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Efferocytosis capacities of blood monocyte-derived macrophages in Systemic sclerosis

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Abstract

A defect in the apoptotic cell clearance (efferocytosis) by phagocytic cells may participate in autoimmunity and chronic inflammation. The mechanisms leading to the emergence of auto-immunity in systemic sclerosis (SSc) are still to be determined. In this study, the efferocytosis capacities of blood monocyte-derived macrophages (MDM) from patients with SSc were

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evaluated. Blood monocytes obtained from patients with SSc and healthy donors (HD) were differentiated *in vitro* into macrophages. The capacities of MDM to engulf CFSE+ apoptotic Jurkat human T lymphocytes were compared between SSc MDM and HD using flow cytometry. The expression of classical engulfing receptors in SSc MDM and HD MDM was also evaluated and their involvement in the modulation of efferocytosis was confirmed using a siRNA approach. The mean phagocytic index (PI) reflecting efferocytosis capacities of SSc MDM (PI = 19.3 $\pm$ 3.0; n=21) was significantly decreased in comparison with the PI of HD MDM (PI = 35.9 $\pm$ 3.0; n=31; $P < 0.001$). In comparison with HD, SSc MDM exhibited a down-regulated expression of SR-B1, SR-A1 and Integrin β5. In HD MDM, the extinction of these receptors was followed by a reduction of efferocytosis only for the repression of Integrin β5, suggesting a possible selective role of this integrin in the impaired efferocytosis observed in SSc. As efferocytosis may be at the crossroads of inflammation, autoimmunity and fibrosis, in showing impaired efferocytosis capacities of blood MDM in SSc, our study offers new pathogenesis considerations for the involvement of macrophages in the autoimmune processes driving this disorder.

INTRODUCTION:

Systemic sclerosis (SSc) or scleroderma is a rare chronic autoimmune disorder involving vascular hyper-reactivity, early inflammation and fibrotic manifestations, especially of the skin and lungs[1]. SSc is also characterized by the presence of autoantibodies targeting intra-nuclear components (anti-nuclear antibodies/ANA). Specific SSc-ANA especially target centromere proteins (anti-centromere antibodies/ACA), topo-isomerase I (anti-Topo I antibodies/ATA) and RNA-polymerase III (anti-RNA pol-III). Although the pathogenic links between autoimmunity and fibrosis in SSc are still unclear, it has been recently highlighted that immune-complex (IC) containing intra-nuclear components and SSc specific ANA could trigger fibroblast activation and fibrosis in SSc[2].

The mechanisms leading to the release of intra-nuclear components with subsequent emergence of auto-antibodies and IC are still to be determined in SSc. Apoptotic cells could be a source of circulating nuclear antigens because, when they are not rapidly and/or efficiently cleared, they can switch from apoptosis to necrosis with subsequent release of their intracellular materials and triggering of pro-inflammatory signals. The clearance of apoptotic cells is a process called efferocytosis[3,4]. Macrophages (MΦ) are one of the key immune cells performing efferocytosis *in vivo* and are essential actors limiting autoimmunity and inflammation[5]. Hence, a deficiency of macrophagic efferocytosis has been found in several systemic autoimmune

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diseases, characterized by positive ANA, such as systemic lupus erythematosus (SLE)[6] and Sjögren syndrome[7]. A defective efferocytosis has also been reported in chronic inflammatory lung diseases[8] such as chronic obstructive pulmonary disease (COPD)[9] and fibrotic disorders such as idiopathic pulmonary fibrosis (IPF)[10]. Efferocytosis may therefore be at the crossroads of inflammation[11,12], auto-immunity[5,12] and fibrosis[10]. Membrane receptors expressed by MΦ are key actors of efferocytosis, and among them, scavenger receptors (SR) could be especially involved[4].

Nonetheless, so far, the efferocytosis capacities of MΦ from patients with SSc have never been studied and the expression of membrane receptors involved in efferocytosis is still to be determined in SSc MΦ. Therefore, the aim of the present study was to evaluate the efferocytosis capacities of MΦ from patients with SSc in comparison with healthy donors (HDs) and to identify key surface markers supposed to modulate engulfment of apoptotic cells in pathological conditions like SSc.

