inc. |
| SeqMan™ | DNASTAR |
| SoftMax Pro v5 | Molecular Devices |
| Microsoft® Office 2004 | Microsoft Corporation |
| Adobe Photoshop CS3 | Adobe |

## Web Resources

| | |
|---|---|
| Ig gene sequence identification by IgBlast | http://www.ncbi.nlm.nih.gov/igblast/ |
| Ig gene sequence information by IMGT | http://www.imgt.cines.fr |

# ACKNOWLEDGMENTS

This PhD thesis would not have been possible without the generous intellectual and emotional support provided by innumerous colleagues, friends and family.

First and foremost, I want to express my deep appreciation to Dr. Hedda Wardemann for her excellent guidance and support, for helpful and inspiring discussions, and her never ending patience. Thank you for having me around!

Moreover, I wish to thank Prof. Dr. Roland Lauster for supervising and reviewing my PhD thesis on behalf of the TU Berlin.

I would like to thank all present and past members of our lab crew at the MPI for Infection Biology for a wonderful time and a great working atmosphere. In particular, I want to thank Dr. Thomas Tiller for introducing me into this exciting field of research and his special sense of humor. Further, I want to express my special gratitude to my lab family Conny, Friederike, Julia, Lydie and Markus.

I am also indebted to Prof. Dr. Falk Hiepe, Dr. Bimba Hoyer and Prof. Dr. Annett Jacobi for providing us with genuine gold- bone marrow aspirates from SLE patients.

I would like to thank Prof. Dr. Michel Nussenzweig and Johannes Scheid for letting us take care of the healthy plasma cell data and also John Pietzsch for helping us whenever needed.

I want to acknowledge Dr. Marc Ehlers and his group at the DRFZ for the incredibly friendly collaboration, for fruitful discussions and technical advice.

Furthermore, I want to thank the laboratory of Prof. Dr. Arturo Zychlinsky, especially Viola, Yvonne and Steven, for being our first aid guardians at any time.

Most importantly, none of this would have been possible without the constant support, encouragement and understanding of my family and friends. I cannot adequately express my gratitude to them. Thank you for everything.