VH4-34 Encoded Antibody in Systemic Lupus Erythematosus: Effect of Isotype

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ABSTRACT. ***Objective.*** To determine the clinical significance of elevated serum levels of VH4-34 encoded IgM and IgG antibodies with respect to the clinical characteristics of systemic lupus erythematosus (SLE).

Methods. VH4-34 encoded IgM and IgG immunoglobulin was measured in 95 patients with SLE by ELISA using antiidiotype monoclonal antibody (Mab) 9G4. SLE disease activity, severity, and damage were assessed by visual analog scales, Systemic Lupus Activity Measure, Lupus Severity of Disease Index, and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index. Presence of VH4-34 encoded antibodies on patients' B lymphocytes was analyzed by flow cytometry using Mab 9G4.

Results. Fifty-two of 95 patients with SLE had elevated levels of VH4-34 encoded antibodies of IgG isotype; 17 patients with VH4-34 IgG had elevated VH4-34 of the IgM isotype. Forty-three of the 95 patients had normal levels of VH4-34 encoded antibodies. When disease severity was correlated to VH4-34 isotype, patients with circulating VH4-34 IgG but without IgM had significantly more severe disease compared to patients who had VH4-34 of both isotypes. Eighty-six percent of patients with SLE nephritis and 100% of those with central nervous system (CNS) lupus had VH4-34 IgG without IgM. *In vivo*, VH4-34 encoded antibodies were found to bind autologous B lymphocytes.

Conclusion. Presence of VH4-34 IgG in the absence of VH4-34 IgM was the finding most strongly associated with severe SLE, nephritis, and CNS lupus, suggesting that isotype switching of VH4-34 encoded antibodies or loss of VH4-34 IgM encoded antibodies may influence the progression of disease in SLE. (J Rheumatol 2002;29:2114–21)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS
VH4-34 GENE
B LYMPHOCYTE
IgM
AUTOANTIBODIES

The VH4-34 gene, previously known as VH4.21 or DP-63, is one of the 53 identified functional VH germline genes[1]. It is a member of the relatively small VH4 family of about 10 functional genes[2]. The VH4-34 gene is present in all haplotypes and no sequence variation has been reported in germline DNA isolated from unrelated individuals[3].

Antibodies derived from the VH4-34 gene have been shown to be autoreactive. Almost all the antibodies directed against the I/i antigens of red blood cells (RBC) are encoded by the VH4-34 gene[4-7]. Generally in the IgM class the anti-I/i antibodies were classically described as cold agglutinins since they agglutinate RBC at low temperature[8,9]. The VH4-34 gene also encodes other autoreactivities, such as anti-Rh antibodies[10] and polyreactive antibodies that bind single stranded DNA, lipid A, cardiolipin, and rheumatoid factor[11].

Another novel self-specificity shown by a subset of VH4-34 derived anti-i monoclonal antibodies (Mab) is binding and toxic towards B lymphocytes[12-14]. With a library of 24 independently derived VH4-34 encoded Mab we have shown that the cytotoxicity of the VH4-34 encoded Mab correlated directly to its B cell binding intensity as measured by fluorescence activated cell sorter (FACS) analysis[15,16]. Besides complement, toxicity is mediated via novel non-apoptotic mechanism of membrane disruption. The mandatory usage of a single VH gene among all anti-B cell antibodies suggests that the heavy chain encoded by the VH4-34 gene is vital, particularly since these antibodies have different VH-CDR3 and are paired with independently derived light chains. *In vivo*, the VH4-34 gene is overrepresented in the normal adult repertoire at the B cell receptor (BCR) level. As detected by antiidiotypic Mab 9G4, 3 to 8% of B cells in human peripheral blood and peripheral lymphoid organs are VH4-34 positive. However, secreted and thus circulating VH4-34 derived immunoglobulins are at low to undetectable levels in the peripheral blood of healthy adults[17,18]. Serum levels of VH4-34 gene derived antibodies increase only in selective clinical conditions, such as cold agglutinin disease, infectious mononucleosis[9,19], acquired immune deficiency syndrome[20,21], and juvenile

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chronic arthritis[22]. The high degree of conservation of the gene, overrepresentation in the B cell repertoire, active secretion only under certain pathological conditions, and B cell binding/cytotoxicity, all suggest that these antibodies may play a role in immune regulation or in host defence against certain antigens or pathogens.

