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Review

Fibrosis and immune dysregulation in systemic sclerosis

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ABSTRACT

Autoimmune and inflammatory phenomena are characteristically present in systemic sclerosis (SSc) and impact on dysregulated fibroblast extracellular matrix deposition, hallmark of the disease in conjunction with fibroproliferative vasculopathy. Oligoclonal T helper 2-like cells are present in the skin and peripheral blood in early diffuse disease. Type 2 cytokines synergize with profibrotic cytokines including transforming growth factor beta, favoring collagen deposition and metalloproteinase inhibition by fibroblasts. Furthermore, chemokine with pro-fibrotic and pro-angiogenic properties are preferentially produced by fibroblasts under the influence of Th2-like cells. The profibrotic monocyte chemotactic protein 1 is also produced by fibroblasts, partially in response to Toll-like receptor 4 (TLR4) recognition, when autoantibodies (autoAb) bind to fibroblast surface. In addition, immune-complex formed by autoAb and ubiquitous antigens including topoisomerase-1 favor the production of interferon-alpha (IFN-α) possibly by interacting with intravesicular TLRs. Consistent with this findings, unbiased gene screening has revealed that SSc peripheral blood cells express genes induced by IFN-α, a characteristic shared with systemic lupus erythematosus and other autoimmune disorders. These findings highlight the complex relationship between adaptive and acquired immune responses, which may participate to the pathogenesis of SSc in manners until now unsuspected, which may help in identifying novel therapeutic targets.

Contents

1. Introduction

Fibrosis is a complex tissue response to various pathological events predominantly characterized by excessive deposition of extracellular matrix (ECM), especially collagen. Excessive ECM deposition results in altered tissue and organ architecture that lead to dysfunction and ultimately pathology. In most circumstances fibrosis may be seen as a process aiming at repair tissue damage, which for various poorly understood reasons is not appropriately controlled and terminated [1]. The role of inflammation in initiating or maintaining the processes resulting in fibrosis is very likely. Indeed, an inflammatory infiltrate is characteristic of the early phases of fibrosis development and inflammatory cells may profoundly affect the ECM production by releasing soluble products or by direct cell-to-cell interactions that modify fibroblast metabolism [2,3].

Systemic sclerosis (SSc) is a disease of unknown origin characterized by fibrosis of the skin and internal organs associated with diffuse fibroproliferative microangiopathy and the presence of autoantibodies (autoAb). An interplay between altered endothelial cells, immune cells and their soluble mediators resulting in characteristic modifications of fibroblast functional phenotype is thought to play a pathogenic role [4,5]. Furthermore complex genetic traits very likely contribute to SSc pathogenesis under the influence of environmental agents [6,7].

Fibroblasts are capable of both synthesis and degradation. ECM degradation is achieved mostly via specific enzymes of which matrix metalloproteinases (MMP) are of major importance. In SSc and other fibrotic conditions excess of synthesis over degradation is thought to take place [8]. Among the many characteristics that distinguish SSc fibroblasts from normal fibroblasts, are: higher collagen synthesis [9], higher TGF-β, connective tissue growth factor (CTGF), interleukin (IL)-6, endothelin-1, plasminogen activator inhibitor-1, and monocyte chemotactic protein-1 (MCP-1) production [10–14], resistance to IFN-γ [15] and to inhibitory signals delivered by T cell contact [15,16],

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altered TGF-β receptor and Smad signaling [17–22], enhanced expression of p300 coactivator of transcription [23], altered responses to endothelin-1 [10], constitutive thrombospondin expression which amplify TGF-β signaling [24].

Cytokines play a major role in regulating ECM deposition by fibroblasts [25]. Among profibrotic cytokines, TGF-β is thought to play a pivotal role as well as CTGF, a member of the CCN family of matricellular proteins [26,27]. Additional pro-fibrotic cytokines are platelet-derived growth factor (PDGF), epithelial growth factor, basic fibroblastic growth factor, TGF-α upon binding to the EGF-receptor [28]. *In vitro*, tumor necrosis factor (TNF)-α inhibits collagen production by inhibiting the transcription of type I and type III procollagen mRNA [29,30], by inhibiting TGF-β signaling in human fibroblasts via activator protein-1 (AP-1) activation [31], and by down-regulating TGF-β RII [32]. From a polarized T cell point of view, it is interesting to note that IFN-γ inhibits [33], while IL-4 and IL-13 enhance collagen synthesis [34–36].

