DcR3 as a diagnostic parameter and risk factor for systemic lupus erythematosus

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Abstract

In this study, we investigated the diagnostic value of serum death decoy receptor 3 (DcR3) for systemic lupus erythematosus (SLE). The possible pathogenic role of DcR3 in SLE was also assessed. Serum DcR3 levels of 90 SLE patients, 11 patients with rheumatic conditions and 123 healthy controls were determined by ELISA. In all, 43% of the SLE patients, 9% of patients with rheumatic conditions and 2.4% of the normal healthy individuals presented elevated serum DcR3 levels. A higher percentage of DcR3-positive SLE patients, compared with DcR3-negative SLE patients, showed abnormally high serum IgE levels, a surrogate marker of Th2-type immune responses. To determine the cause and effect relationship of DcR3 expression and a Th2-prone status, we studied young DcR3 transgenic (Tg) mice, whose transgene was driven by an actin promoter. These mice had IL-4 overproduction and augmented serum IgE levels, signs of dominant Th2 immune responses. To determine possible SLE pathogenic roles of DcR3, the T-cell-depleted bone marrow of DcR3 Tg mice was transplanted into lethally irradiated syngeneic C57BL/6 female mice. The recipients developed an SLE-like syndrome. They presented anti-dsDNA and anti-nuclear antibodies, along with renal and liver pathology compatible with that of SLE. In total, 90% of Tg bone marrow-transplanted mice, compared with 20% of wild-type bone marrow-transplanted mice, perished within 12 months after the transplantation. Our results showed that serum DcR3 could serve as an additional parameter for SLE diagnosis and that DcR3 secreted from cells of hematopoietic origin was SLE pathogenic in mice.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease inflicting damage to multiple organs. The disease prevalence is ~0.05% in the general population, with 80–90% of patients being women (1, 2). The exact etiology of SLE has not been elucidated, but it is obvious that genetics, gender, and environment are involved in its pathogenesis. Regarding genetics, SLE is under polygenic control (3). Multiple genomic loci containing SLE risk genes have been identified in humans and mice (4, 5). Certain class II MHC genes are known to contribute to SLE risk (6). Among others, molecules in the apoptosis pathways are implicated in SLE pathogenesis (7). In mice, mutations in the prototype pro-apoptotic molecules Fas or Fas ligand (FasL) lead to the occurrence of an SLE-like syndrome (8, 9), but human SLE patients rarely have mutations in Fas or FasL (10, 11). However, it is possible that molecules in the Fas/FasL pathway are SLE risk factors, but they have yet to be identified as such.

Decoy receptor 3 (DcR3) is a member of the tumor necrosis factor (TNF) receptor family. As it lacks transmembrane and intracellular sequences in its peptide, it is a secreted protein (12). It can bind to three TNF family members, i.e. FasL, LIGHT and TL1A, and interferes with the interaction of these ligands with their respective receptors, i.e. FasL with Fas, LIGHT with HVEM and LTβR and TL1A with DR3 (12–15). Because these receptors are all capable of...
inducing cell death (15–17), one of DcR3’s biological functions is to act as a death decoy, preventing cell death under certain circumstances (15). In total, ~50–60% of various tumors secrete DcR3 (18), which is probably a strategy evolved by tumors to gain a survival advantage over immune surveillance. Activated T cells also produce DcR3 (19). The biological significance of such DcR3 production could be (i) to modulate activation-induced cell death after clonal expansion and, hence, influence memory T-cell development (20) and (ii) to regulate T-cell migration after their activation as we have found that soluble DcR3 can inhibit T-cell chemotaxis (21).

T cells in SLE patients are abnormally activated. As activated T cells produce DcR3 (19), we examined serum DcR3 in these patients as a possible diagnostic marker. We further investigated a possible role of DcR3 in SLE pathogenesis.