Systemic lupus erythematosus (SLE) is a multisystem disease with a variable course and prognosis. The quest for the underlying primary immune defect that allows the manifestation of forbidden self-reactivities has been the focus of many studies. We and others have described elevated levels of VH4-34 derived antibodies in patients with SLE using the antiidiotype Mab 9G4[23-25]. Our study revealed that 52 of 95 patients with SLE had elevated levels of VH4-34 antibodies compared to 18/344 controls, giving a sensitivity of 55% and a specificity of 95% for elevated VH4-34 antibodies as a serologic test for SLE[23]. The study also showed a significant correlation between levels of VH4-34 encoded antibodies and disease severity.

In this study we tested the biological properties of VH4-34 encoded immunoglobulins in SLE at the isotype level. We observed that VH4-34 encoded antibodies bind autologous B lymphocytes in a subset of patients with VH4-34 IgG and in all patients with detectable circulating VH4-34 of the IgM isotype. Moreover, patients with VH4-34 IgM have significantly less severe disease than patients with circulating VH4-34 encoded immunoglobulin of only the IgG isotype. These results suggest that isotype switching of VH4-34 encoded immunoglobulins from IgM to IgG may correlate with disease progression in SLE.

MATERIAL AND METHODS

Human subjects. Peripheral blood specimens were obtained from healthy donors 20–50 years of age and from patients with SLE, and aliquoted and frozen at –70˚C as described[23]. Blood specimens were obtained with the approval of the Committee for the Protection of Human Subjects at Stanford University. At the time of specimen collection, patients were clinically assessed for 3 separate disease dimensions. (1) SLE activity was measured using the Systemic Lupus Activity Measure (SLAM)[26] and a visual analog scale (VAS) completed by the treating physician. (2) Chronic and irreversible damage as the result of the disease and its treatment was measured by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index (SLICC/ACR)[27]. (3) Overall disease severity over the entire course of the disease was estimated, irrespective of the current disease activity level. This measure, while intuitively appealing, has not been studied in as much detail as disease activity. One instrument for ascertaining SLE severity was validated, the Lupus Severity of Disease Index (LSDI)[28], but this measure has not been used in additional studies. However, we have reported that the LSDI correlated strongly with physician's assessment of disease severity by VAS, and less with disease activity or damage, suggesting that disease severity does indeed represent a separate dimension of SLE[23]. Therapy was based on physician assessment of ongoing severity and activity of disease in patients followed in the Stanford Immunology and Rheumatology Clinic.

ELISA for VH4-34 encoded antibody isotype. In 1986 Stevenson, *et al* developed the rat Mab 9G4, subsequently shown to be specific for VH4-34 encoded antibodies[29]. The VH4-34 idiotype identified by Mab 9G4 is conformation restricted and dependent on a unique amino acid sequence at amino acid 7 and amino acids 23–25 in the framework 1 (FW1) region of the variable heavy chain[30,31]. Sequence analysis of 58 IgM and 11 IgG Mab derived from the VH4-34 gene showed that only 2 IgM Mab have mutations in this region that prevent them binding Mab 9G4[4,10,11,19,30,32-34]. Mab 9G4 is estimated to bind > 98% of secreted antibodies derived from the VH4-34 gene. VH4-34 BCR expression on normal peripheral blood B cells and B cell lymphomas has also been studied using 9G4 and VH region sequencing[18,35]. No non-VH4-34-expressing cells were detected with 9G4.

Nunc ELISA plates were coated with purified 9G4 overnight at 10 μg/ml. The plates were blocked and washed as described[23]. Sera were serially diluted starting with 1:400 for VH4-34 IgG and 1:50 VH4-34 IgM and binding was detected with goat anti-human IgG or IgM, respectively, conjugated with horseradish peroxidase (CalTag, South San Francisco, CA, USA). VH4-34 derived IgG Mab C7[36] and VH4-34 derived IgM Mab 216[16] were included as standards on each plate. Levels greater than the normal mean + 3 SD were considered elevated for each isotype. This assay gives a relative value of VH4-34 isotype in each serum specimen. High levels of VH4-34 encoded IgG in serum can compete with elevated VH4-34 IgM and vice versa, so the amount of each isotype detected remains an approximation, proportional to their respective levels[23]. To determine the actual concentration of individual VH4-34 isotypes, separation of total serum IgG from total serum IgM is necessary and was not undertaken due to the variability involved with the procedure.

