Serum DNase I, soluble Fas/FasL levels and cell surface Fas expression in patients with SLE: a possible explanation for the lack of efficacy of hrDNase I treatment

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Keywords: DNase I, human recombinant DNase I, SLE, soluble Fas/FasL

Abstract

The objectives of the study are to evaluate DNase I serum levels and their correlation with soluble Fas (sFas) and soluble Fas ligand (sFasL) and with cell surface Fas expression in patients with systemic lupus erythematosus (SLE), thus contributing to the dysregulated apoptosis typical of the disease. The methods include the following: Serum DNase I levels in patients and in controls were detected using the dot blot method and quantified by densitometry; sFas and sFasL were quantified using an ELISA system. Cell surface Fas expression was evaluated by FACS analysis. Apoptosis was studied by means of internucleosomal DNA degradation using a commercially available kit. The results are as follows: We found a significant difference in DNase I, sFas and sFasL serum levels between patients and controls. Levels of DNase I <7.79 ng ml\textsuperscript{-1} are more represented in patients with SLE. Active SLE is strongly associated with high sFas levels and detectable sFasL. DNase I does not correlate with sFas or sFasL, whereas it correlates with T cell surface Fas expression that is higher in patients with active SLE than in healthy controls. Finally, administration of exogenous human recombinant DNase (hrDNase) I to freshly isolated T cells up-regulates cell surface Fas expression and induces increased susceptibility to Fas-mediated apoptosis. In conclusion, our findings confirm that DNase I is low in SLE and suggest that it may play a role in apoptosis in SLE by regulating the surface expression of the cell death molecule Fas. This role may contribute to explain the inefficacy of hrDNase I in SLE, a treatment proposed for the ability of DNase I to remove DNA from auto-antigenic nucleoprotein complexes.

Introduction

Systemic lupus erythematosus (SLE) is characterized by the presence of anti-nuclear antibodies (ANAs) directed against naked DNA and nucleosomes. The etiology of SLE is unknown, but several studies suggest that increased production and/or inadequate clearance of nuclear DNA–protein complexes after cell death may initiate and/or propagate the disease (1). DNases are the primary enzymes involved in the metabolism and clearance of DNA. Low serum and urine DNase activity has been observed in some patients with SLE (2–4), as well as in lupus-prone mice (5); indeed DNase I-deficient mice have been shown to develop a lupus-like phenotype, with nephritis and serum auto-antibodies directed against nuclear antigens (6). Reduction or loss of DNase I activity, such as in case of gene

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mutation of DNase I (7), may result in a high risk to produce ANAs as a potential prerequisite to develop a SLE-like disease.

Besides the removal of DNA from auto-antigens, the enzyme complex DNase I is responsible for internucleosomal DNA degradation in human cells undergoing apoptosis in response to pharmacological stimuli (11) and that endonuclease behaves as a transcription factor that regulates cell surface Fas expression (12). Moreover, exogenous human recombinant DNase (hrDNase) I is endocytosed into cells up-regulating cell surface Fas expression (12).

Fas is an apoptosis signaling receptor belonging to the tumor necrosis factor (TNF) receptor family, while FasL is a type II membrane protein of the TNF family, which behaves as a death factor, transducing death signals to Fas-positive cells. The hypothesis that perturbation in the Fas/FasL system may be responsible for the development of autoimmunity has prompted studies in patients with autoimmune disorders leading to controversial results (13-15). An abnormal regulation of the Fas/FasL apoptotic signaling pathway has been associated with the pathogenesis of SLE (16, 17). In particular, the extensive apoptosis of T lymphocytes (18), of bone marrow CD34+ cells (19), of neutrophils (20) and of monocytes/maturing macrophages (21), as well as the resistance of autoimmune Th cells driving pathogenic auto-antibody production (22), seem to result from impaired activation of Fas/FasL system.

In light of the results of our previous study showing a direct link between DNase I and fag gene expression (12), we decided first to evaluate DNase I serum levels in SLE patients, second to evaluate whether DNase I correlates with soluble Fas (sFas) and soluble Fas ligand (sFasL) and third whether DNase I correlates with cell surface Fas expression. Moreover, we wanted to evaluate whether these molecules may be useful in predicting either the development of SLE or the organ involvement or in discriminating different clinical stages of the disease.