RESULTS AND DISCUSSION

Comparison of efferocytosis capacities of MDM from HD and patients with SSc

The process of efferocytosis was evaluated through the comparison of the mean PI for living Jurkat cells (J) with the mean PI for apoptotic Jurkat cells (apoJ), using the functional assay depicted in Supplementary Figure 1. The clinical characteristics of patients with SSc are reported in Supplementary Table 1. In unselected HD (n=31), the mean PI for apoJ (PI=35.9 $\pm$ 3.0) was significantly increased when compared to the mean PI for J (PI=13.8$\pm$1.9) ($P < 0.001$) demonstrating an efficient efferocytosis in HD MDM. On the contrary, in patients with SSc (n=21), the mean PI for apoJ (PI=15.3$\pm$3.0) was comparable to the mean PI for J (PI=8.0$\pm$2.2) ($P < 0.05$), highlighting an impaired efferocytosis in SSc MDM. Moreover, the mean PI for apoJ in SSc (PI=15.3$\pm$3.0) was significantly decreased when compared to the mean PI for apoJ in HD (PI=35.9 $\pm$ 3.0)($P < 0.001$) (Figure 1a). The same results on PIs were obtained when patients with SSc (n=14) and HD (n=14) were selected to be similar in terms of age and sex, excluding a direct influence of these parameters to explain the impaired efferocytosis in SSc patients (Figure 1b and Supplementary Table 2). In accordance with these results, the MFI ratio of CFSE+ MDM cells, representing MDM which engulfed Jurkat cells, was significantly decreased in SSc MDM when compared to HD MDM (Figure 1c).

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In order to characterize the heterogeneity in the efferocytosis capacities of SSc MDM observed on Figure 1a, we conducted sub-group analyses based on clinical and epidemiological characteristics in patients with SSc. We did not find any difference in PI based on age or sex considerations (Supplementary Figure 2). Similarly, the difference in efferocytosis capacities of MDM from SSc was not explained by any following characteristics of the disease: limited (lSSc) versus diffused (dSSc), presence of SSc associated interstitial lung disease (ILD), disease duration and autoantibodies status and treatment by immunosuppressive drugs (IS) or not (Supplementary Figure 2). Nonetheless the absence of difference in the PI among subgroups of patients with SSc might be explained by a lack of statistical power due to the limited sample size of groups defined by each disease characteristic, constituting therefore one limitation of our study.

As described in Methods, the only varying parameter in our experimental conditions was the origin of the MDM suggesting that the low efferocytosis capacities of SSc MDM was intrinsic to macrophages. As previously demonstrated by our group, this cannot be attribute to a defect of monocyte differentiation in SSc since the expression of CD206, specific to differentiated MΦ was similar between MDM from HD and SSc patients[13]. However, we cannot exclude an influence of sera components of SSc patients on monocytes before *in vitro* differentiation, even if MDM were maintained for at least 6 days in the same culture conditions as HD MDM.

Role of scavenger receptors SR-B1, SR-A1 and of ITGβ5 in efferocytosis capacities of MDM.

Therefore, we investigated to decipher the links between this decreased efferocytosis in SSc MDM and the analysis of engulfment receptor expressions on MDM. The "eat me" signals such as phosphatidylserine of apoptotic cells are recognized by receptors called "find-me" signals, which participate in their clearance. Among them, receptors like integrins, CD44, CD91 and receptors from the scavenger family (SR) such as SR-B1, SR-A1 and CD36 (SR-B2) play an important role. Because a defect or loss of these receptors may lead to a decreased efferocytosis, we compared their expression in MDM from HD and patients with SSc.

The membrane expressions of CD36 and CD44 expressed in ratio of MFI were significantly higher in MDM from patients with SSc when compared to MDM from HD whereas the percentages of positive cells for these two markers were similar in SSc and HD (Figure 2a and b). Therefore, this over-expression of CD36 and CD44 could not explain the altered efferocytosis

capacities in SSc. However, such CD44 overexpression were in accordance with previous data showing an increased expression of CD44 in skin MΦ from patients with SSc[14]. CD36 overexpression in SSc might have a role in the mechanism of fibrosis[15] since CD36 is involved in the transformation of latent to active form of TGF-beta1, a major actor in the development of lung fibrosis[16]. Our analysis of engulfment receptors also revealed that CD91 may not be involved in the observed defect, since its membrane expression was found similar in HD and SSc MDM (Figure 2c). Beyond these markers, we investigated two other scavenger receptors, SR-B1 and SR-A1, which were both down-regulated in SSc MDM in comparison with HD MDM. More precisely, the percentages of SR-A1 positive cells and its expression in MFI were significantly decreased in MDM from patients with SSc when compared to MDM from HD (Figure 2e), whereas only the percentages of SR-B1 positive cells was significantly reduced in MDM from patients with SSc when compared to MDM from HD (Figure 2d). In parallel, we demonstrated that both, the percentages of ITGβ5 positive cells and the expression of this integrin were significantly decreased in SSc when compared to HD (Figure 2f).