Methods

Patients and healthy controls

Sera from SLE patients, patients with other autoimmune diseases and healthy controls were collected at the Department of Immunology, Instituto Nacional de Cardiología ‘Ignacio Chávez’, Mexico City. Informed consent was obtained from serum donors, and this study was approved by the local Ethic Committee. All SLE patients (86 females and 4 males; age between 16 and 63, 37.3 ± 12.9) met the American College of Rheumatology classification criteria (22). The systemic lupus erythematosus disease activity index (SLEDAI) of each patient was obtained at the time of serum collection. All the patients were under one to four different medications, which were duly recorded and illustrated in relevant figures. Eleven patients (nine females and two males) between 20 and 59 years of age (38.8 ± 13.5) with rheumatic conditions [three with rheumatoid arthritis, one with idiopathic juvenile arthritis, one with anti-phospholipid syndrome, two with Sjögren syndrome, one with thrombophilia, two with undifferentiated connective tissue disease, one with overlap syndrome (systemic sclerosis plus polymyositis)] were included for comparison. Control sera were from healthy donors (92 females and 31 males; age between 18 and 64, 35.6 ± 10.5) approximately matched for age and gender of SLE patients. All the patients and healthy controls were Mexican Mestizo.

DcR3 ELISA

DcR3 ELISA has been described elsewhere (18). An mAb specific to DcR3 was used for coating, and a biotinylated affinity-purified rabbit antibody against DcR3 served as detecting antibody. The sensitivity of the assay was 6 pg ml⁻¹. Human samples were tested in duplicate, and mouse samples, in triplicate. The mean + 1.64 SD (95% confidence interval, one-sided test) of the control serum DcR3 levels was used as the threshold (25 pg ml⁻¹), levels equal or above which were considered DcR3 positive.

Clinical serological tests

Serum anti-nuclear antibodies (ANAs) were detected by indirect immunofluorescence on HEP-2 cells slides (NOVA Lite, INOVA Diagnostics, San Diego, CA, USA). Anti-dsDNA antibodies were detected by indirect immunofluorescence on Crithidia luciliae substrate (NOVA Lite, INOVA Diagnostics). Anti-SSA, anti-SSB, anti-RNP and anti-SM antibodies were all detected by ELISA (QUANTA Lite, INOVA Diagnostics). All the assays were performed according to manufacturer’s instructions.

Human serum IgE assay

Human serum IgE was quantified using an ELISA kit from Bethyl Laboratories (Montgomery, TX, USA), according to the manufacturer’s instructions. The sensitivity of the assay was 16 ng ml⁻¹.

Cell culture

Mouse spleen cells were cultured in 96-well plates at a density of 4 × 10⁵ cells per 200 μl per well and stimulated with phorbol myristate acetate (PMA, 10 nM) and ionomycin (2 μg ml⁻¹) for 4 h in the presence of brefeldin A (10 μg ml⁻¹) for the detection of intracellular IL-4 and IFN-γ.

Flow cytometry

Two-color flow cytometry was performed to determine the expression of CD4 and intracellular IL-4 and IFN-γ in mouse spleen T cells, as described before (23).

Measurement of Ig isotype levels in mouse sera

Mouse serum Ig isotype levels were quantified with Ig-isotyping ELISA and OptEIA kits from BD Biosciences (Mississauga, Ontario, Canada), according to the manufacturer’s instructions.

Whole-body irradiation and bone marrow transplantation

C57BL/6 female mice were whole-body irradiated at 900 Rad. After 16 h, they received intravenously (i.v.) 10 million T-cell-depleted bone marrow cells from DcR3 or wild-type (WT) mice. T-cell depletion from bone marrow cells was carried out with EasySep Mouse CD90 (Thy1.2) Positive Selection Kit according to the manufacturer’s instructions (StemCell Technologies Inc., Vancouver, BC).

Immunofluorescence for the detection of ANA

HeLa cells were fixed with a BD Cytofix/Cytoperm kit (BD Biosciences, San Diego, CA, USA) at 4°C for 60 min and then incubated overnight at 4°C with sera from mice having received WT bone marrow transplantation (BMTx) or transgenic (Tg) BMTx (1:500 dilution in wash buffer from the kit). After washing, the cells were reacted overnight at 4°C with FITC-conjugated goat anti-mouse IgG antibody (1:1000 dilution; Bethyl Laboratories). The cells were examined under fluorescence microscopy. The method was similar to that as described by Vinuesa et al. (24).

ELISA for anti-dsDNA

Costar 96-well high-affinity ELISA microplates were coated with 250 μg ml⁻¹ salmon sperm DNA (Invitrogen, Burlington, Ontario, Canada) in coating buffer (0.05 M sodium bicarbonate solution, pH 9.5) overnight at 4°C. After washing with PBS containing 0.05% Tween 20, the plates were incubated
with blocking buffer (PBS containing 1% BSA) at room temperature for 1 h. WT and Tg mouse sera were diluted in blocking buffer at 1:50 and incubated overnight in the wells at 4°C. After washing, the plates were reacted with HRP-conjugated sheep anti-mouse IgG antibody (1:2000 dilution; Amersham Biosciences, Piscataway, NJ, USA) for 1 h at room temperature. Signals were revealed by adding tetramethylbenzidine substrate (BD Biosciences), followed by 20-min incubation at room temperature. The reaction was stopped by 50 μl 4 N HCl, and the plates were read at a wavelength of 450 nm.

