Anti-Tyro3 IgG associates with disease activity and reduces efferocytosis of macrophages in new-onset systemic lupus erythematosus

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Abstract

Backgroud: To investigate the role of Tyro3 receptor in macrophages' efferocytosis of apoptotic cells in systemic lupus erythematosus (SLE), we aimed to reveal the clinical relevance and impact of anti-Tyro3 antibody on SLE. Methods : The serum levels of IgG-type autoantibody against Tyro3 receptor were detected in new-onset, treatment-naïve SLE patients (n =70) and healthy controls (HCs) (n =70) using enzyme-linked immunosorbent assay (ELISA). The correlation of the levels of autoantibodies against Tyro3 receptor with clinical and laboratory characteristics were analyzed by Spearman correlation analysis. Receiver operating characteristic (ROC) curve was used to assess the sensitivity and specificity of anti-Tyro3 IgG for the diagnosis of SLE. The effects of purified Tyro3 autoantibody from SLE patients on the efferocytosis of human monocyte-derived macrophages were measured by flow cytometry and immunofluorescence. Results : The serum levels of IgG-type autoantibody against Tyro3 receptor were significantly elevated in patients with SLE compared to HCs ($p < 0.0001$). The levels of anti-Tyro3 IgG were negatively associated with haemoglobin (Hb) ($r =-0.294$, $p = 0.014$), and positively correlated with the presence of oral ulcers ($r = 0.254$, $p = 0.034$), SLE disease activity index (SLEDAI) score ($r = 0.254$, $p = 0.034$), erythrocyte sedimentation rate (ESR) ($r = 0.430$, $p = 0.000$), C-reactive protein (CRP) ($r = 0.246$, $p = 0.049$) and immunoglobulin G (IgG) ($r = 0.408$, $p = 0.001$). Higher levels of anti-Tyro3 antibody were observed in patients with oral ulcers than paitents without oral ulcers ($p = 0.035$). Further flow cytometry demonstrated that purified anti-Tyro3 IgG inhibited the efferocytosis of macrophages ($p = 0.004$). Immunofluorescence assay also showed a decreased engulfment of apoptotic cells in the macrophages incubated with purified anti-Tyro3 IgG ($p = 0.044$) compared with control IgG. Conclusions: These observations indicated that autoantibody against Tyro3 was associated with disease activity and impaired efferocytosis of macrophages, which might be involved in the pathogenesis of SLE.

Background

Systemic lupus erythematosus (SLE), which is characterized by the production of a large number of autoantibodies [1, 2], is a chronic autoimmune disease that affects almost all organs [3, 4]. The overproduced autoantibodies circulate in the blood and specifically affect the antigen-presenting cells. Numerous lines of evidence have revealed that the overproduction of autoantibodies is attributed to the impaired efferocytosis of apoptotic cells and debris [1, 5, 6]. However, the mechanism underlying the impairment of efferocytosis in SLE is not fully understood.

Macrophages belong to the largest subset of immune cells that are principally responsible for efferocytosis [2, 7]. Generally, efferocytosis in macrophages is initiated by the recognition of apoptotic cells via receptors on the cell surfaces of macrophages, which then trigger the phagocytic process [8, 9]. A 3-member transmembrane kinase receptor family named Tyro3/Axl/Mertk (TAM) is responsible for the recognition of apoptotic cells during efferocytosis by macrophages [10]. TAM family receptors include the Tyro3, Axl, and Mertk receptor tyrosine kinases and can be activated by interaction with the 'eat-me' phosphatidylserine on the surfaces of apoptotic cells, which rely on the assistance of the ligands growth arrest-specific growth arrest specific 6 (GAS6) and protein S [11, 12]. Emerging evidence has revealed that

a deficiency in TAM receptors results in the accumulation of apoptotic cells [5, 13]. For instance, Mertk knockdown or knockout mice showed the delayed clearance of infused apoptotic cells [14]. Not only Mertk but also Axl and Tyro3 function in the phagocytosis of apoptotic cells. $Axl^{-/-}$, $Tyro3^{-/-}$ macrophages had ~40–50% reduction in their abilities to phagocytose apoptotic thymocytes [15]. Furthermore, triple knockout Tyro3/Axl/Mertk mice developed SLE-like autoimmunity and overproduced autoantibodies, such as anti-double-stranded DNA (dsDNA) [16], indicating that the dysfunction of the TAM receptors on macrophages contributed to the development of SLE. Among TAM receptors, Tyro3 is the least characterized member. In the hematopoietic system, Tyro3 receptor is expressed in dendritic cells, natural killer cells, monocytes and macrophages, platelets and megakaryocytes and osteoclasts [17]. It was reported that primary peritoneal macrophages isolated from Tyro3-/- mice had decreased ability to phagocytose apoptotic cells compared to macrophages from wild-type mice [17]. Clinical studies revealed that the levels of the soluble forms of Tyro3 (sTyro3) and Mertk receptor were increased in plasma from SLE patients, and sTyro3 showed the most significant positive linear correlation with SLE disease activity index (SLEDAI) ($p = 0.001$). In addition, only sTyro3 had an increase in the high SLEDAI group, while there were no differences for soluble forms of Axl and Mertk [18, 19]. It might be responsible for the defective function of efferocytosis in the disease [20]. However, whether Tyro3 receptor could serve as autoantigen to further affect the function of macrophages has remained unexplored. Here, we systematically investigated the profiles and clinical relevance of the Tyro3 autoantibody in new-onset and treatment naïve SLE patients and further explored the implications of this for the efferocytosis by macrophages.