A siRNA approach was next used to validate a potential implication of SR-B1, SR-A1 and ITGβ5 in the altered efferocytosis capacities of SSc MDM. SR-B1, SR-A1 and ITGβ5 siRNAs significantly reduced the expression of their respective receptor in HD MDM (Figure 3 abc). The PI of SR-B1 and SR-A1 siRNA transfected cells was not significantly different to the PI of control siRNA transfected cells (Figure 3 ab). This result regarding SR-A1 in human MDM is concordant with previous data showing no impaired efferocytosis in SR-A1 knock-out mice[17]**.** Nonetheless, although the suppression of SR-B1 or SR-A1 separately did not seem to alter the process of efferocytosis in our work, the simultaneous and concomitant decrease of this two markers may have an impact on efferocytosis. We did not explore this hypothesis and this may constitute a limitation of our work.

By contrast, the PI was significantly reduced when ITGβ5 was repressed by siRNA in HD MDM (Figure 3c), suggesting that ITGβ5 down-expression in SSc could directly participate in the impairment of efferocytosis. Integrin-dependent efferocytosis is indirect and mediated by MFG-E8, a protein secreted by MΦ and dendritic cell, which acts as a link between "eat me" signals on apoptotic cells and integrins on phagocytic cells[18]. It was recently described a decrease of MFG-E8 in the serum of SSc patients and more severe fibrotic manifestations in mouse models of SSc knock-out for MFG-E8[19]. Interestingly, MFG-E8-knockout mice also present autoimmune features including an increased production of autoantibodies such as ANA[19,20].

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Therefore, these results in mouse models suggest that integrin-mediated efferocytosis by phagocytic cells might play a key role in the prevention of autoimmunity.

To additionally support the hypothesis of ITGβ5 implication in efferocytosis, we evaluated whether the putative modulation of ITGβ5 expression by macrophagic polarization may affect efferocytosis. Indeed, MΦ can adopt distinct phenotypes and activation states depending on the nature of the surrounding environmental signals. Macrophagic polarization states are commonly divided into two categories: the pro-inflammatory "classically activated MΦ" or M1 type and the "alternative activated MΦ" or M2 types[21]. Polarization can impact the expression of cell-surface receptors and is also supposed to modulate efferocytosis[12]. Moreover, polarization of MΦ is also disrupted in SSc[22–24]. We therefore tested the hypothesis that the variation of cell expression of ITGβ5 among *in vitro* polarized MDM subtypes from HD could also result in a variation of efferocytosis capacities among them. ITGβ5 expression was significantly decreased in M1 pro-inflammatory MDM in comparison with M0 unpolarized MDM and M2a alternatively activated MDM (Figure 4a). Similarly, the PI of MDM was significantly decreased in M1 HD MDM in comparison with M0 and M2a (Figure 4b), confirming some previous data[12]. This down-expression of ITGβ5 both in M1 HD MDM and in SSc support the recent results from our group and others, suggesting that blood monocytes and MDM from patients with SSc not only display pro-fibrotic properties but also share some characteristics with pro-inflammatory M1 MΦ[13,22,25].

From a pathogenic view point, the decreased efferocytosis capacities of MΦ may play a key role in the autoimmune processes associated with SSc and connective tissue diseases in general. Impaired efferocytosis is indeed a shared characteristic between SLE, Sjögren syndrome[6,7] and as suggested by our work, SSc. Interestingly, this reduced efferocytosis was more marked in our experiments in SSc than previously described for other autoimmune diseases like SLE[7,26]. These three connective tissue disorders are characterized by a common expression of autoantibodies targeting intra-nuclear components (ANA such as anti-ribonucleoprotein and anti-DNA antibodies in SLE, anti-SSA or SSB in SLE or Sjögren syndrome, and anti-centromere proteins, anti-topoisomerase I or anti-RNA polymerase III antibodies in SSc). The hypothesis of an increase of autoantigens leading to an excess of autoantibodies has been proposed to explain these ANA, especially in SLE[5,20]. Interestingly, apoptotic cells express at their membrane surface antigens that are normally expressed in the nucleus[27]. Therefore, the decreased efferocytosis capacities of MΦ in these connective tissue diseases may lead to an accumulation of apoptotic

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cells expressing nuclear components at their surface. Moreover, un-engulfed apoptotic cells may secondary undergo necrosis with release of intracellular and nuclear components that could also activate the immune system to produce autoantibodies[20]. It has also been recently highlighted that IC containing intra-nuclear components and SSc-specific ANA could trigger fibroblast activation and fibrosis in SSc[2]. Therefore, the observed defect in efferocytosis of MDM in SSc could participate in the pathogenesis of autoimmunity and fibrosis via an increase release of antigen with subsequent formation of IC. Although the role of IC has been largely studied in mice models of SLE, their relevance in mice models of SSc is still to be determined. Addressing this issue in the future might help to better understand the place of the inefficient efferocytosis of blood MDM in SSc.