Whole blood staining. Presence of VH4-34 encoded antibodies on patients' B lymphocytes (n = 63) was evaluated by whole blood staining. This analysis began midway through the study of VH4-34 encoded antibodies in SLE sera and patient samples were obtained in chronological order; 100 μl of whole blood from patients or controls (n = 15, ages 25–60 yrs) was washed twice with phosphate buffered saline (PBS) to remove serum Ig and incubated with biotin conjugated antiidiotype 9G4 and phycoerythrin conjugated anti-CD20 (Becton Dickinson, Immunocytometry Systems, San Jose, CA, USA). Cells were washed twice and then incubated with FITC-streptavidin (CalTag). The RBC were lysed and WBC fixed with Cal-Lyse solution according to manufacturer's instructions (Caltag). As an isotype control for 9G4 (a rat IgG), normal and SLE samples were also incubated with biotin labeled monoclonal rat IgG2 (Caltag) and anti-CD20 as described above. The staining was uniformly negative and is not shown as contour plots. The cells were resuspended and analyzed on FACScan (Becton-Dickinson, Mountain View, CA, USA) interfaced with a Vax 6300 computer (Digital Equipment, Maynard, MA, USA) running FACS/Desk software[37]. Data were collected on 100,000 cells.

To differentiate between a B cell receptor that was VH4-34 encoded and exogenous binding of VH4-34 antibodies to its B cell ligand, whole blood was washed twice with PBS and incubated overnight at 37˚C in 10% fetal calf serum (FCS). Cells were subsequently stained as above, the RBC lysed, and the fixed WBC analyzed on FACS as described. The process of washing and longterm incubation in the absence of patient serum favors the dissociation of surface bound antibody due to the decline in the concentration of free antibody. Percentage of B cells expressing VH4-34 encoded BCR would remain unchanged. Data were collected only on patients showing ± 1% change in total CD20+ B cells compared to the original value.

Statistics. Statistical comparisons between groups were performed using the one way ANOVA. Values < 0.05 were considered significant. Bartlett's test was performed for testing equality of variance prior to analysis by one way ANOVA. Analysis was performed using Prism software.

RESULTS

Isotype determination for VH4-34 encoded antibodies in patients and controls was performed by ELISA. Levels greater than the normal mean + 3 SD were considered elevated for each isotype (Figure 1). VH4-34 encoded antibody was found to be elevated in 52 of 95 patients. VH4-34 IgM was elevated in 17 patients that were a subset of the 52 patients with VH4-34 IgG. Thus, 35 patients had elevated VH4-34 of only the

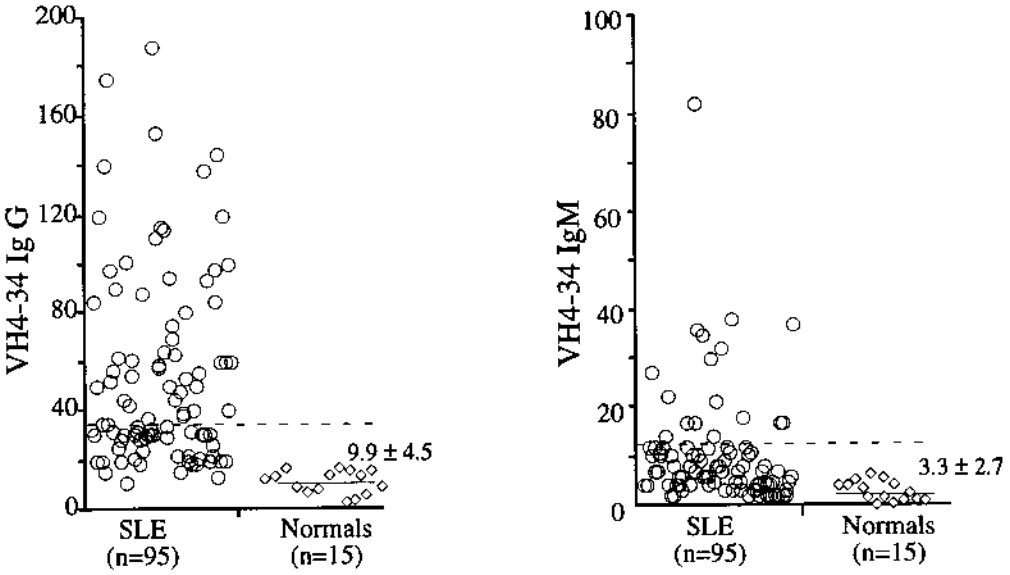

Figure 1. Relative levels of VH4-34 IgM and IgG antibodies in patients and controls. Isotype levels in patient serum were measured by ELISA using the standard curve generated by a known VH4-34 IgM and IgG. This assay does not measure the absolute values of VH4-34 isotype but gives a relative value of the levels of each VH4-34 isotype (see Materials and Methods). Mean ± SD values for VH4-34 IgM and IgG in normal serum are shown. Patients with levels 3 SD above the mean were considered positive (broken line).