Methods

Patients and healthy controls

The study included consecutive 70 new-onset and treatment naïve patients with SLE (including 60 females and 10 males) who met the 1997 American College of Rheumatology (ACR) classification criteria for the diagnosis of SLE confirmed by two qualified rheumatologists (Jialin Teng and Chengde Yang) [21]. Demographic data, clinical characteristics, and laboratory findings such as anti-dsDNA IgG levels, erythrocyte sedimentation rate (ESR), white blood cell counts in blood (WBC), haemoglobin (Hb), platelets (PLT), C-reactive protein (CRP), immunoglobulin G (IgG), complement 3 (C3) and complement 4 (C4) of SLE patients were collected. In addition, samples from 70 sex- and age-matched healthy donors with neither autoimmune nor infectious diseases were collected as healthy controls (HCs) (including 58 females and 12 males). All of the sera samples were stored at -80 °C until use. Disease activity was measured using SLEDAI score [22]. The study was performed in accordance with the Declaration of Helsinki and the Principles of Good Clinical Practice. Biological samples were obtained under a protocol approved by the Institutional Research Ethics Committee of Ruijin Hospital (ID: 2016-62), Shanghai, China.

Detection of anti-Tyro3 autoantibody

Recombinant human Tyro3 (Abnova, Taiwan) was prepared by wheat germ expression system. The protein was fused with a GST-tag at N-terminal and purified by glutathione sepharose 4 fast flow. Antibody against human Tyro3 receptor in the sera of SLE patients and HCs was determined by an enzyme-linked immunosorbent assay (ELISA). Ninety-six-well high binding plates (Corning, New York, USA) were coated with recombinant human Tyro3 protein in 0.05 mol/L carbonate buffer sodium (pH=9.6) overnight at 4 °C. The antigen-coated wells were washed three times with PBST (PBS plus 0.05% Tween-20) and blocked with PBST containing 5% bovine serum albumin (BSA) for 2 h at 37 °C. The blocking buffer was removed, and the plates were washed as described above before the addition of 100 µl of serum sample (1:100 diluted in 1% BSA). The human sera were incubated for 2 h at room temperature followed by incubation with HRP-conjugated goat anti-human IgG (Abcam, Cambridge, UK) for another 1 h at room temperature. Then, the plates were washed, and 100 µl of tetramethylbenzidine substrate solution was added. The color development was stopped by the addition of 50 µl of 0.5 M H_2SO_4. The absorbance was measured at a wavelength of 450 nm in a microplate reader (Bio-Rad Laboratories, Richmond, USA).

IgG purification

Total IgG was isolated from the serum of new-onset SLE patients by Thiophilic Adsorbent reagent (Pierce, Thermo Scientific, Rockford, USA) according to the manufacturer's instructions and concentrated in a centrifuge tube (Amicon, Millipore, Eschborn, Germany). The total protein content was estimated using a spectrophotometer (NanoDrop, Thermo Scientific, USA).

Purification of anti-Tyro3 IgG

The purification of the specific antibody was performed using AminoLink Plus Coupling Resin (MicroLink, Thermo Scientific, USA). We purchased recombinant human Tyro3 protein (Abnova, Taiwan) and coupled the protein to the resin. Then, we used the purified total IgG from SLE patients to form the resin-bound complex and incubated them with gentle end-over-end mixing. The eluted antibody was neutralized with 1 M Tris (pH=9.0). After sterile filtration, the autoantibody was stored as small aliquots at -80 °C.

Preparation of macrophages

Peripheral blood mononuclear monocytes (PBMCs) were isolated from the blood of healthy volunteers using Ficoll density gradient centrifugation (GE Healthcare, Madison, USA). The $CD14^+$ monocytes were isolated by positive selection using CD14 microbeads (Miltenyi Biotec, Auburn, USA) according to the manufacturer's instructions. The selected cells were cultured in RPMI 1640 medium containing 10% fetal calf serum (FCS; Gibco, NY, USA), 100 units/ml penicillin, and 100 µg/ml streptomycin that was supplemented with 100 ng/ml of macrophage colony stimulating factor (M-CSF) (R&D Systems,

Minneapolis, USA) in a humidified 5% CO_2 incubator. On day 4, the medium was replaced with RPMI 1640 medium containing M-CSF, and on day 7, the mature macrophages were harvested.