In this review we will highlight evidence indicating that the immune response, and in particular the adaptive immune response, may participate in initiating and/or maintaining fibroblast modifications that are characteristically observed in SSc, and therefore it could participate to and/or control fibrosis development.

T cells in SSc and fibrosis. Histological examination of early SSc-skin lesions has demonstrated that an inflammatory infiltrate precedes fibrosis and development of the vasculopathy, including ultrastructural changes affecting endothelial cells [37]. Of interest, collagen synthesis determined by in situ localization of procollagen Iα(1) appears to be higher in fibroblasts adjacent to inflammatory cells [38,39]. These findings led to the hypothesis that inflammatory cells and in particular T cells provide important stimuli that drive collagen synthesis in fibroblasts. Transcriptome analysis in animal models has shown that genes involved in wound healing and fibrosis are associated to Th2 polarized responses, characterized by the production of IL-4, IL-5, IL-13, and IL-21 as opposed to Th1 polarized responses characterized by IFN-γ production [40,41]. Considerable evidence indicates that indeed type 2 polarized responses are important for fibrosis development [42,43]. In SSc, T cells infiltrating the skin and other organs are functionally heterogeneous. Some reports indicate recruitment of polarized T cells preferentially producing IFN-γ and therefore belonging to the Th1-like subset [44,45]; and other reports, particularly the most recent, point to a preferential accumulation of T cells producing high levels of IL-4, belonging to the Th2-like subset [46–53]. Since, Th2 cytokines directly favor collagen production by fibroblasts, there is good correlation between the presence of Th2-like cells and fibrosis. It is however unknown what drives the Th2 differentiation in SSc. An additional point of interest is the demonstration of a restricted usage of T cell receptor (TCR), indicating antigen-driven T cell expansion in SSc skin and lung [51,54–56]. However, no recent progress has been made in identifying the responsible antigen(s). Finally, two reports indicate that IL-17 is increased in SSc serum and in the bronchoalveolar lavage fluid of SSc individuals with lung involvement thus spurring interest on the role of Th17 cells in SSc [57–59]. IL-17A has been reported to induce IL-6 and IL-8 production and ICAM-1 expression in human fibroblasts [60] and IL-17F is known to induce endothelial cells to produce the pro-fibrotic growth factor TGF-β [59]. As to now, a single report awaiting confirmation has shown that Th17 are increased in the peripheral blood of SSc individuals [61].

We have conducted studies aimed at investigating the regulatory mechanisms exerted by T cells on ECM metabolism of skin fibroblasts obtained from involved skin of SSc patients or normal individuals. We have observed that cell membranes of activated Th1 and Th2 cells inhibit collagen I production. The molecular mechanisms are however different since IFN-γ neutralization reversed type 1 (Th1)-mediated inhibition, while Th2 cell-dependent inhibition was essentially mediated by TNF-α. T cell contact inhibition was dominant over profibrotic IL-4 and TGF-β cytokines and was specific for collagen I, since mRNA levels of COL1A1 were decreased while mRNA levels of MMP-1 were strongly increased [15,16]. However, SSc fibroblasts were not inhibited by Th2 cells and Th1 cells were more potent inhibitors of collagen synthesis than Th2 cells [16]. An intriguing observation while characterizing T cells infiltrating the skin of early SSc was that some of the α/β TcR + T cells expressed simultaneously the lineage-specific markers CD4 and CD8, usually mutually exclusive in peripheral compartments. In double-positive (DP) T cells the CD8 molecule was formed by the classical alpha-beta heterodimer. DP T cells actively transcribed both accessory molecules, were endowed with clonally distributed cytolytic and helper activity and expressed TcR clonotypes distinct from CD4+ and CD8+ single positive (SP) T cells. In SSc, DP compared to CD4+ SP T cells produced very high levels of IL-4. Furthermore, DP T cells were directly identified in SSc skin, thus arguing for the existence of DP T cells as a distinct subset *in vivo* [51]. The preferential high production of IL-4 by CD8+ and CD4 + CD8+ DP skin-homing T cells may indeed have a pathogenic potential since, as mentioned here above, IL-4 enhance collagen deposition and fibroblast proliferation by itself and has synergistic activities with TGF-β (Chizzolini et al. unpublished).