Results

Elevated serum DcR3 levels in SLE patients

Sera from 90 SLE patients (86 females and 4 males) and 123 approximately age- and gender-matched healthy individuals (92 females and 31 males) were tested for DcR3. As shown in Fig. 1(A), SLE patients presented elevated serum DcR3, with 39 of them (i.e., 43.3% of total patients; 75% of males and 41.9% of females) being serum DcR3 positive, compared with only 3 out of the 123 healthy controls (2.4%). The difference was highly statistically significant (P < 0.01, Student’s t-test). In these patients, who were all under medication, DcR3 levels had no obvious correlation with SLEDAI, according to linear regression analysis (r = 0.119, P = 0.26) (Fig. 1B). No significant correlation was found between age (ranging from 16 to 64 years) and serum DcR3 levels (r = −0.1, P = 0.92; Fig. 1C). Further analysis showed that serum DcR3 positiveness was not correlated to positiveness of ANA, anti-dsDNA antibody, anti-RNP antibody, anti-Sm antibody, anti-Ro/SSA antibody or anti-La/SSB antibody (P > 0.05, χ² test; Fig. 1D).

For comparison, DcR3 levels in 11 patients with rheumatic conditions were determined. Only 1 out of 12 (9%) of these patients were positive in serum DcR3. The DcR3-positive patient in this group was diagnosed with overlap syndrome (systemic sclerosis plus polymyositis). Although a large sample size of each disease will be needed to assess the DcR3-positive incidence, our data at least suggest that DcR3-positive incidence in rheumatic conditions is not as high as that in SLE.

![Fig. 1](image-url). Serum DcR3 levels and their relationship with SLEDAI in SLE patients. Serum DcR3 was assayed by ELISA in duplicate. DcR3 levels of 90 SLE patients (86 females and 4 males), 11 patients with rheumatic conditions (two males and nine females) and 123 healthy controls (92 females and 31 males) were measured. The threshold of DcR3 positiveness (25 pg ml⁻¹) was the mean + 1.64 SD of control serum levels. (A) SLE patients had elevated serum DcR3 levels. The serum DcR3 levels of SLE patients, patients with rheumatic conditions and healthy controls were 32 ± 32, 22.7 ± 35.9 and 5 ± 12 pg ml⁻¹, respectively (means ± SD). The difference between SLE patients and healthy controls and between patients with rheumatic conditions and healthy controls were highly significant (P < 0.01 for both comparisons; Student’s t-test), although the difference between SLE and rheumatic patients was not (P > 0.05, Student’s t-test). (B) SLE patient serum DcR3 levels plotted against SLEDAI. Serum DcR3 levels of 90 SLE patients are plotted against their SLEDAI (ranging from 0 to 24). No correlation is found between these two parameters (r = 0.119, P = 0.26; Pearson correlation test). (C) SLE patient serum DcR3 levels plotted against their age. Serum DcR3 levels of 90 SLE patients are plotted against their age (16–64 years). No correlation is found between these two parameters (r = −0.100, P = 0.92; Pearson correlation test). (D) No correlation between positiveness of DcR3 and auto-antibodies in SLE patients. Serum DcR3 of SLE patients was determined as positive or negative using 25 pg ml⁻¹ as a threshold (36 patients were DcR3 positive and 52 patients were DcR3 negative) and analyzed against positiveness of their serum ANA, anti-dsDNA, anti-RNP antibody, anti-Sm antibody, anti-Ro/SSA antibody and anti-La/SSB antibody, using χ² test. P-values were >0.05 in all the comparisons. The serum DcR3 positiveness was marked as ‘+’ or ‘−’ under each comparison. Solid black and slashes indicate serum positiveness and negativeness of a certain auto-antibody, respectively.
All SLE patients were under medication, such as cyclophosphamide (CFM), prednisone (PDN), hydroxychloroquine (HCQ), azathioprine (AZA) and/or statins, at the time of serum collection. Only 13 patients were on a single medication (11 on HCQ and 2 on PDN); all the others were treated with two to four drugs. We attempted to determine whether any particular drug would influence serum DcR3 and whether, in the absence of the said drug, there was a correlation between SLEDAI and DcR3 levels. Patients without a particular medication but on one to three other medications were compared with those taking the said medication along with zero to three other drugs. As shown in Fig. 2(A), regimens containing CFM, PDN and AZA did not seem to affect serum DcR3 levels. On the other hand, patients on regimens without HCQ appeared to have higher DcR3 levels. This was probably due to that patients taking HCQ were those with a lower SLE activity. Indeed, SLEDAI of patients with a regimen containing HCQ tended to be lower than that without HCQ (Fig. 2B), although the difference did not reach a statistically significant level. We also noticed that patients on a regimen containing statins presented higher DcR3 levels (Fig. 2A; \( P < 0.004 \)), and they also had higher SLEDAI (Fig. 2B), albeit without reaching statistically significance.