Preparation of apoptotic cells

To generate apoptotic cells, Jurkat cells (purchased from ATCC, Manassas, USA) were cultured in RPMI 1640 medium without FCS, and apoptosis was induced with 0.5 μg/mL staurosporine (BBI Life Science, Shanghai, China) for 3 h, as previously reported that the inhibitor of protein kinases staurosporine showed remarkable activity in inducing apoptosis in a wide variety of mammalian cells [23]. Afterwards, the cells were washed three times with PBS and resuspended in RPMI 1640 medium. Staurosporine treatment yielded a population of 90% apoptotic cells, which was verified by staining with annexin V and 7-amino-actinomycin D (7-AAD, Tianjin Sungene Biotech Co., China). Before being fed to the macrophages, the apoptotic cells were labeled with iFL Green dye (pHrodo, Invitrogen, Thermo Scientific, USA), which could be detected with FITC (fluorescein), protected from light at room temperature for 20 minutes.

Efferocytosis assays

Human CD14 positive monocyte derived macrophages were incubated with fresh medium containing 60 μg/ml purified human Tyro3 antibody from SLE patients or normal human IgG (BBI Life Science, Shanghai) for 1 h at 37 °C in an incubator.

Then, the macrophages were incubated with staurosporine induced apoptotic Jurkat cells labeled with iFL Green dye (ratio of macrophages : apoptotic cells = 1:5) in RPMI 1640 medium without FCS in an incubator for 30 minutes. After washed with PBS, the macrophages were collected using trypsin. Efferocytosis was determined according to the percentage of macrophages that phagocytosed apoptotic cells labeled with FITC using a FACS Canto II cytometer (BD Biosciences, San Jose, USA). The data were analyzed using FlowJo software (Tree Star Inc., Ashland, USA).

For immunofluorescence, the apoptotic cells were labeled with 5 μM 5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester (CFSE) (eBioscience, Invitrogen, Thermo Scientific, USA), protected from light at room temperature for 10 minutes and washed with PBS. Then, the macrophages were incubated with induced apoptotic Jurkat cells for 30 minutes. After washing with PBS, macrophages were fixed with 4% formaldehyde for 20 minutes. After three rinses with PBS, the cells were permeabilized with Triton X-100 (Beyotime, Shanghai, China) for 5 minutes. The cytoskeleton was dyed with phalloidin (Servicebio, Shanghai, China), and incubated for 1 h protected from light. DNA was stained with 10 μg/ml 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (hoechst 33324) (Thermo Scientific, USA) for 5 minutes protected from light. After rinsing 3 times with PBS, the cells were viewed by confocal fluorescence microscope LSM 800 (ZEISS, Oberkochen, Germany).

Statistics

Continuous variables were tested for normality by One-Sample Kolmogorov-Smirnov Test. Continuous variables were expressed as mean ± SD or median (interquartile range) as per distribution type, and categorical data were expressed as frequency and percentages. Receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC) were used to assess the sensitivity and specificity of anti-Tyro3 IgG for the diagnosis of SLE. Statistical analysis was performed using independent samples *t*-test for normal data and Mann-Whitney *U*-test for non-normal data. The analyses were carried out under the two-sided principle. Correlations between groups were evaluated by the Spearman correlation analysis. A *P*-value less than 0.05 was considered statistically significant. Graphs were drawn using Graphpad Prism (version 7, GraphPad Software Inc, San Diego, USA) and data were analyzed using the SPSS software for Windows (Version 23; SPSS Inc., Chicago USA).

Results

Increased serum levels of Tyro3 receptor autoantibody in patients with SLE

As TAM proteins are well known kinase receptors that are crucial for macrophage efferocytosis for the clearance of apoptotic cells [10], to investigate the profile and clinical relevance of autoantibody against Tyro3 receptor in SLE, we determined the serum levels of anti-Tyro3 IgG in patients with SLE by ELISA. We recruited a total of 70 new-onset patients with SLE, and the clinical data were listed in Table 1. The results showed that the levels of anti-Tyro3 IgG were significantly higher in SLE patients than in HCs ($p < 0.0001$) (Fig. 1A), suggesting that Tyro3 receptor antibody might be involved in the pathogenesis of SLE.