IgG isotype and 17 had circulating VH4-34 of both the IgM and IgG isotypes, and 43 patients had VH4-34 antibody levels comparable to those in healthy controls, which will be termed the no-VH4-34 antibody patient cohort.

Clinical indices for disease activity (SLAM and VAS-A), severity (LSDI and VAS-S), and damage (SLICC/ACR) were determined on the day serum was drawn. Our earlier study showed significant correlation between serum levels of total VH4-34 encoded antibodies (not distinguishing between IgM and IgG isotypes) and SLE disease severity[23]. Here, we investigated the relationship between VH4-34 isotypes and disease activity, severity, and damage. Disease severity, measured by VAS-S or by LSDI, was significantly lower in those patients who had both IgM and IgG circulating VH4-34 encoded antibodies than in patients who had VH4-34 of the IgG isotype only (Figure 2). Indeed, the level of severity in patients with both isotypes was not significantly different from that in patients with no detectable VH4-34 encoded antibodies.

Disease activity measured by SLAM and VAS-A, although lower in patients with no VH4-34 and VH4-34 IgM than in patients with only VH4-34 IgG, was not significantly different within these 3 groups (Table 1). When the 95 lupus patients were compiled according to organ involvement, VH4-34 encoded IgM was absent in 28 of 29 patients with nephritis (one patient had mild nephritis) and 6 patients with central nervous system (CNS) disease. In contrast, 25 of 29 patients (86%) with nephritis and all 6 patients with CNS lupus (100%) had circulating VH4-34 of the IgG isotype (Table 1). Choice of cytotoxic and immunosuppressive therapy was determined by the patient's clinical course. When VH4-34 isotype expression was correlated with immunosuppressive therapy, there was no significant difference in the percentage of patients receiving immunosuppression between the patient cohorts denoted as VH4-34 IgG and VH4-34 IgM/IgG (Table 1). Within the cohort of patients with no VH4-34, as expected, a significantly greater percentage of patients were in the no-treatment group.

VH4-34 encoded Mab of the IgM isotype have been shown to bind human B lymphocytes *in vitro*[12,13,15]. We studied the ability of the VH4-34 encoded antibody fraction in SLE serum to bind autologous B lymphocytes. Whole blood from patients (n = 63) and controls (n = 15, ages 25–60 yrs) was stained with antibodies to pan-B cell marker, CD20, and the anti-VH4-34 idiotype 9G4 as described above. The 63 SLE patient cohort included 15 patients with VH4-34 encoded IgM and IgG, 24 with VH4-34 encoded IgG alone, and 24 with no VH4-34 encoded antibody.

In peripheral blood of 15 controls and the 24 SLE patients with no VH4-34 encoded antibody, 3–9% of the B lymphocytes were stained with antiidiotype Mab 9G4, consistent with the reported frequency of B cells expressing the VH4-34 gene encoded BCR[18]. Of the 24 patients with circulating VH4-34 of the IgG isotype only, 16 had < 15% 9G4+ B cells, whereas 8 had a much higher frequency of 9G4+ B cells, ranging from 20 to 60%. In the 15 patients with elevated VH4-34 encoded IgM, 14 patients again had 20 to 95% 9G4+ B cells. The remaining VH4-34 IgM patients had no detectable B lymphocytes within the mononuclear population. Thus, 22 of 63 SLE patients had greater than the expected BCR based frequencies of 9G4+ B cells.