Possible explanations for such an observation are given in the Discussion. Another observation was that patients treated with a regimen containing PDN or AZA had significantly higher SLEDAI (\( P = 0.03 \) in both cases). This is not unexpected as these two drugs are normally administered to patients with elevated SLEDAI.

**Elevated serum IgE levels in SLE patients**

SLE is an autoimmune disease with an underlying Th2 immune response, and physiological conditions such as pregnancy that boosts the Th2 response tend to aggravate SLE immune response, and physiological conditions such as pregnancy that boosts the Th2 response tend to aggravate SLE. The 90 SLE patients were divided into DcR3-positive and DcR3-negative groups, with 25 pg ml\(^{-1}\) serum DcR3 as the threshold. The results (Fig. 3) showed that DcR3-positive patients had an elevated serum IgE concentrations (with a median IgE concentration of 120 ng ml\(^{-1}\)) compared with DcR3-negative patients (with a median IgE concentration of 60 ng ml\(^{-1}\)). A higher percentage of DcR3-positive patients presented serum IgE levels above the normal range using 240 ng ml\(^{-1}\) (100 IU ml\(^{-1}\)) as the upper limit of the range (26), compared with DcR3-negative patients (\( P < 0.05 \); \( \chi^2 \) test). These results suggest the existence of a correlation between DcR3 levels and the Th2 phenotype.

**Th2-prone immune responses in young DcR3 Tg mice**

To establish the cause and effect relationship between DcR3 expression and Th2-prone immune responses, we generated actin promoter-driven human DcR3 Tg mice (20). The mouse does not have an orthologue of human DcR3, but human DcR3 could effectively interact with mouse FasL, LIGHT and TL1A (12, 27). Tg mice younger than 5–6 months of age, thymus, spleen and lymph node weight and cellularity were in the normal range, as were cell sub-populations in these organs (data not shown). Their T-cell proliferation in response to solid-phase anti-CD3 alone or in combination with anti-CD28 was variable and did not have consistent changes in comparison to WT T cells [data not shown; methods detailed in Luo et al. (23)]. However, when Tg CD4 cells experienced a short-term ionomycin and PMA stimulation, a higher percentage of them became intracellular IL-4 but not IFN-\( \gamma \)-positive, compared with WT CD4 cells (Fig. 4A). Because such a stimulation regimen triggered previously activated rather than resting T cells to secrete these lymphokines, this result suggests that Tg mice at this age already experienced a Th2-dominated immune response history.

The serum antibody isotypes of these Tg mice were determined by ELISA. As illustrated in Fig. 4(B), Tg and WT mice had similar levels of IgA, IgM, IgG2A and IgG3, but Tg mice produced higher amounts of IgG1 and IgG2b than WT mice. Tg mice also generated increasingly significantly higher...
concentrations of IgE, a typical Th2-dependent isotype, from 2 to 6 months of age (Fig. 4C). The overproduction of IgG1 and IgE indicated that the immune responses in Tg mice were skewed to the Th2-type response and was consistent with increased intracellular IL-4-positive CD4 cells found in the spleens of young Tg mice. These results suggest that DcR3 over-expression is a cause of Th2-prone immune responses.