Table 1

The clinical and laboratory characteristics of new-onset and treatment naïve SLE patients

Variable	SLE patients
Gender (female/male, n)	60/10
Age (mean ± SD, years)	39 ± 16
Disease duration (months, median, IQR)	3.5(1, 24)
SLEDAI (mean ± SD)	12 ± 5
Fever (n, %)	32(45.7)
Arthritis (n, %)	42(60.0)
Rash (n, %)	36(51.4)
Oral ulcer (n, %)	16(22.9)
Alopecia(n, %)	16(22.9)
Vasculitis(n, %)	5(7.1)
Serositis (n, %)	16(22.9)
Photosensitivity (n, %)	10(14.3)
Raynaud's phenomenon (n, %)	13(18.6)
Hematological (n, %)	54(77.1)
Lupus nephritis (proteinuria ≥ 0.5 g/24 h) (n, %)	31(44.3)
Neuropsychiatric manifestations (n, %)	1(1.4)
Anti-dsDNA positive (n, %)	63(90.0)
Anti-Sm positive (n, %)	17(24.3)
Anti-SSA positive (n, %)	38(54.3)
Anti-SSB positive (n, %)	8(11.4)
Anti-U1RNP positive (n, %)	27(38.6)
Anti-Rib-P positive (n, %)	29(41.4)
Anti-nucleosome-A positive (n, %)	24(34.3)

Note: SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index; IQR, interquartile range; dsDNA, double-stranded DNA.

Association between anti-Tyro3 IgG and the clinical manifestations in SLE patients

Next, we analyzed the association of serum anti-Tyro3 IgG levels with the clinical data. The levels of anti-Tyro3 IgG were negatively associated with Hb (r =-0.294, p = 0.014), and positively correlated with SLEDAI score (r = 0.254, p = 0.034), ESR (r = 0.430, p = 0.000), CRP (r = 0.246, p = 0.049) and IgG (r = 0.408, p = 0.001) (Fig. 1B-L). Furthermore, the differences of levels of anti-Tyro3 antibody in SLE patients with and without clinical characteristics were determined. As shown in Table 2, higher levels of anti-Tyro3 antibody were observed in patients with oral ulcers than patients without oral ulcers (p = 0.035).

Table 2
Comparison of anti-Tyro3 antibody according to disease manifestations in 70 new-onset and treatment naïve SLE patients

	anti-Tyro3 IgG (OD 450)		p value
Photosensitivity	(+), n = 10 (—), n = 60	0.645 ± 0.185 0.578 ± 0.177	0.185
Raynaud's phenomenon	(+), n = 13 (—), n = 57	0.594 ± 0.187 0.586 ± 0.178	0.892
Fever	(+), n = 32 (—), n = 38	0.618 ± 0.228 0.562 ± 0.121	0.402
Serositis	(+), n = 16 (—), n = 54	0.606 ± 0.153 0.582 ± 0.187	0.640
Oral ulcer	(+), n = 16 (—), n = 54	0.698 ± 0.264 0.555 ± 0.131	0.035*
Rash	(+), n = 36 (—), n = 34	0.605 ± 0.214 0.569 ± 0.132	0.953
Alopecia	(+), n = 16 (—), n = 54	0.599 ± 0.157 0.584 ± 0.186	0.781
Arthritis	(+), n = 42 (—), n = 28	0.607 ± 0.178 0.558 ± 0.179	0.148
Vasculitis	(+), n = 5 (—), n = 65	0.634 ± 0.166 0.584 ± 0.180	0.552

Note: Anti-Tyro3 IgG (OD 450) are shown as mean ± SD, and differences between two groups were analyzed using the independent samples t-test for normal data and the Mann-Whitney U test for non-normal data. SLE, systemic lupus erythematosus. * $p < 0.05$.

Receiver operating characteristic curve of anti-Tyro3 IgG in SLE

To determine the efficacy of the measurement of autoantibody against Tyro3 receptor for diagnosing SLE, we calculated the ROC curve to determine the sensitivity and specificity of the autoantibody in distinguishing patients with SLE from HCs. As shown in Figure 2, the AUC of anti-Tyro3 IgG was 0.8182 ($p < 0.0001$).

SLE-derived autoantibody against Tyro3 receptor inhibited the efferocytosis of macrophages

Since TAM receptors mainly function in macrophage-associated efferocytosis, we further determined the role of Tyro3 receptor autoantibody in the efferocytosis of macrophages. First, we purified specific IgG-type antibody against Tyro3 from the serum of new-onset SLE patients, which were further confirmed by silver staining compared to different quantity of IgG (Supplementary figure 1). Then, primary human monocyte-derived macrophages and staurosporine-induced apoptotic Jurkat cells were incubated with purified anti-Tyro3 IgG or control IgG, and macrophage efferocytosis was analyzed by flow cytometry and immunofluorescence. As shown in Figure 3, macrophage efferocytosis was significantly decreased after anti-Tyro3 IgG ($p = 0.004$) treatment compared with control IgG treatment detected by flow cytometry. As well, immunofluorescence assay showed a decreased engulfment of apoptotic cells in the macrophages incubated with purified anti-Tyro3 IgG ($p = 0.044$) compared with control IgG. These data suggested that Tyro3 receptor autoantibody reduced the efferocytosis of macrophages by blocking Tyro3 receptor and may result in the accumulation of cell debris.