Finally, we wanted to confirm whether exogenous hrDNase I is able to up-regulate cell surface Fas expression and facilitate Fas-mediated apoptosis in freshly isolated peripheral blood (PB) T cells obtained from SLE patients and from healthy subjects, as already shown in human cell lines (12).

Materials and methods

Patients

Seventy-three SLE patients fulfilling the revised criteria for SLE of the American College of Rheumatology (23) and 60 age- and sex-matched healthy controls were enrolled. Twenty-two patients were in remission, 29 had moderate active and 22 had active SLE according to the SLE disease activity index (24).

Blood samples were drawn from patients and controls after informed oral or written consent.

Serum levels of DNase I

Patients’ and controls’ sera were blotted onto a nitrocellulose membrane (Hybond-C Extra, Amersham Biosciences, Freiburg, Germany), incubated overnight with a rabbit anti-DNase I antibody developed in our laboratory (11) and then revealed with a peroxidase-conjugated mouse anti-rabbit IgG antibody (Sigma, Milan, Italy). DNase I level in serum samples was calculated comparing the density of each dot with dots containing known concentrations of hrDNase I (purchased from Hoffman–La Roche, Grenzach-Wyhlen, Germany) using a VersaDoc image analyzer apparatus (Bio-Rad, Hercules, CA, USA).

Measurement of human sFas and sFasL

Serum levels of sFas and sFasL were quantified using commercially available ELISA kits (Bender MedSystems, Wien, Austria) following the manufacturer’s instructions. The detection limit was of 20 pg ml⁻¹ and 0.1 ng ml⁻¹ for sFas and sFasL, respectively.

Cells preparation and culture

Peripheral blood mononuclear cells (PBMC) were isolated from freshly heparinized PB of 18 patients and 10 controls by density-gradient centrifugation on Lymphoprep (Nycomed Pharma, Oslo, Norway) and analyzed by flow cytometry.

In four patients and two controls, PBMC were suspended at 2 × 10⁶ ml⁻¹ in RPMI-1640 + 10% FCS incubated with 80 ng ml⁻¹ of hrDNase I for 96 h at 37°C. Then, they were analyzed by flow cytometry.

Flow cytometry analysis

FITC-conjugated anti-CD3 (anti-CD3–FITC), R-PE-conjugated anti-CD95/Fas (anti-CD95–PE) mAb or isotype/fluorochrome-matched controls were purchased from Becton Dickinson (San Jose, CA, USA). Data were collected on a FACSCalibur (Becton Dickinson) using CellQuest software and analyzed by FlowJo software (TreeStar).

Measurement of apoptosis

The extent of internucleosomal DNA fragmentation was quantified using a commercially available kit (Roche Biochemical, Indianapolis, IN, USA) according to the manufacturer’s instructions. The principle of this test is based on the detection of mono- and oligonucleosomes in the cytoplasmic fractions of cell lysates. The enrichment of mono- and oligonucleosomes released into the cytoplasm is calculated by dividing the absorbance value obtained in the cells exposed to the apoptotic stimuli with the absorbance value of the untreated cells (absorbance of sample cells/absorbance of control cells). The enrichment factor was used as an index of apoptosis (apoptotic index) and is shown on the vertical axis as mean ± SD of triplicates.

For the induction of apoptosis, cells were incubated with either the anti-Fas IgG3 mAb (Alexis, Launsen, Switzerland) or the recombinant human sFasL (Alexis) and cell death assessed at several time points. To inhibit Fas-mediated apoptosis, cells were incubated with the blocking anti-Fas IgG2b mAb (Alexis).

Statistical analysis

Calculations were performed with SPSS 14 statistical package. Quantitative data with a normal distribution were expressed as mean ± SD and were analyzed with Student’s t-test. DNase I and sFas, which presented a not-normal distribution, were expressed as median with 25–75th percentile distribution, were expressed as median with 25–75th percentile.
DNase I (ng ml$^{-1}$)

Age (years) 38

Male/female 9/51 (19.1%) 7/66 (11%)

DNase I levels were lower in SLE compared with controls 

Serum DNase I levels are low in SLE

The characteristics of the study population and the levels of DNase I, sFas, sFasL status and also sex and age was performed to evaluate the independent association between these variables and SLE. The study population was then stratified into many groups on the basis of DNase, sFas and sFasL status and the association of these subgroups and SLE was analyzed.