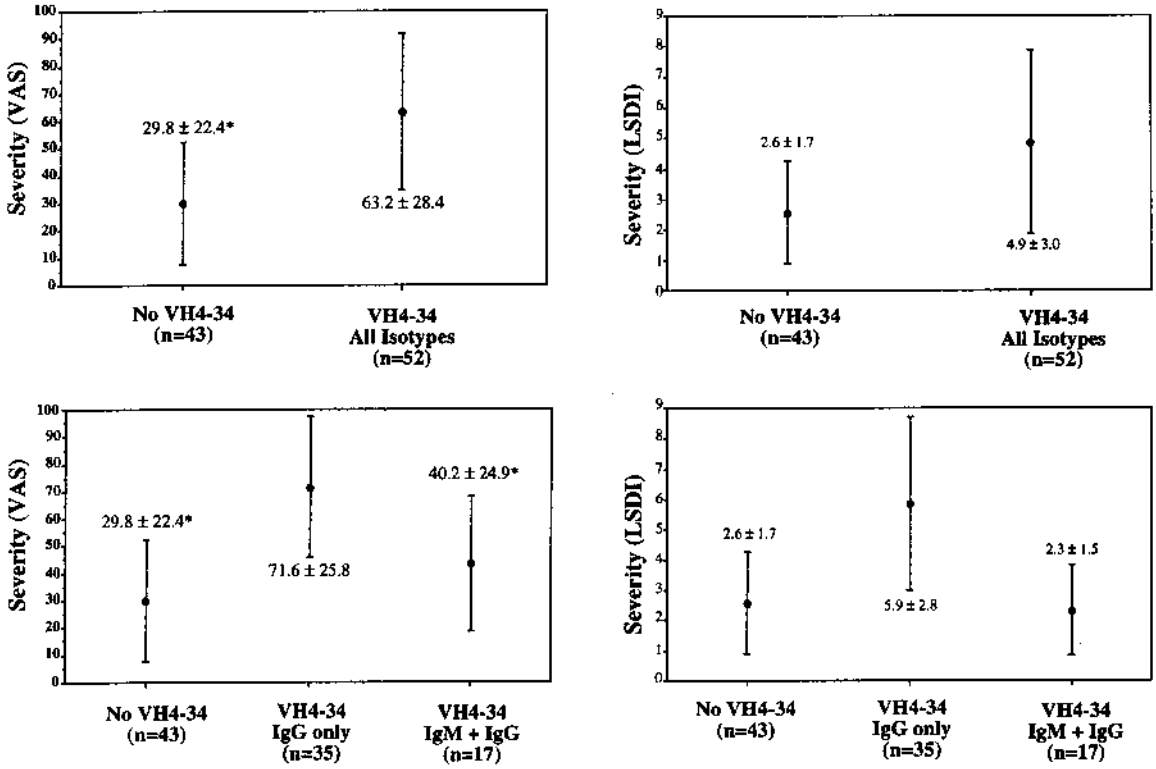

Figure 2. Levels of VH4-34 encoded IgG isotype correlate with severity of disease. Patients with no VH4-34 antibodies and patients with IgM and IgG isotype had significantly less severe disease than patients with VH4-34 of IgG isotype only. Top panel shows the mean ± SD of disease severity measured by VAS-S and LSDI among patients with and without VH4-34 antibody. VAS-S and LSDI are significantly different between the 2 groups. Bottom panel splits patients with VH4-34 antibodies into VH4-34 IgG-only and VH4-34 IgM + IgG. The VAS-S and LSDI of the no-VH4-34 and VH4-34 IgM + IgG cohorts were significantly different than those of the VH4-34 IgG cohort. Statistical analysis by one way ANOVA; p values < 0.05 were considered significant.

Table 1. VH4-34 isotype and disease activity, organ involvement, and treatment.

VH4-34 Encoded Antibodies	SLAM	VAS-A	Organ Involvement		Treatment***	
	Mean ± SD*		Nephritis, n = 29 (%)	CNS, n = 6 (%)	No Cytotoxic Rx (%)	Cytotoxic Rx (%)
No VH4-34, n = 43	5.6 ± 3.2	15.2 ± 13.9	n = 3 (11)**	n = 0	n = 22 (51)**	n = 21 (49)**
IgM + IgG, n = 17	5.3 ± 3.6	13.5 ± 11.8	n = 1 (3)**	n = 0	n = 5 (30)	n = 12 (70)
IgG only, n = 35	7.8 ± 5.8	20.9 ± 26.6	n = 25 (86)	n = 6 (100)	n = 12 (34)	n = 23 (66)

* Statistical analysis was performed using one way ANOVA. Although a trend to lower disease activity is seen in patients with no VH4-34 Ab and the VH4-34 IgM and IgG group, statistically the 2 groups were not significantly different than the patient cohort with VH4-34 IgG only.
** Significant difference between groups by 2 tailed Fisher's exact test ($p < 0.01$).
*** Patients were divided into 2 groups, one receiving no immunosuppressive or cytotoxic therapy and another receiving single drug or various combinations of prednisone (≥ 5 mg/day), cytoxan, methotrexate, and/or imuran. No significant difference was seen between the VH4-34 IgM and IgG and VH4-34 IgG-only cohorts. Treatment in the no VH4-34 Ab cohort was significantly different than in both the cohorts with VH4-34 Ab. Patients with no VH4-34 Ab have less severe disease, hence the percentage of patients receiving no treatment is higher.