METHODS

Subjects

Patients fulfilling the 2013 ACR/EULAR classification criteria for SSc[28] were consecutively included after written informed consent. Patients with overlapping syndrome with Sjögren or SLE were not included. Patients were also sub-classified in the sine scleroderma/limited cutaneous (lSSc) or diffused cutaneous (dSSc) subsets according to LeRoy classification[29]. The lSSc subset had skin thickening involving the extremities distal to the elbows and knees whereas the dSSc subset had proximal skin thickening. Interstitial Lung disease (ILD) was diagnosed by a senior radiologist in all patients based on the results of thoracic HRCT, according to current guidelines. SSc- ILD was defined as ground-glass opacities and reticular abnormalities, predominantly in lower lobes and subpleural area. The radiologist was blinded from the results of the efferocytosis assay. Blood buffy coats of HD were provided by Etablissement Français du Sang (Rennes, France) as controls after consent. All control donors included in this study answered a questionnaire evaluating the presence of any pathologic condition (acute or chronic); donors therefore had to be healthy to be included as HD. This study was approved by the local ethics committees (Committees for protection of persons (CPP) Ouest-V France, CPP approval N°: 2015-A01221-48; study N°.15/26-988).

Preparation of monocyte-derived MΦ (MDM)

In order to properly compare the function of MDM, the cultures of HD and SSc MDM were always performed in the same experimental conditions. As previously described[30], peripheral blood mononuclear cells were obtained from blood buffy coats of HD or from blood of SSc patients through Ficoll gradient centrifugation. Monocytes, selected after a 1-h adhesion step,

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were differentiated into MΦ for 6 days using M-CSF (50 ng ml^{-1}) (Miltenyi Biotec SAS, Paris, France) in RPMI 1640 medium GlutaMAX (Gibco, Life technologies SAS, Courtaboeuf, France) supplemented with antibiotics and the same 10% heat-inactivated fetal bovine serum (FBS, Lonza, Levallois-Perret, France) to inactivate complement which can interfere with phagocytosis of apoptotic cells.

Polarization of macrophages

After 6-7 days of differentiation, MDM were placed in medium with 5% of heat-inactivated FBS in the presence of M-CSF (10 ng ml^{-1}); this first state of differentiation corresponded to unpolarized MΦ (or M0), presented in all experiments until Figure 4. For polarization (Figure 4), MDM were activated for additional 24 h by the addition of 20 ng ml^{-1} of IFNɣ and 20 ng ml^{-1} of LPS (M1 type) or by the addition of 20 ng ml^{-1} of IL-4 and 20 ng ml^{-1} of IL-13 (M2a type)[30]. This method of *in vitro* polarization has been previously validated by our team in this model of MDM[13]. Human recombinant cytokines IFNɣ, IL-4, IL-13 were purchased from Peprotech (Neuilly sur Seine, France) and LPS from E.coli (serotype: 055:B5) from Sigma-Aldrich (St-Quentin Fallavier, France).

Induction of apoptosis in Jurkat cells

The human Jurkat cells CD4 T lymphocyte cell line was cultured in RPMI 1640 Glutamax culture medium supplemented with 10 % of heat-inactivated FBS and antibiotics. Apoptotic cells were generated through the exposure of Jurkat cells (1.10^6 ml^{-1}) to 10 µM of camptothecin for 4 h at 37°C in 5% CO_2 humidified incubator. Cells were then washed and early apoptosis was confirmed with FITC-Annexin V/iodide propidium (IP) staining using flow cytometry. Camptothecin and IP were purchased from Sigma-Aldrich. FITC-Annexin V was purchased from BD Biosciences (Le Pont de Claix, France).

Phagocytosis assay

Jurkat cells (1.10^6 cells ml^{-1}) were stained for 15 min with 100 ng ml^{-1} CellTrace™CFSE (Invitrogen, ThermoFisher scientific, Courtaboeuf, France), washed and then exposed to campothecin to induce apoptosis. CFSE-stained apoptotic and non-apoptotic Jurkat cells were then added to MDM plated in 12-well tissue culture plates, in 10:1 ratio (Jurkat cells/ MΦ) for 90 min at 37°C. After co-culture, Jurkat cells were removed, MΦ were washed at least 6 times with phosphate-buffered saline (PBS) and were then detached using Accutase™ (BioLegend, Paris, France). The staining of Jurkat cells with CD3 antibody was used to exclude MΦ with

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unengulfed lymphocytes bound to their surface. Engulfment efficiency was measured by flow cytometry on a LSRII cytometer with FACSDiva software (BD Biosciences, San Jose, CA, USA). The phagocytic index (PI) was calculated as follows: (number of $CFSE^{Pos}$ /$CD3^{neg}$ MΦ /number of total MΦ) x100 (supplementary Figure 1). To be sure that the variations of efferocytosis capacities were not related to experimental procedures, the preparation of apoptotic Jurkat cells was performed at the same time and in the same conditions before their co-culture with MDM from HD and patients with SSc.