Discussion

The contribution of the dysregulation of macrophages to autoantibody overproduction in SLE has been widely studied, but the effect of the resultant autoantibodies on macrophages in SLE has rarely been reported. Here, we demonstrated that patients with SLE produced high levels of autoantibody against Tyro3, which was one of the three key tyrosine kinases involved in the macrophage-mediated elimination of apoptotic cells. Further functional studies revealed that the anti-Tyro3 IgG inhibited the efferocytosis of macrophages, which might further promote the production of autoantibodies in SLE.

The overproduction of autoantibodies is a hallmark of the progression of SLE. The targets of these antibodies are variable, and thus, these antibodies could affect many cells via direct binding to antigens on cells [24]. The functions of these autoantibodies are also variable and include the activation or inhibition of downstream signaling and the blocking of interactions between targets and other proteins [25]. Previous studies of autoantibodies have mainly focused on the effects of circulating autoantibodies on inflammatory damage in multiple organs, including the skin, lung, and kidney [1]. Antibodies related to disease progression have been identified, such as anti-dsDNA, anti-ribosomal P, anti-cardiolipin, and anti-RNP antibody [2]. Recently, we also found that the overproduced antibodies in SLE included antibodies

recognizing proteins specifically expressed in immune cells that were responsible for key immune responses, such as anti-PD-1 [26]. These immune response related antibodies might further disturb the balance of the immune system and contribute to the progression of SLE. In this study, we demonstrated that antibody against Tyro3 was increased in patients with SLE and was associated with SLEDAI score, ESR, CRP, indicating that the antibody was related with the disease activity and might contribute to the altered function of macrophages.

The dysfunction of macrophages has been observed in SLE for many years [27]. The majority of the studies on macrophages in SLE have mainly focused on the function of dysregulated efferocytosis resulting in the accumulation of apoptotic cells [27–29], but the mechanism underlying macrophage dysregulation remains largely unexplored. The major factor involved in the regulation of macrophage mediated efferocytosis is TAM receptors [11, 13, 30]. Our study provided evidence that the overproduced autoantibodies included antibody against Tyro3 receptor. The antibody inhibited the activity of the Tyro3 receptor and resulted in the inhibition of macrophage efferocytosis. These observations revealed autoantibody overproduction's influence on dysregulation of macrophage that might be involved in the pathogenesis of SLE.

It was interesting that the statistic analyses showed that patients with the presence of oral ulcers had high levels of anti-Tyro3 IgG, and there was little known about the relationship between TAM autoantibodies and oral ulcers. It has been reported that CD68 positive macrophages are one of the main inflammatory cells relevant to the pathogenesis of the ulcers [31]. In oral ulcers, the apoptosis of epithelium remains in large quantity and pose a great pressure of scavenging cells such as macrophages. The rapidly apoptotic epithelial cell may exceed the ability of phagocytosis of macrophages, which lead to sloughing of the dead epithelial cells [32]. Furthermore, autoantibodies related defective phagocytosis of macrophages might in turn worsen the symptom of oral ulcers. In addition, our study discovered that the levels of anti-Tyro3 IgG were negatively associated with Hb ($r = -0.294$, $p = 0.014$). It was reported that significant negative correlations were detected between sAxl levels and glycated hemoglobin ($r = -0.4$, $p = 0.003$) in type 2 diabetes [33].

The identification of pathological antibodies is key to understand the abnormal events that occur in patients with SLE. Our study is the first to report the enrichment of pathological anti-Tyro3 antibody in SLE. Our study indicated that autoantibody against Tyro3 might be responsible for the reduced efferocytosis of macrophages, which might be involved in the pathogenesis of SLE.

Conclusions

This study showed elevated level of anti-Tyro3 IgG in patients with SLE compared to HCs and was associated with with SLE disease activity. Taken together, our results indicated that autoantibody against Tyro3 impaired efferocytosis of macrophages, which might be involved in the pathogenesis of SLE.

List Of Abbreviations

TAM, Tyro3, Axl, and Mertk; GAS6, growth arrest specific 6; SLE, systemic lupus erythematosus; HC, healthy control; ACR, American College of Rheumatology; SLEDAI, SLE disease activity index; ESR, erythrocyte sedimentation rate; dsDNA, double-stranded DNA; ELISA, enzyme-linked immunosorbent assay; WBC, white blood cell; Hb, haemoglobin; PLT, platelet; CRP, C-reactive protein, IgG, immunoglobulin G; C3, complement 3; C4, complement 4; PBMC, peripheral blood mononuclear monocyte; M-CSF, macrophage colony stimulating factor; FCS, fetal calf serum; BSA, bovine serum albumin; ROC, receiver operating characteristic; AUC, area under the curve; sTyro3, soluble form of Tyro3.