The 9G4 Mab staining of patients' B cells could be due to detection of VH4-34 encoded BCR or the binding of circulating VH4-34 encoded antibodies to its B cell ligand. To distinguish between these 2 possibilities, whole blood from patients (n = 63) and controls (n = 15) was washed twice with PBS, incubated overnight at 37˚C in Iscove's medium with 10% FCS, and then stained again for 9G4 reactivity. Removal of patients' lymphocytes from serum reduces the concentration of free antibody, favoring dissociation of lymphocyte bound antibody. If the BCR is VH4-34 encoded no change in 9G4+ B cells is expected.

The percentage of CD20+ (total B cells) within the mononuclear population was monitored before and after overnight incubation. In all 63 samples the percentage of B

cells within the mononuclear population (5–15%) remained the same (± 1%) for each individual before and after overnight incubation. In controls and SLE patients with the expected frequency of 9G4+ B cells no significant change (within ± 1%) in 9G4+ B cells was observed after overnight incubation, remaining at 3–9% of total B cells. However, in all 22 patients with a high frequency of 9G4+ B cells a dramatic decrease from 20 to 95% to 0.5 to 6% in 9G4+ B cells was observed using electronic gates. Contour plots of 9G4 reactivity are shown before and after overnight incubation for 4 representative patients (Figure 3). If the 9G4 reactivity of SLE B cells was due to VH4-34 encoded BCR the overnight incubation would not alter the frequency of 9G4+ B lymphocytes. Thus, the decrease in 9G4+ B cell frequency was due to the release of VH4-34 antibodies from its B cell ligand during incubation in the absence of patient serum, since the percentage of CD20+ cells in the samples remained unchanged.

When the LSDI was plotted against the percentage of 9G4+ B cells (n = 63), an inverse correlation was found, i.e., the greater the percentage of 9G4+ B cells the less severe the disease as measured by LSDI (Figure 4).

DISCUSSION

SLE, like many other autoimmune disorders, is heterogeneous in clinical presentation and course. Diagnosis of SLE requires some but not all clinical features to be present and shows evidence of polygenic inheritance, suggesting it may be a collection of similar but molecularly distinct diseases. The hallmark of the disease is the elevation of myriad antibodies, particularly autoantibodies. The elevation of autoantibodies is conceivably a response to antigens and/or cross reactive epitopes that are typically ignored in the non-disease state. Alternatively, it may be due to a basic dysfunction in the immune circuitry that allows unpoliced production of autoantibodies, independent of specific lymphocyte stimulation by cross reactive autoantigens[38]. The search for the latter has led to a long list of antigens, such as DNA, PCNA, Sm, etc. Nevertheless, their link to SLE development, in terms of cause or effect, remains controversial. Some of the specificities described in the lupus antibody repertoire are used as diagnostic markers for lupus[39,40].

We have shown that VH4-34 encoded antibodies occur in 55% of patients with SLE[23]. With a specificity of 94–95% this autoantibody assay may provide a valuable confirmatory diagnostic test for SLE. The study also showed a significant correlation between disease severity and serum levels of total VH4-34 encoded antibodies (not distinguishing IgM and IgG isotype). The current study extends this observation and shows that association between disease severity and VH4-34 antibodies is due to the presence of the IgG isotype. In contrast, VH4-34 encoded IgM appears to confer protection, suggesting a sharp distinction between the roles of the 2 isotypes.

It is possible that the varied isotype expression could be due to differences in patient therapy, perhaps due to the differential effect of treatment on IgM or IgG-secreting plasma cells. When patients were divided according to therapy, the no-VH4-34 cohort had a significantly greater percentage of patients in the no-cytotoxic treatment group, as expected, since they have less severe disease. No significant difference in cytotoxic treatment was observed within the VH4-34 IgG + IgM and the VH4-34 IgG-only groups. Further studies taking