Cell surface marker analyses by Flow cytometry

After detachment from plastic support, MDM were first blocked in PBS supplemented with 2% FBS and with Fc block (Miltenyi Biotec SAS) for 10 min at room temperature to avoid nonspecific binding, and then re-suspended and incubated with specific antibodies or appropriate isotype controls for 30 min at 4°C. Surface markers were evaluated using the following antibodies: PE anti-SR-A1 (from R&DSystems, Abington, UK), PeVio770 anti-CD36, vioBright FITC anti-CD91, vioBlue anti-CD44 and vioBright FITC anti-integrin β5 and APC anti-SR-B1 (Miltenyi Biotec SAS). Data were analyzed on a LSR II cytometer and FACSDiva software. Results were expressed as percentage of positive cells or as the ratio of mean of fluorescence intensity (MFI) calculated as follows: mean fluorescence (antibody of interest)/mean fluorescence (antibody of isotype control).

Transfection of siRNA

SMARTpool of individual siRNAs directed against human SCARB1 (SR-B1) (L-010592-00-0005), human MSR1 (SR-A1) (L-008035-00-0005), human ITGβ5 (L-004125-00-0005) and a non-targeting pool (siRNA Ct), used as control, were purchased from Dharmacon (GE Healthcare Europe GmbH-FR, Velizy-Villacoublay, France). Human MΦ from HD were transfected using Lipofectamine RNAiMax reagent (Invitrogen) with siRNA SR-B1 (5 pmol) for 24h and siRNA SR-A1 or ITGβ5 (25 pmol) for 48h. Efficient silencing of targeted markers was confirmed by flow cytometry-based immune-labelling using appropriate antibodies. MΦ were then exposed to Jurkat cells for efferocytosis assays.

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Statistical analyses

Data are presented as means ± standard error on the mean (SEM). Differences were evaluated using *t*-test or one-way ANOVA followed by Newman-Keuls multiple comparison post-hoc tests, as appropriate. Statistical significance was considered at $P < 0.05$. Data analyses were performed with GraphPad Prism 5.03 (GraphPad Software, San Diego, USA).

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CONFLICT OF INTEREST:

A.Ballerie, A. Lescoat, Y. Augagneur, M. Lelong, C. Morzadec, C. Cazalets, S. Jouneau, O. Fardel, L. Vernhet and V. Lecureur declare no conflict of interest. P.Jégo received speaking fees from Actelion Pharmaceuticals Ltd. and Bayer (< 10 000 € each) outside of the current study.

SUPPLEMENTARY INFORMATION

Supplementary information is available at *Immunology & Cell Biology*'s website

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FIGURES LEGENDS

Figure 1 Flow cytometry-based efferocytosis assay showing decreased efferocytosis capacities of MDM from patients with SSc in comparison with MDM from healthy donors (HD).

Unpolarized MDM (M0) from HD or patients with SSc were exposed to living Jurkat cells (J) or to apoptotic Jurkat cells (apoJ) for 90 minutes in the same conditions. Efferocytosis was evaluated by flow cytometry as detailed in Methods. The efferocytosis capacities were assessed by the Phagocytic Index (PI) for apoJ in HD and SSc MDM. **(a)** The PI of each samples and the mean of PI $\pm$ SEM of unselected HD (n=31) and patients with SSc (n=21) are represented. **(b)** The PI of each samples and the mean of PI $\pm$ SEM of HD (n=14) and SSc patients (n=14) after matching on age and sex are also presented. **(c)** The engulfment of CFSE+ Jurkat cells by MDM was also expressed as MFI relative to CFSE- Jurkat (ratio) $\pm$ SEM of the same HD (n=14) and SSc patients (n=14) as (b). ANOVA followed by Newman-Keuls' multiple comparison test, ** $P < 0.01$, *** $P < 0.001$; ns: not significant.

Figure 2 Expression of cell surface molecules classically involved in macrophagic efferocytosis in MDM from HD and from patients with SSc

Primary human monocytes from HD and patients with SSc were differentiated into MDM *in vitro* in the presence of M-CSF for 6 days. MDM were then stained and the expressions of **(a)** CD36, **(b)** CD44 and **(c)** CD91 **(d)** SR-B1, **(d)** SR-A1 and **(f)** ITGβ5 were analyzed by flow cytometry as detailed in Methods. Data are expressed as mean fluorescence intensity (MFI) relative to isotype control (ratio) $\pm$ SEM and % $\pm$ SEM of positive cells, from 9 to 14 HD (a-f) and 10 patients with SSc in (a-e), and 6 patients with SSc in (f). Student unpaired *t*-test, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. ns: not significant.