DNase I, sFas and sFasL status were also analyzed in SLE subjects divided on the basis of disease activity or renal involvement.

Correlations between DNase I, sFas, sFasL, cell surface Fas levels, erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) were analyzed with Kendall’s tau test.

A value of $P<0.05$ was considered significant.

Results

Serum DNase I levels are low in SLE

The characteristics of the study population and the levels of DNase I, sFas and sFasL are shown in Table 1. Serum DNase I levels were lower in SLE compared with controls (11.75 ng ml$^{-1}$ in SLE patients versus 13.11 ng ml$^{-1}$ in control subjects; $P = 0.002$ by Mann-Whitney test). Moreover, considering low levels of DNase I (<7.79 ng ml$^{-1}$; below the arbitrary threshold value of the 20th percentile in the healthy controls), subjects with low DNase I levels were significantly more represented in SLE patients than in controls (42.4 versus 18.3%; $P = 0.003$ by Chi-square test; odds ratio (OR) 3.34 with confidence interval (CI) 95% 1.40–7.49). These data indicate that serum DNase I is reduced in patients with SLE when compared with healthy donors and that levels of DNase I <7.79 ng ml$^{-1}$ are associated with SLE.

sFas and sFas ligand levels in SLE

Also sFas levels were lower in SLE patients than in control subjects (sFas: 134.7 versus 211.7 pg ml$^{-1}$; $P = 0.002$ by Mann-Whitney test) and subjects with low sFas levels (<176.9 pg ml$^{-1}$) were more represented in SLE patients (63 versus 18.3% of controls; $P < 0.001$ by Chi-square test; OR 7.22 with CI 95% 2.8–18.6).

Considering sFasL, 67.1% of patients and only 20% of controls have a dosable sFasL ($P < 0.001$ by Chi-square test; OR 8 with CI 95% 3.07–20.88). Therefore, low sFas levels and dosable sFasL are typical features of SLE.

We then stratified the study population on the basis of low sFas status and of dosable sFasL. A highly significant asymmetry was observed (total Chi-square = 49.46, $P < 0.001$; Chi-square for linear trend = 43.04, $P < 0.001$; remaining Chi-square = 6.42, $P = 0.04$): remarkably, only one of the 73 SLE patients presented high levels of sFas and not dosable sFasL. On the other hand, only two of 60 control subjects had low levels of sFas and dosable sFasL. If we consider the SLE diagnosis on the basis of the presence of either low sFas levels or dosable sFasL, we have a test with high sensibility (98.1%) and low specificity (67.5%). If we consider the SLE diagnosis on the basis of the concomitant presence of both sFas low levels and dosable sFasL, we have a test with high specificity (97.5%) and low sensibility (31.5%).

When low DNase I status, low sFas status, dosable sFasL and also age and sex were simultaneously controlled in a multiple logistic regression analysis, low sFas levels ($P < 0.001$; OR 69.7 with CI 95% 9.2–526.2) and dosable sFasL ($P = 0.001$; OR 18.4 with CI 95% 3.1–111.1) maintained a significant association with SLE, whereas this association was lost for low DNase I ($P = 0.517$; OR 1.76 with CI 95% 0.32–9.76). Moreover, there was a significant direct correlation between sFas and sFasL (correlation coefficient 0.54; $P = 0.003$ by Kendall’s rank correlation), while there was no correlation between sFas or sFasL and DNase I. These data indicate that, among the three variables studied (sFas, sFasL and DNase I), sFas and sFasL have a better ability than DNase I in discriminating SLE patients from controls.