Declarations

Ethics approval and consent to participate

The study was performed in accordance with the Declaration of Helsinki and the Principles of Good Clinical Practice. Biological samples were obtained under a protocol approved by the Institutional Research Ethics Committee of Ruijin Hospital (ID: 2016-62), Shanghai, China.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZZ, AX participated in carrying out the experiments and wrote the manuscript. FW and YT provided the figures and tables and performed statistical analysis. JT, HL and XC helped to revise the manuscript. YS, HS, QH, HC, JL, and JH collected the clinical samples and data. YS, CY and JY designed experiments and revised the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no conflicts of interest.

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Supplementary Figure

Supplementary figure 1. Silver Staining of purified anti-Tyro3 IgG from SLE patients. The heavy chain and light chain of IgG were 50 kD and 25 kD, respectively. The quantity of IgG-1, IgG-2, IgG-3 were 1 μg, 0.3 μg, 0.1 μg, respectively. The quantity of anti-Tyro3 IgG was 0.3 μg.

Figures

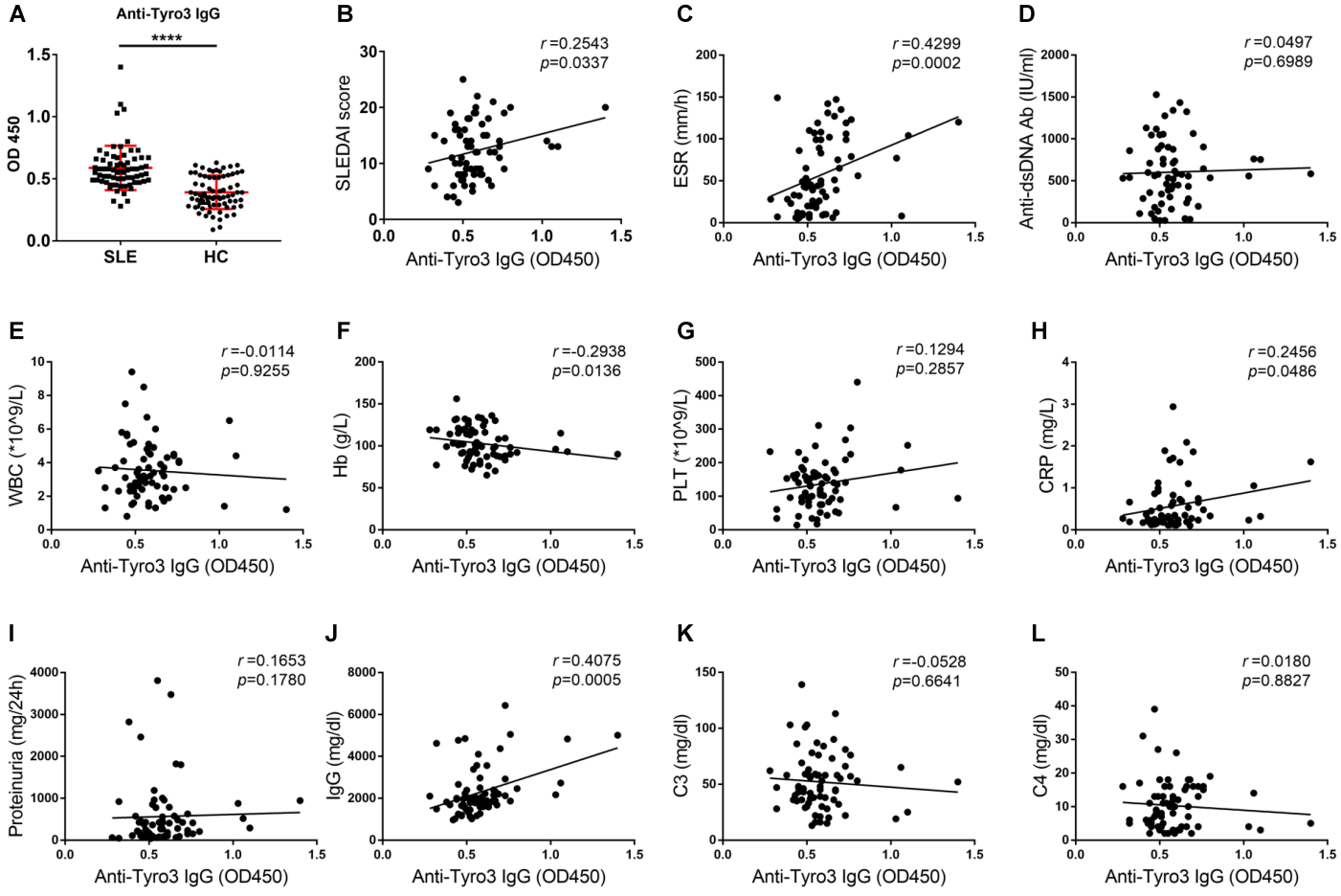

Figure 1

Detection of anti-Tyro3 IgG and correlation of anti-Tyro3 IgG levels and clinical manifestations in SLE patients. (A) The levels of anti-Tyro3 IgG in SLE patients and HCs were examined by ELISA. **** $p < 0.0001$. (B-L) The correlation between anti-Tyro3 IgG levels and SLEDAI score, ESR, Anti-dsDNA Ab, WBC, Hb, PLT, CRP, Proteinuria, IgG, C3, C4 in SLE patients. $p < 0.05$ represents a significant difference. SLE, systemic lupus erythematosus; HC, healthy control; ESR, erythrocyte sedimentation rate; SLEDAI, SLE disease activity index; dsDNA, double-stranded DNA; WBC, white blood cell; Hb, haemoglobin; PLT, platelet; CRP, C-reactive protein, IgG, immunoglobulin G; C3, complement 3; C4, complement 4.

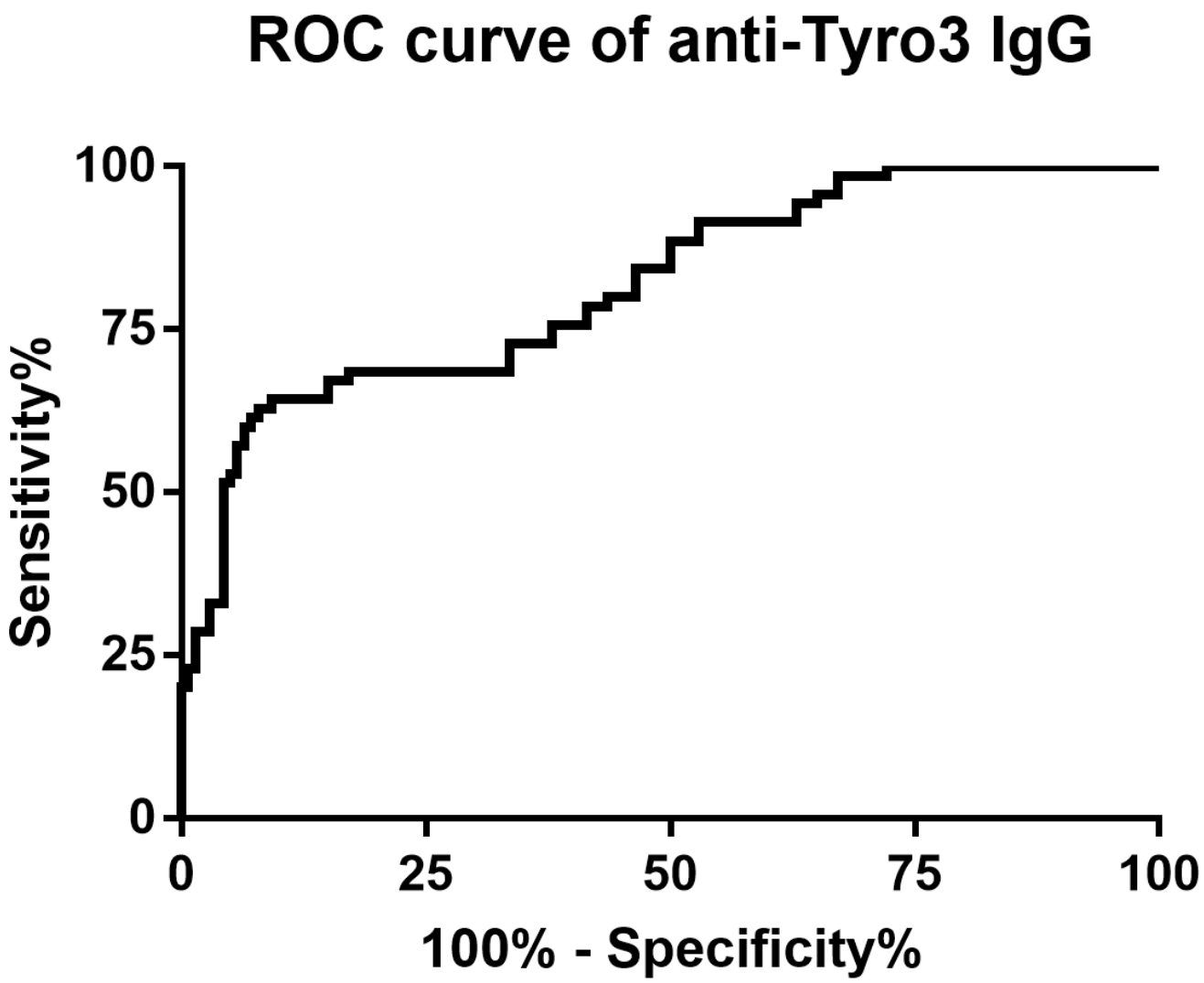

Figure 2

The receiver operating characteristic (ROC) curve of anti-Tyro3 IgG in SLE patients and HCs. The AUC of anti-Tyro3 IgG was 0.8182 ($p < 0.0001$).