Figure 3 Role of scavenger receptors SR-B1 and SR-A1 and of ITGβ5 in efferocytosis capacities of human MDM.

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Primary human monocytes from HD were differentiated into MDM *in vitro* in the presence of M-CSF for 6 days and were next lipotransfected as described in Methods. The cell surface expression of **(a)** SR-B1, **(b)** SR-A1 and **(c)** ITGβ5, down-regulated using siRNA transfection, was analyzed by flow cytometry. Impact of SR-B1, SR-A1 and ITGβ5 down-expression on efferocytosis was evaluated **(a)** 24h or **(b and c)** 48h after lipotransfection. MDM from HD were exposed to apoptotic Jurkat (apoJ) for 90 minutes. The capacities of efferocytosis, represented by the Phagoctytic Index (PI) for apoJ, were evaluated with or without siRNA-mediated down-regulation of each receptor. The PI of each sample and the mean of PI ± SEM of 6 HD are represented in (a) and 7 HD in (b) and (c). Student paired *t*-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns: not significant.

Figure 4 Impact of the modulation of ITGβ5 expression by macrophagic polarization on efferocytosis capacities in polarized human MDM.

Primary human monocytes from HD were differentiated into MDM *in vitro* in the presence of M-CSF for 6 days. This first state of differentiation corresponded to unpolarized macrophages (or M0). For *in vitro* polarization, MDM were secondly activated for additional 24 h by the addition of 20 ng ml^{-1} of IFNɣ and 20 ng ml^{-1} of LPS (M1 type), by 20 ng ml^{-1} of IL-4 and 20 ng ml^{-1} of IL-13 (M2a type). **(a)** MDM were then harvested, stained and the expression of ITGβ5 was analyzed by flow cytometry as described in Methods. Data are expressed as mean fluorescence intensity (MFI) relative to isotype control (ratio) ± SEM of 7 independent experiments. ANOVA followed by Newman-Keuls' multiple comparison test, ** $P < 0.01$; *** $P < 0.001$. **(b)** Efferocytosis capacities of unpolarized MΦ (M0), M1 and M2a MDM were evaluated as described in Methods. M0 and polarized MΦ were exposed to living Jurkat cells (J) or to apoptotic Jurkat cells (apoJ) for 90 minutes. The phagocytosis index (PI) of each sample and the mean of PI ± SEM of 6 HD are represented. ANOVA followed by Newman-Keuls' multiple comparison test, *** $P < 0.001$; ns: not significant.