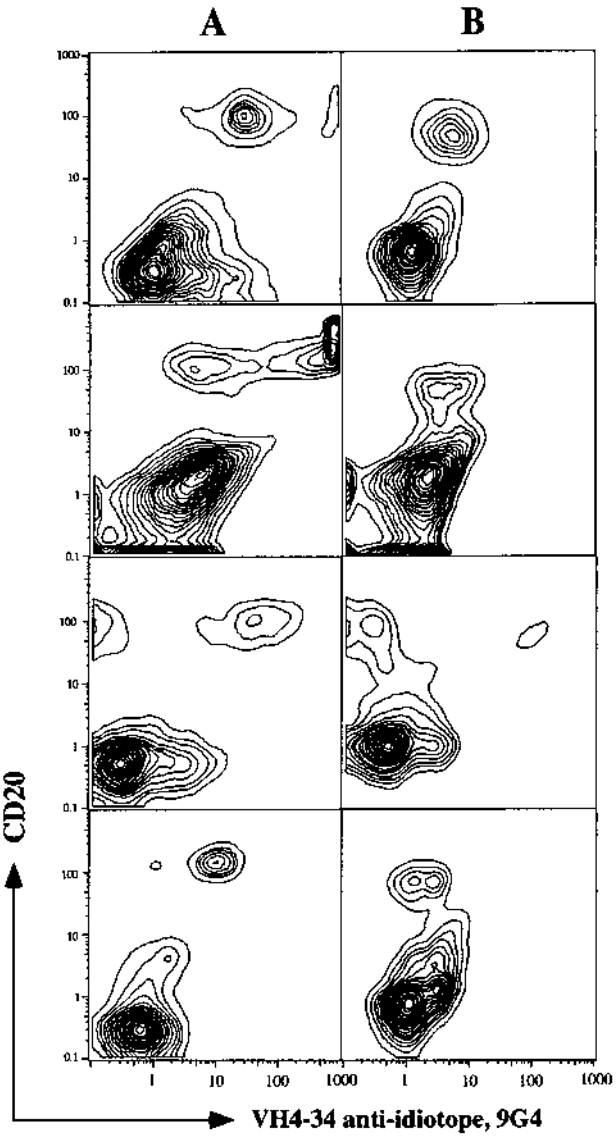

Figure 3. Mab 9G4 anti-VH4-34 reactivity of B lymphocytes after overnight incubation. Patient whole blood was washed and stained with anti-VH4-34 antibody 9G4 and anti-CD20 (A). Flow cytometry profiles of 4 representative patients with > 20% 9G4+ B cells are shown. An electronic gate to include mononuclear cells (MNC) and exclude neutrophils and residual RBC was used. The percentage of CD20+ cells of MNC changed < 1% after overnight incubation. In most patients a continuum of 9G4 staining was seen on autologous B lymphocytes. Column B depicts 9G4 and CD20 staining of the same patient after overnight incubation. In each patient the percentage of 9G4+ CD20+ cells decreased after overnight incubation. Data are presented as 5% probability contour map.

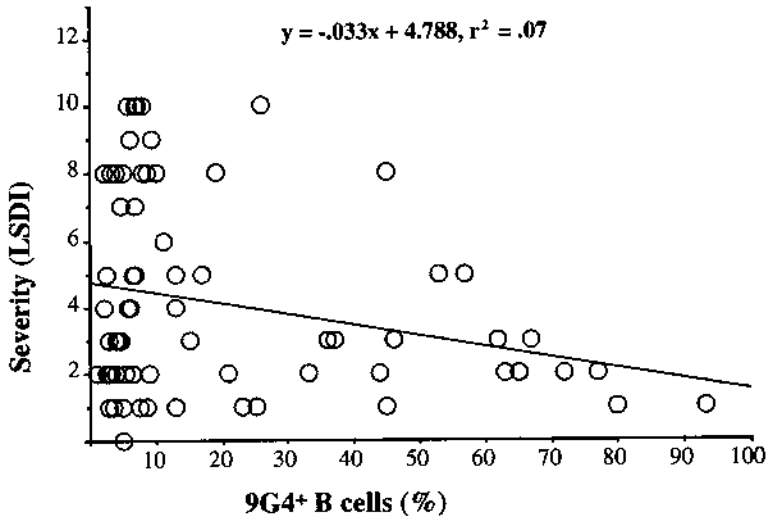

Figure 4. Disease severity decreases with increase in percentage of VH4-34+ autologous B cells. Severity by LSDI for 63 SLE patients is plotted against the percentage of antiidiotype Mab 9G4+ B cells of total B cells. Correlation coefficient between LSDI and 9G4+ B cells, r = –0.26, calculated using StatView.

into account the dosage and length of treatment will clarify this issue.