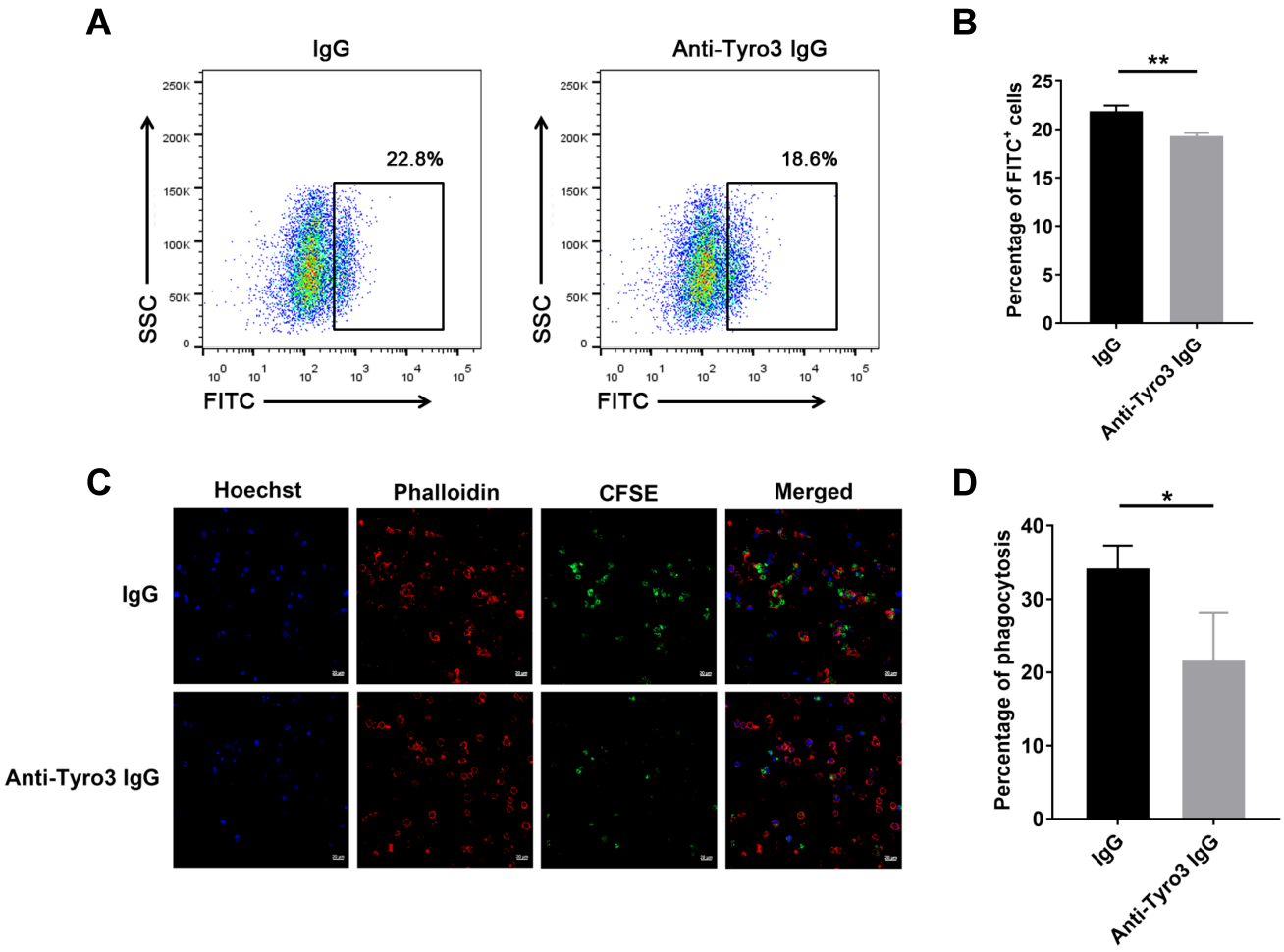

Figure 3

Autoantibody against Tyro3 receptor reduced macrophage efferocytosis by flow cytometry and immunofluorescence. (A) The efferocytosis of macrophages was analyzed by flow cytometry. (B) The statistical graph showing the flow cytometry data (n = 4). The values represent the mean ± SD. **P < 0.01. (C) Representative photograph of the efferocytosis of macrophages monitored by immunofluorescence, presented as merged pictures of Hoechst (blue), phalloidin (red) and CFSE (green). Bar, 20 μm. (D) Statistical data of the percentage of efferocytosis in 100 × views of the confocal microscope (n = 3). The values represent the mean ± SD. *P < 0.05.

Supplementary Files

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- supplfig1.tif