Table 1. General characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Controls ($n = 60$)</th>
<th>SLE subjects ($n = 73$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>9/51 (19.1%)</td>
<td>7/66 (11%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38 ± 10.9</td>
<td>40.5 ± 12.9</td>
</tr>
<tr>
<td>Disease activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quiescent</td>
<td>—</td>
<td>22/73 (30.1%)</td>
</tr>
<tr>
<td>Mild active</td>
<td>—</td>
<td>29/73 (39.7%)</td>
</tr>
<tr>
<td>Active</td>
<td>—</td>
<td>22/73 (30.1%)</td>
</tr>
<tr>
<td>Renal involvement</td>
<td>—</td>
<td>25/73 (34.2%)</td>
</tr>
<tr>
<td>ANA positive</td>
<td>0</td>
<td>65/73 (89%)</td>
</tr>
<tr>
<td>Anti-nativeDNA+</td>
<td>0</td>
<td>23/73 (31.5%)</td>
</tr>
<tr>
<td>DNase I (ng ml$^{-1}$)$^a$</td>
<td>13.11 (8.99–23.2)</td>
<td>11.75 (3.69–18.53)$^b$</td>
</tr>
<tr>
<td>Subjects with DNase I level &lt;7.79 ng ml$^{-1}$</td>
<td>11/60 (18.3%)</td>
<td>31/73 (42.4%)$^c$</td>
</tr>
<tr>
<td>sFas (pg ml$^{-1}$)$^a$</td>
<td>211.7 (183.7–262.4)</td>
<td>134.7 (91–276.6)$^b$</td>
</tr>
<tr>
<td>Subjects with sFas level &lt;176.9 pg ml$^{-1}$</td>
<td>11/60 (18.3%)</td>
<td>46/73 (63%)$^c$</td>
</tr>
<tr>
<td>Subjects with dosable sFasL</td>
<td>12/60 (20%)</td>
<td>49/73 (67.1%)$^c$</td>
</tr>
</tbody>
</table>

$^a$Data are expressed as median with 25–75$^a$ percentile range.

$^b$P < 0.05 by Mann-Whitney test.

$^c$P < 0.05 by Chi-square test.

DNase I and SLE disease activity

Next we evaluated whether the three variables studied (DNase I, sFas and sFasL) correlate with SLE serological and clinical parameters.

DNase I, sFas and sFasL levels did not correlate with either inflammation markers (ESR and CRP), presence or absence of anti-double strand DNA antibodies and of renal involvement (data not shown).

SLE patients were divided into three groups on the basis of disease activity (quiescent, mild active and active): the correlation between sFas or sFasL and DNase I. These data indicate that, among the three variables studied (sFas, sFasL and DNase I), sFas and sFasL have a better ability than DNase I in discriminating SLE patients from controls.


DNase I and SLE

Table 2. Distribution of sFas and sFasL status stratification subgroups in SLE and controls

<table>
<thead>
<tr>
<th></th>
<th>High sFas and not dosable sFasL</th>
<th>High sFas and dosable sFasL</th>
<th>Low sFas and not dosable sFasL</th>
<th>Low sFas and dosable sFasL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 60)</td>
<td>40 (66.6%)</td>
<td>11 (18.3%)</td>
<td>7 (11.7%)</td>
<td>2 (3.3%)</td>
</tr>
<tr>
<td>SLE (n = 73)</td>
<td>1 (1.4%)</td>
<td>26 (35.6%)</td>
<td>23 (31.5%)</td>
<td>23 (31.5%)</td>
</tr>
</tbody>
</table>

Total Chi-square = 49.46; P < 0.001; Chi-square for linear trend = 43.04; P < 0.001; remaining Chi-square = 6.42; P = 0.04.

Fig. 1. Distribution of subjects with low sFas levels (<176.9 pg ml⁻¹; gray bars) and dosable sFasL (white bars) in controls and in SLE patients divided on the basis of disease activity. *: Significant difference from control group; P < 0.05 by Chi-square test. #: Significant difference from SLE patients with active disease; P < 0.05 by Chi-square test. §: Significant difference from SLE patients with quiescent disease; P < 0.05 by Chi-square test.

Remarkably, SLE patients with quiescent disease resulted to have lower levels of serum sFas (108.5 pg ml⁻¹; P < 0.05 by Kruskal–Wallis all pairwise comparisons) when compared not only with control subjects (211.7 pg ml⁻¹) but also with SLE patients with active disease (286.1 pg ml⁻¹). No significant difference was found between controls and SLE patients with active disease; however, the latter group presented a trend toward higher sFas levels. Low levels of sFas (<176.9 pg ml⁻¹) was therefore mainly a characteristic of SLE patients with quiescent disease (21/22–95.5% versus 8/22–36.4% in SLE patients with active disease; P < 0.001 by Chi-square test). On the other hand, the proportion of subjects with dosable sFasL increased significantly and progressively from quiescent to active disease (18/22–81.8% versus 8/22–36.4% in SLE patients with quiescent disease; P = 0.011 by Chi-square test) (Fig. 1). These data show that the combined utilization of sFas and sFasL levels allows a better discrimination among quiescent, mild active and active SLE.