Recipients of DcR3 Tg bone marrow developed an SLE-like syndrome

Tg mice with actin promoter-driven DcR3 expression developed an SLE-like syndrome after 5 months of age (20). They produced auto-antibodies against dsDNA and Smith antigen. The kidneys of these Tg mice showed pathological changes indicative of glomerular nephritis and IgG and C3 deposition; kidney dysfunction, such as proteinuria, leukocyturia, and hematuria, were obvious. Aged Tg mice also developed skin lesions and lymphocyte infiltration in the liver and suffered from leukopenia, anemia and thrombocytopenia. SLE-like syndrome penetrance in DcR3 Tg mice was gender dependent, with ~60% in females versus 20% in males. These findings have been reported by us recently (20). The results suggest that DcR3 might be SLE pathogenic. In actin promoter-driven DcR3 Tg mice, DcR3 was produced almost universally by various tissues. Because activated T cells

Fig. 3. Elevated serum IgE levels in SLE patients. The serum IgE of 90 SLE patients were determined by ELISA, for which sera were tested in duplicate. The median IgE level in DcR3-negative patients was 60 ng ml$^{-1}$ (short horizontal bar) and that in DcR3-positive patients was 120 ng ml$^{-1}$ (short horizontal bar). Using 240 ng ml$^{-1}$ serum IgE concentration (the upper limit of normal serum IgE levels of healthy individuals [Bueno et al. (26)]) as a threshold, a significantly higher percentage of DcR3-positive SLE patients presented elevated serum IgE levels (41.0%), compared with that of DcR3-negative SLE patients (19.6%; $P < 0.05$, $\chi^2$ test).

Fig. 4. Th2-prone immune responses in young DcR3 Tg mice. The experiments were conducted in 2- to 4-month-old DcR3 Tg and WT littermates. (A) Increased intracellular IL-4 but not IFN-γ in Tg CD4 cells. Spleen cells were either not stimulated (resting) or stimulated with PMA (10 nM) and ionomycin (2 μg ml$^{-1}$) for 4 h. Intracellular IL-4 and IFN-γ were analyzed by two-color flow cytometry (CD4/IL-4 or CD4/IFN-γ). The experiments were repeated two times and data from a representative experiment are shown. (B) Serum IgM, IgA and IgG levels in 4-month-old Tg and WT littermates ($n = 5$ pairs) were assayed by ELISA. Asterisks over IgG1 and IgG2b indicate statistically significant differences ($P < 0.05$, two-tailed Student’s t-test). (C) Increased serum IgE levels in Tg mice. Serum IgE levels of DcR3 Tg and WT littermates at ages of 2, 4 and 6 months were assayed with ELISA. Median levels are indicated by horizontal bars. Each dot or triangle represents an individual mouse. Tg mice at all age groups present higher IgE levels than their littermates ($P < 0.05$, two-tailed Student’s t-test).
secrete DcR3 (19), we wondered whether DcR3 from such a source was sufficient to cause SLE. To explore this possibility, we irradiated C57BL/6 female mice at a lethal dosage (900 Rad) and transplanted i.v. T-cell-depleted bone marrow cells from DcR3 Tg mice. For controls, C57BL/6 female mice underwent whole-body irradiation (WBI), followed by BMTx from WT mice. Sera of Tg BMTx but not WT BMTx recipients contained human DcR3 as shown in Fig. 5A. Four months after transplantation, five of the eight Tg BMTx recipients developed anti-dsDNA antibody, a hallmark of SLE, according to ELISA, while none of the five controls receiving WT BMTx had such antibody (Fig. 5B). However, there was no correlation between the levels of DcR3 and anti-dsDNA antibody (Fig. 5C). Those Tg BMTx recipients also produced ANA (Fig. 5D), another typical laboratory finding in SLE.

Human SLE is associated with glomerulonephritis. Renal pathology was thus assessed in mice 6 months after Tg BMTx, according to hematoxylin-eosin staining (Fig. 5E, top panel). The kidneys showed mild interstitial congestion and edema, with moderate interstitial cell infiltration, particularly around the blood vessels. Some glomeruli were extended in size, and capillary loops were markedly thickened and obliterated. Epithelial cells of Bowmen’s capsule proliferated focally. These pathological findings were compatible with glomerulonephritis.

In the liver of mice 6 months after Tg BMTx, periarterial lymphocyte infiltration (Fig. 5E, lower panel) was similar to the liver pathology seen in SLE patients (28).

The survival rate of WBI–BMTx mice is shown in Fig. 6. Twelve months after WBI–BMTx, 80% WT bone marrow recipients were still alive, but only 10% DcR3 Tg bone marrow recipients survived after this period. This finding was similar to that in DcR3 Tg female mice without WBI–BMTx (20).