In partial agreement with our findings, others have identified in the peripheral blood of SSc individuals CD8+ T cells producing high levels of IL-13 also considered as an important pro-fibrotic cytokine [62]. In this respect, it is interesting to note that a significant association between the IL-13 receptor IL-13Rα2 gene polymorphisms and SSc has been found and may constitute an indirect evidence of the importance of IL-13 in the disease [63]. Indeed, this receptor might act as a decoy receptor since mice deficient in IL-13Rα2 show a phenotype of enhanced IL-13 responsiveness [64]. Of interest, the level of Th2 cytokines tends to decrease as skin sclerosis regresses and one study suggests that a shift from Th2 to Th1 response correlates with improvement in skin fibrosis in SSc [65,66].

We also explored the capacity of dermal fibroblasts to produce inflammatory chemokines, potentially involved in fibrosis, in response to contact with polarized human T cells. Th1 cells preferentially induced the production of CXCL10/IP-10 and Th2 cells the production of CXCL8/IL-8, while CCL2/MCP-1 was equally induced by both subsets. Of interest, Th1 and Th2 cells induced different signaling pathways in fibroblasts [67]. These results indicate that fibroblasts have the potential to participate in shaping the inflammatory response through the activation of flexible programs of chemokine production that depend on the T helper subset eliciting their response [67]. It is also important to stress that IP-10 and IL-8 may have opposite roles in fibrosis, IP-10 being anti-angiogenic and anti-fibrotic, while IL-8 possessing pro-angiogenic and pro-fibrotic properties [68]. MCP-1 a CC chemokine that binds to CCR2, has attracted keen interest in the fibrosis field since it appears to have direct roles on collagen and MMP-1 production in fibroblasts and is present at sites undergoing fibrosis [69–71]. In SSc, MCP-1 mRNA has proven to be the most abundant mRNA when broncho-alveolar lavage (BAL) cells from SSc-lung were compared to controls using microarray technology and testing a total of 4507 genes [50]. Moreover, it is produced in large amount by SSc skin fibroblasts [72]. Of interest, IL-4 triggers MCP-1 production by human lung fibroblasts [17], and MCP-1 may polarize T cells toward Th2 subset in the mouse [73]. In a rodent model of fibrotic versus nonfibrotic pulmonary granulomas, procollagen production was associated with Th2 cells and MCP-1 production [74]. Furthermore, CCR2−/− mice were resistant to development of lung fibrosis induced by transgenic IL-13 [75] and bleomycin [76].

In summary, polarized T cells have distinct roles in respect to their influence on the metabolisms of fibroblasts (Fig. 1). Th1 cells inhibit collagen deposition and enhance MMP production directly by releasing IFN-γ and/or by direct cell-contact with fibroblasts, while Th2 enhance collagen deposition and inhibit MMP by releasing IL-4 and IL-13. Furthermore, chemokines produced by fibroblasts in

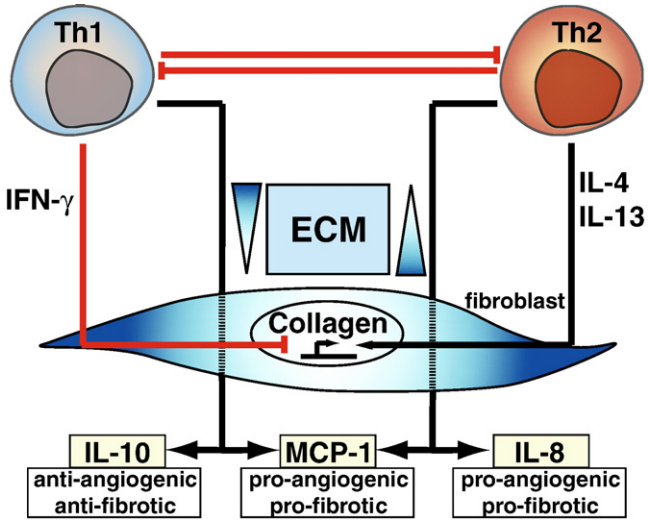

Fig. 1. T cells interaction with fibroblasts in SSc. Schematic representation of the influence of polarized T cells and their products on fibroblasts for their capacity to depose or digest extracellular matrix (ECM) and chemokines with relevant fibrotic properties. Arrows indicate stimulation, and blunted ends inhibition.