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FIGURE 1

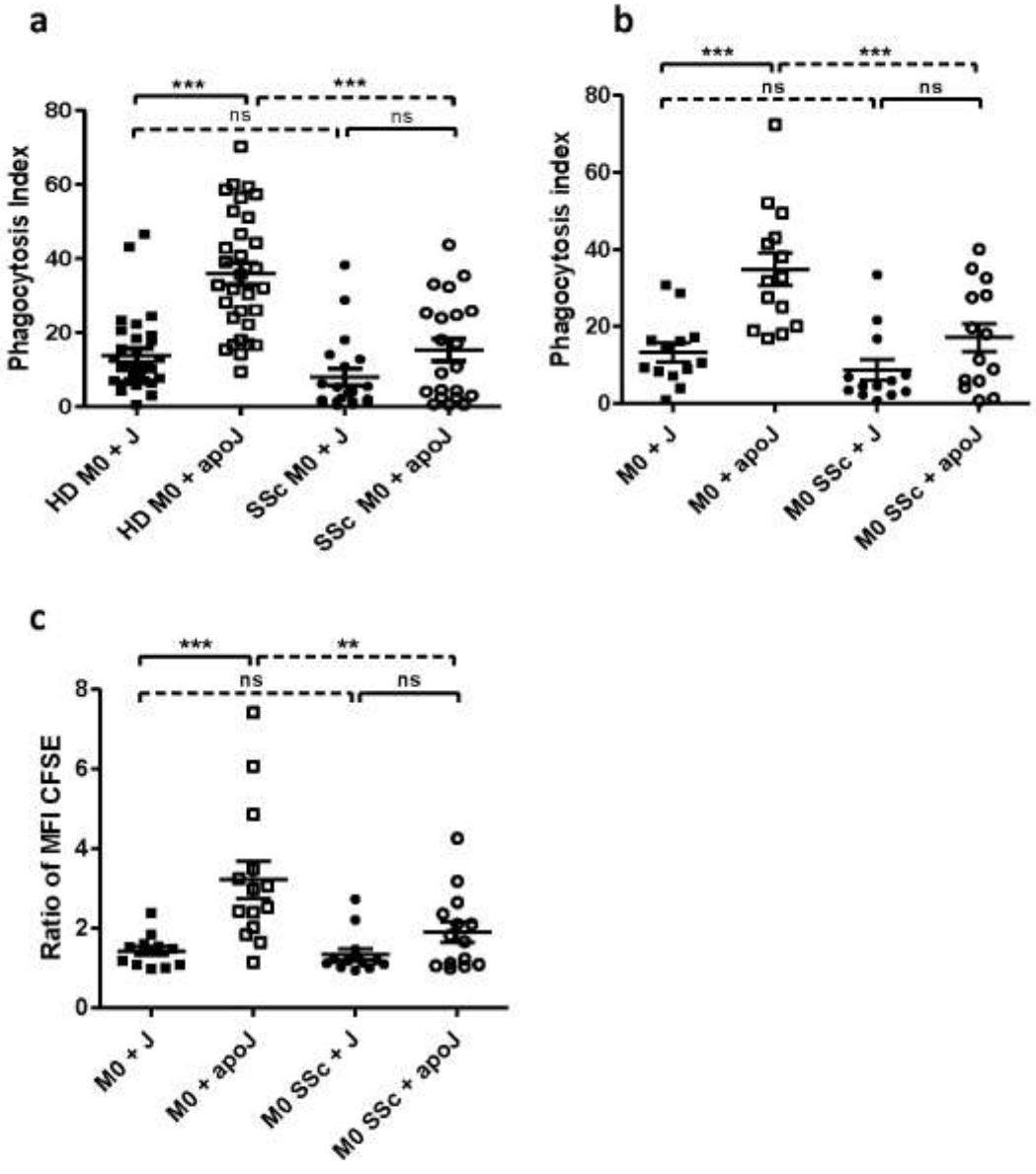

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FIGURE 2