DNase I levels, cell surface Fas expression and apoptosis

Since we have previously shown that over-expressed or exogenously administered DNase I up-regulates Fas expression in different cell lines, we analyzed the cell surface expression of Fas (Fig. 2A) in a group of 28 subjects (18 patients with active SLE and 10 controls). There was a mild significant direct correlation between DNase I level and Fas-positive-CD3⁺ lymphocytes (correlation coefficient 0.275; P = 0.045 by Kendall’s rank correlation). Interestingly, the percentage of Fas-positive-CD3⁺ lymphocytes was significantly higher in SLE patients than in controls (P < 0.05) (Fig. 2B).

Finally, we wanted to verify whether exogenous hrDNase I can modulate Fas expression in freshly isolated cells. For this purpose, PBMC obtained from four patients with SLE and two healthy donors were incubated with hrDNase I and stained with anti-Fas mAb. Indeed, an increased expression of Fas was detectable on CD3⁺ cells by FACS analysis after incubation with hrDNase I in cells obtained from both SLE patients and healthy donors (Fig. 3A). These data show that exogenously administered hrDNase I is able to modulate Fas expression also in freshly isolated cells.

Since Fas/FasL-mediated pathway is particularly important in T cells undergoing apoptosis in SLE (18), we evaluated whether cells exposed to hrDNase I had an increased susceptibility to Fas-mediated apoptosis. We observed that mononuclear cells, obtained from the same subjects analyzed for cell surface Fas induction and incubated with hrDNase I as above described, showed an increased internucleosomal DNA degradation upon engagement of Fas using either an anti-Fas IgG3 mAb or recombinant FasL (Fig. 3B and C).

The apoptosis observed was truly Fas dependent since this phenomenon was not observed in the presence of an anti-Fas-blocking antibody (data not shown). These results show that exogenously administered hrDNase I can up-regulate Fas expression and induce Fas-mediated apoptosis also in freshly isolated cells derived from both normal donors and SLE patients.

Discussion

Clearance of nucleosomes from the body is crucial for the development of SLE; DNase I is responsible for nucleosome degradation and therefore low enzyme activity may contribute to the anti-nuclear auto-antibody production typical of SLE (25, 26). The decreased activity may be related to mutation in the DNase I gene as shown in 2 of 200 Japanese patients studied (7). However, this mutation is not present in the three major ethnic groups: Caucasian, African and Asian (27). Single-nucleotide polymorphism (SNP) may affect or not the serum DNase I activity (28, 29) and from this effect may depend the described association of a SNP with colorectal (30) and gastric carcinomas (31) and with myocardial infarction (32).

The measurement of serum DNase I activity may be influenced by a number of factors such as (i) the presence of natural inhibitors of the enzyme (actin), (ii) the presence of anti-DNA antibodies able to bind the catalytic site of DNase I and to interfere with DNase I activity (33) and (iii) the
Table 3. DNase I, sFas and sFasL levels in SLE patients divided on the basis of disease activity

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 60)</th>
<th>Quiescent (n = 22)</th>
<th>Mild active (n = 29)</th>
<th>Active (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNase I (ng ml$^{-1}$)$^a$</td>
<td>13.11 (8.99–23.2)</td>
<td>12.3 (6.6–18.5)</td>
<td>7.5 (2.9–18.3)</td>
<td>12.1 (3.5–18.1)</td>
</tr>
<tr>
<td>Subjects with DNase I &lt;7.79 ng ml$^{-1}$</td>
<td>9/47 (19.1%)</td>
<td>7/22 (31.8%)</td>
<td>15/29 (51.7%)</td>
<td>7/22 (31.8%)</td>
</tr>
<tr>
<td>sFas (pg ml$^{-1}$)$^b$$^c$</td>
<td>211.7 (183.7–262.4)</td>
<td>108.5 (78.6–129.8)$^d$</td>
<td>154.3 (88.9–251.4)</td>
<td>286.1 (120.8–317.1)</td>
</tr>
<tr>
<td>Subjects with sFas&lt;176.9 pg ml$^{-1}$</td>
<td>12/60 (20%)</td>
<td>21/22 (95.5%)$^e$</td>
<td>18/29 (62%)$^e$</td>
<td>8/22 (36.4%)</td>
</tr>
<tr>
<td>Subjects with dosable sFasL$^d$</td>
<td>12/60 (20%)</td>
<td>8/22 (36.4%)</td>
<td>22/29 (75.9%)$^e$</td>
<td>18/22 (81.8%)$^e$</td>
</tr>
</tbody>
</table>