These findings establish that DcR3 over-expression by cells of hematopoietic origin suffices to induce the SLE-like syndrome.

Discussion

In this study, we demonstrated that SLE patients had elevated serum DcR3 levels, compared with healthy individuals. We further proved that DcR3 over-expressed by cells of hematopoietic origin was sufficient to induce the SLE-like syndrome in mice.

SLE has complex clinical manifestations, and additional laboratory parameters are certainly needed for more accurate diagnosis. As 43.3% of the SLE patients but only 2.4% healthy controls in our cohort were DcR3 positive, serum DcR3 could be employed as a new parameter in addition to the current ones for SLE differential diagnosis. It should be mentioned that ~9% patients with rheumatic conditions (this

![Figure 5](image-url)

**Fig. 5.** Development of an SLE-like syndrome in WBI-BMTx mice. WBI female C57BL/6 mice received T-cell-depleted BMTx from DcR3 Tg or WT mice. Four months after BMTx, their serum DcR3, anti-dsDNA antibody and ANA were assessed. Tg BMTx recipients of Tg BMTx; WT BMTx, recipients of WT BMTx. (A) Serum DcR3 levels of WBI-BMTx mice. Serum DcR3 levels of Tg BMTx or WT BMTx recipients 6 months after BMTx are shown. The latter had no detectable DcR3. (B) Anti-dsDNA antibody in Tg BMTx recipient sera according to ELISA. Sera from Tg BMTx or WT BMTx recipients were measured for anti-dsDNA 6 months after WBI-BMTx. The positive threshold was determined according to the mean ± 2 SD of serum DcR3 levels of WT BMTx recipients. The DcR3 level difference between Tg BMTx and WT BMTx recipients is highly significant (P < 0.01, Student’s t-test). (C) No correlation between serum DcR3 and anti-dsDNA levels in Tg BMTx recipients. For Tg BMTx recipients, their serum DcR3 levels were plotted against anti-dsDNA levels. No obvious correlation was observed. (D) ANA in Tg BMTx recipient sera according to immunofluorescence. Sera from Tg and WT BMTx recipients 6 months after WBI-BMTx were reacted against permeabilized HeLa cells, and the cells were examined by immunofluorescence microscopy. (E) Kidney and liver pathology of Tg BMTx recipients. Hematoxylin-eosin staining of kidney and liver sections from 6-month-old Tg BMTx recipients. Original magnification: ×100.
study), 60% of tumor patients and some liver cirrhosis patients are serum DcR3 positive (18), and these conditions should be taken into consideration if DcR3 is employed for SLE differential diagnosis.

All the patients in our cohort were treated with one to four medications. The fact that elevated serum DcR3 was detected in these patients suggests that serum DcR3 positivity can be used for SLE diagnosis, even if they are treated with symptom-relieving medications before a final diagnosis of SLE is made.

No correlation between SLEDAI and quantitative DcR3 levels was found in our SLE cohort, but it is quite possible that the medications might suppress DcR3 production and abolish possible correlations. A prospective study on the relationship between DcR3 levels and SLEDAI in patients without medication is underway to address this question. Still, by analyzing the current data, we attempted to extract some information on the relationship between DcR3 levels and SLEDAI. We note in Fig. 2(A) that the 15 patients on a drug regimen containing no HCQ presented higher DcR3 levels. A careful look at these patients revealed that 12 of the 15 were on a regimen containing CFM and/or AZA, an indication of more severe disease activity, while 11 of the 75 patients on a regimen containing HCQ were under single-drug therapy, an indication of milder disease activity only involving skin lesions. Indeed, patients receiving a regimen containing no HCQ showed a higher SLEDAI than that with HCQ, although the difference was not statistically significant (Fig. 2B). This raises the possibility that the reduced DcR3 levels in patients treated with HCQ are not a consequence of drug administration but are due to lower disease severity in this sub-population to start with.

Statins are used to lower blood cholesterol to control cardiovascular complications in SLE (29). Patients treated with a regimen containing statins had statistically significantly higher DcR3 levels (Fig. 2A); their SLEDAI was also higher than those on a regimen without statins, although the difference was not statistically significant (Fig. 2B). In our SLE cohort, statins were not particularly used for a selected group of patients with a severer or less severe form of disease. Statins, particularly second-generation statins, such as simvastatin and atorvastatin, have unexpected immunoregulatory functions, in addition to its cholesterol-lowering effect (30). An increasing number of cases with statin-induced lupus-like syndrome have been reported (31, 32). Taking such literature and our findings as a whole, an intriguing possibility is that statins increase DcR3 levels and aggravate SLE disease activity. Additional perspective studies including non-SLE patients taking statins will be necessary to verify this possibility.