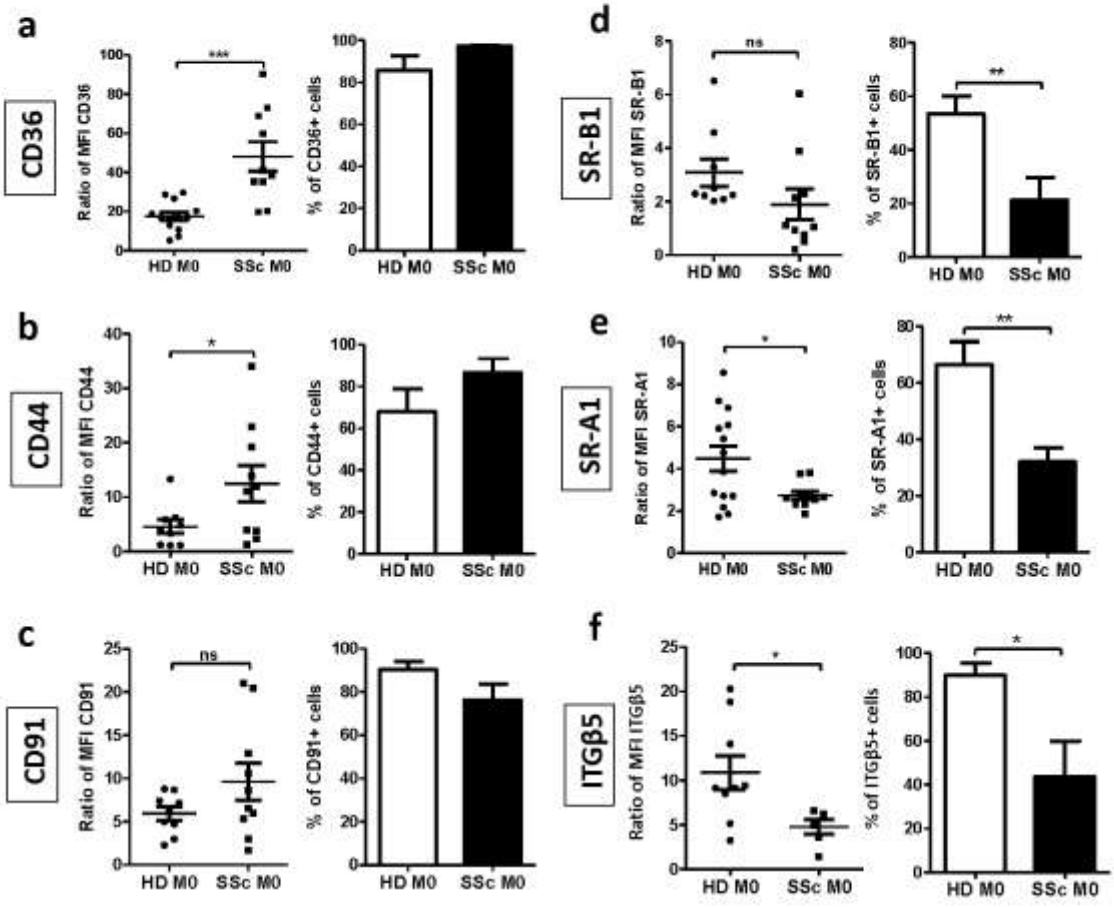

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FIGURE 3

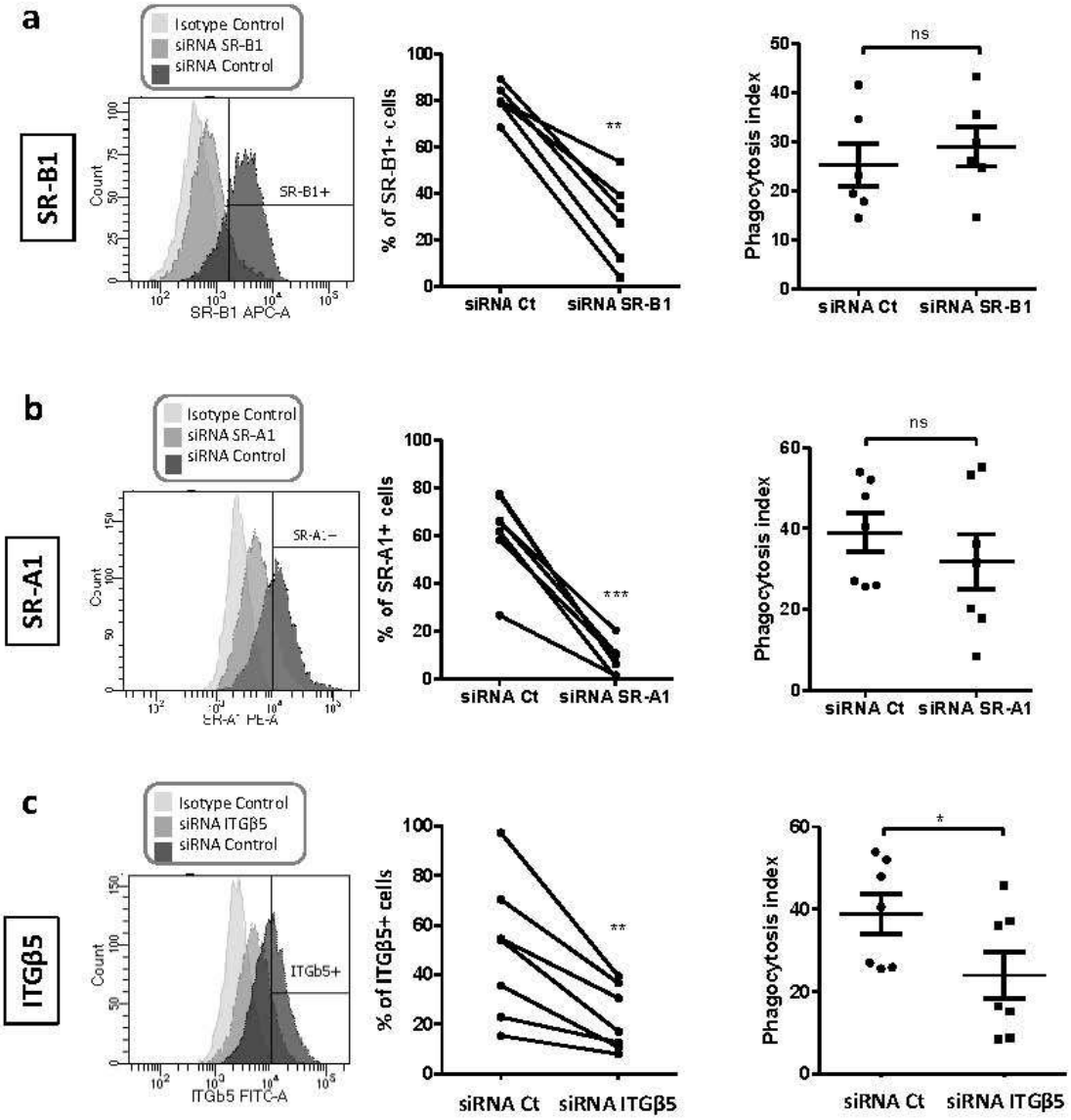

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FIGURE 4