$^a$Data are expressed as median with 25th–75th percentile range.
$^b$P < 0.001 by Kruskal–Wallis.
$^c$Significant difference from controls (P < 0.001) and from SLE patients with active disease (P = 0.03) by Kruskal–Wallis all pairwise comparisons (Dwass–Steel–Chritchlow–Fligner).
$^d$P < 0.001 Chi-square test.
$^e$Significant difference from control group; P < 0.05 by Chi-square test.

Most importantly, we confirmed the ability of hrDNase I to enter into freshly isolated T cells and to increase surface Fas expression in PB T cells as already shown in the erythroleukaemia cell line K562 (12). A possible explanation for the direct correlation between DNase I levels and cell surface Fas expression in SLE patients is that circulant DNase I enters into the cells through the binding to the cation-independent mannose 6-phosphate/insulin-like growth factor (CI-MPR) receptor and induces surface expression of the death receptor Fas, behaving as a transcription factor for the fas gene. In this way, DNase I would contribute to the pathogenesis of SLE not only through removal of DNA from auto-antigent nucleoprotein complexes but also by modulating Fas expression and ultimately cell apoptosis. Indeed, the results obtained incubating PBMC from patients and controls with hrDNase I on the expression of Fas and on increased susceptibility to apoptosis suggest that such a mechanism may be present also in vivo.

hrDNase I has been proposed as a therapeutic agent in SLE (26, 36); however, no changes both in clinical manifestations and in serum markers of disease activity have been observed in humans. Moreover, mice over-expressing DNase I showed a reduction in auto-antibodies but were not protected from developing lupus nephritis (37).

We believe that the lack of effect of DNase I treatment in SLE may be related to its internalization into cells and to the increased expression of cell surface Fas essentially leading to increased Fas-mediated apoptosis. Therefore, we think that DNase I is not a beneficial therapy in SLE because of its ability to increase cell susceptibility to apoptosis. On the contrary, this treatment could be detrimental, since CI-MPR is an endocytosis receptor ubiquitously expressed in any cell types and particularly abundant in phagocytes. Therefore, DNase I delivery within cells like neutrophils and CD34+ cells may even result in exacerbation of some disease features like neutropenia and leukopenia (19–21). This aspect would be useful in cancer therapy since DNase I increases tumor cells’ susceptibility to apoptosis induced by chemotherapeutical agents (11). Such hypothesis is sustained by the association of a SNP with low DNase I activity with particular types of carcinoma (30, 31).

In conclusion, our findings show that DNase I enzyme level is low in patients with SLE and that it can be considered a marker of the disease but not an indicator of disease severity. Moreover, our data using PBMC from patients and
controls confirm previous data obtained with cell lines on the ability of DNase I to modulate Fas expression and cell susceptibility to apoptosis. This finding, together with the measurements of DNase I and Fas expression \textit{ex vivo}, suggests that a similar mechanism may occur also \textit{in vivo}, providing one possible explanation for the lack of efficacy of hrDNase I in the treatment of SLE.

**Abbreviations**

- ANA: anti-nuclear antibody
- CI: confidence interval
- CI-MPR: cation-independent mannose 6-phosphate/insulin-like growth factor
- CRP: C reactive protein
- ESR: erythrocyte sedimentation rate
- hrDNase: human recombinant DNase
- OR: odds ratio
- PB: peripheral blood
- PBMC: peripheral blood mononuclear cells
- sFAS: soluble Fas
- sFASL: soluble Fas ligand
- SLE: systemic lupus erythematosus
- SNP: single-nucleotide polymorphism
- TNF: tumor necrosis factor

**References**