While this article DcR3 was under review, Lee et al. (33) reported that serum DcR3 levels in oriental SLE patients were elevated, compared with healthy controls. The finding corroborated our report using a different ethnic population. In Lee’s article, serum DcR3 levels were found to be positively correlated to SLEDAI. However, medication was not described in their patient cohort, and it will be necessary to confirm whether such correlation only occurs in the absence of anti-inflammatory drugs or immunosuppressants.

Aberrant apoptosis has been cited as a possible cause of SLE. Several genes involved in apoptosis are considered SLE risk factors. Mice with mutations in Fas and FasL, which are in the prototype apoptosis pathway, manifest an SLE-like syndrome. Human SLE patients rarely have Fas or FasL mutations, but DcR3 could be a culprit blocking the Fas apoptosis pathway and induce SLE. Because of DcR3 elevation in SLE patients, its known roles in blocking apoptosis and the putative relationship between disease severity and DcR3 levels in HCQ- and statin-treated patients, we studied DcR3 Tg mice to assess DcR3’s role in the pathogenesis of SLE. In our Tg mice, DcR3 expression was driven by an actin promoter. To avoid complications due to universal DcR3 expression from cells other than those of hematopoietic origin, we transplanted T-cell-depleted Tg bone marrow into irradiated syngeneic recipients. These mice developed an SLE-like syndrome in 4 months after BMTx. They had anti-dsDNA and ANA and presented renal and liver pathology compatible with SLE. Thus, we proved that DcR3 secreted from cells of hematopoietic origin was SLE pathogenic in an animal model.

Why do a large percentage of tumor patients (~60%) have DcR3 levels comparable to those of SLE patients (18) in the range of 30–200 pg ml\(^{-1}\), but they rarely develop SLE? It seems that serum DcR3, at least at concentrations present in tumor patients, is not SLE pathogenic. In mice receiving WBI followed by Tg BMTx, the serum DcR3 levels were much higher at a range of 2–80 ng ml\(^{-1}\). The biologically active local DcR3 concentrations in lymphoid organs in both patients and Tg BMTx are probably much higher than their serum levels, and the presence of such high local DcR3 concentrations is a more likely culprit.

As not all SLE patients are DcR3 positive, DcR3 is obviously only pathogenic or facilitates SLE development in a sub-population of SLE patients. The final proof of DcR3 in SLE pathogenesis will need to come from human genetic studies examining single-nucleotide polymorphisms in the DcR3
gene or genes regulating its expression in DcR3-positive SLE patients versus DcR3-negative healthy controls.

It is interesting that, in lpr/lpr and gld/gld mice (with Fas and FasL mutations, respectively) and in our actin promoter-driven DcR3 Tg mice, a large percentage (>65% of females and 20% of males) develops lymphadenopathy along with their SLE-like syndrome (20). On the other hand, none of the WBI–BMTx female mice manifested lymphadenopathy, in spite of their SLE-like syndrome. Similar to that in our WBI–BMTx mice, SLE patients rarely develop lymphadenopathy. It appears that our WBI–BMTx model more closely resembles human SLE, compared with lpr/lpr, gld/gld and DcR3 Tg mice. All the latter three SLE mouse models, in contrast to the WBI–BMTx mice, have one thing in common, i.e. compromised Fas-mediated apoptosis in non-hematopoietic cells. This raises an intriguing possibility that the development of lymphadenopathy needs interaction between T cells and Fas pathway-defective cells of non-hematopoietic origin.

Our study has demonstrated that serum DcR3 can be considered as an additional SLE diagnostic parameter. Further genetic study in humans is needed to confirm its role in SLE pathogenesis as mice have no genes orthologous to human DcR3.

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Abbreviations

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<th>Abbreviation</th>
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<tr>
<td>ANA</td>
<td>anti-nuclear antibody</td>
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<tr>
<td>AZA</td>
<td>azathioprine</td>
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<td>BMTx</td>
<td>bone marrow transplantation</td>
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<td>CFM</td>
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<td>DcR3</td>
<td>decoy receptor 3</td>
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<td>FasL</td>
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References