The self-specificities of VH4-34 encoded immunoglobulins include cord/adult RBC, B lymphocytes, and autoantigens such as ssDNA and cardiolipin. Reactivity to the latter group of antigens appears to be an essential characteristic of the immune system, with possibly little relevance to disease development. The existence of such "natural autoantibodies," generally considered nonpathogenic, is a well established phenomenon in both mice and humans[41,42]. Using assays capable of distinguishing between ssDNA and dsDNA, our previous study showed that the VH4-34 gene does not encode anti-dsDNA reactivity[23]. Although other studies do report 6–10% of anti-dsDNA activity to be derived from the VH4-34 gene[25,33], it is clear that the bulk of the dsDNA reactivity found in SLE is not derived from the VH4-34 gene. It does, however, encode a fraction of the anti-ssDNA reactivity in normal and lupus serum. Besides VH4-34, a number of other human VH genes encode anti-ssDNA reactivity[43-45].

In contrast, reactivity to cord/adult RBC and B lymphocytes is encoded exclusively by the VH4-34 gene[4-7,46]. The anti-B cell antibodies are generally germline in configuration and IgM in isotype. This study revealed autologous B cell binding by VH4-34 encoded Ig in SLE. The 22 patients that showed B cell binding by VH4-34 encoded Ig included 14 of the 15 patients with detectable VH4-34 of the IgM isotype. The assay used for the detection of the VH4-34 IgM and IgG gives a relative value for each isotype. High levels of VH4-34 encoded IgG in serum compete with VH4-34 IgM, so the amount of each isotype detected remains an approximation proportional to their respective levels. Given the constraints of the assay, the remaining 8 patients with increased frequencies of 9G4+ B cells could have circulating VH4-34 IgM below the ELISA limit of detection. It thus appears that B cell binding segregates predominantly to the IgM isotype of VH4-34 encoded immunoglobulins.

The *in vivo* cytotoxicity of VH4-34 anti-B cell antibody on patients' B lymphocytes has been difficult to assess. Correlating the frequency of peripheral B cells to VH4-34 encoded IgM is ineffective due to the various corticosteroid/cytotoxic regimes that affect patient WBC count, including mononuclear cells. Our *in vitro* studies have shown that not all VH4-34 encoded IgM bind B lymphocytes and not all VH4-34 encoded Mab that bind B lymphocytes are cytotoxic[16,46]. The polyclonality of the SLE serum hinders the assessment of *in vivo* binding/cytotoxicity until sequencing of individual VH4-34 BCR-expressing B cells is undertaken. Such a study will also assist in understanding the wide range of B cell binding (20–95%) among VH4-34 IgM+ patients with SLE. Even then, the degree of antibody secretion from each parent B lymphocyte will not be determined. Thus data obtained from *in vitro* studies using Mab cannot be easily translated to a clinical setting. Further studies addressing the effect of various VH4-34 IgM in murine models of SLE may resolve this issue. Regardless, this study shows that VH4-34 encoded immunoglobulins, generally of the IgM isotype, coat autologous B lymphocytes of patients with SLE, and disease in these patients is significantly less severe.

Besides SLE, serum levels of VH4-34 immunoglobulins are significantly elevated in infectious mononucleosis and cold agglutinin disease. In neither clinical condition has the effect of circulating VH4-34 anti-i/B cell antibodies on patient B lymphocytes been investigated.

Various groups have described lymphocytotoxic antibodies in SLE sera[47-50]. Our studies ascertain the identity of a subset of these antibodies at the gene level. Our earlier studies have

shown that B cell reactivity is predominantly exhibited by VH4-34 encoded Mab in germline configuration[16]. Isotype switch and affinity maturation lead to the decrease or loss of B cell binding. Thus, it is tempting to speculate that this autoreactivity conferred by the IgM isotype is relevant to disease development and progression. The IgM type VH4-34 antibodies may attenuate disease manifestations by downregulatory/cytotoxic effects on autoreactive B lymphocytes.

The IgG isotype VH4-34 antibodies may aggravate the immunopathological mechanisms in SLE, for instance by direct binding of target antigen and complement activation. Alternatively, VH4-34 IgG may simply represent a marker of immune dysfunction due to isotype switching. The regulation, secretion, and isotype switch of VH4-34 antibodies can thus be exploited to analyze the loss of organization and/or regulation of the immune system associated with SLE.

Our significant clinical finding is that virtually all patients with the most severe SLE manifestations, nephritis and CNS lupus, lack the IgM isotype of VH4-34 antibodies, while all these patients do have IgG VH4-34. This observation supports our hypothesis that VH4-34 antibodies play an important role in the pathogenesis of certain SLE manifestations, and that isotype switching can be a critical event in the pathophysiological process. In view of the clear distinction between the groups with and without nephritis/CNS lupus, measurement of VH4-34 antibodies by isotype may have a practical role in the clinical diagnostic process of these SLE manifestations. It may also allow segregation of SLE into 2 distinct molecular diseases.

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