response to Th1 cells have anti-angiogenic and anti-fibrotic potential, while those induced by Th2 cells have pro-fibrotic and pro-angiogenic properties. However, both T helper cell subsets induce MCP-1, which plays pivotal roles in fibrotic processes (Fig. 1).

Autoantibodies (autoAb) in SSc and fibrosis. The presence of autoAb since long has been used to identify individuals affected by SSc and overlapping conditions. AutoAb directed against distinct ubiquitous autoantigens (centromeric protein B, Scl-70, RNA-polymerase III, etc.) segregate with specific clinical subsets. While the titer of Scl-70 may correlate with clinical severity and B cell epitopes of Scl-70 correlate with clinical manifestations [77,78], their contribution to pathogenesis remains elusive. Additional autoAb, not directed against ubiquitous autoantigens, with possible implication in SSc pathogenesis have recently been identified. They include: antibodies directed against MMP1 and MMP3, which appear to inhibit the enzymatic activities of these enzymes [79,80]. Antibodies against the collagen-specific molecular chaperone heat-shock protein 47 (HSP47), which point to fibroblasts as target of autoimmunity [81]. In addition, autoAb anti-fibrillin-1, a matrix protein with primary architectural function, have been detected in the sera of SSc patients of various geographical origin [82,83], in other pathologies such mixed connective tissue disease (MCTD), primary pulmonary hypertension [84,85], and in tight skin-1 mouse (TSK1/+) sera [86]. Anti-fibrillin-1 autoAb were shown to activate fibroblasts and induce collagen synthesis, likely by enhancing the biological activity of TGF-β bound to fibrillin-1 [87]. By immunoscreening of a HepG2 cDNA library, a novel protein overexpressed in SSc fibroblasts and targeted by autoAb has been identified as PHET (protein highly expressed in testis). Its localization appears to be primarily cytoplasmatic [88]. IgG of SSc individuals with pulmonary artery hypertension (PAH) were reported to recognize fibroblast components distinct from those with primary PAH [89]. Other authors found that anti-fibroblast antibodies specifically recognized Scl-70 expressed on the surface of fibroblasts and not of endothelial cells [90,91]. In addition, autoAb binding to the adhesion molecule NAG-2 expressed in endothelial cells and fibroblasts were described in SSc. They are capable of inducing apoptotic cell death in endothelial cells while activating fibroblasts to produce profibrotic cytokines and collagen [92,93]. Furthermore, autoAb reacting with PDGF receptor expressed on fibroblasts and resulting in their activation with increased collagen synthesis were described in SSc and chronic graft versus host disease (GVHD) [94,95]. While PDGF-R specific antibodies have attracted enormous interest, confirmation of their presence in SSc by independent laboratories is missing [96,97]. Overall these findings indicate that humoral components of the immune response may be involved in the pathogenesis of SSc to an extent that was previously unthought-of and justify further investigations.

We have undertaken the functional characterization of autoAb present in the sera of SSc patients and directed against the surface of fibroblasts (AFA). Operationally, we defined AFA positive (AFA + IgG) the IgG purified from SSc patients sera when binding to fibroblasts was observed in a fibroblast-based cell ELISA, and AFA negative (AFA-IgG) when it was not. AFA + IgG were detected in 20 to 40 % of tested SSc individuals and proved capable of activating dermal fibroblasts. They upregulated ICAM-1 expression, enhanced IL-6 production, and IL-1 mRNA levels. These seminal data indicate that autoAb reacting with fibroblast surface molecules act *in vitro* as an extrinsic stimulus inducing a pro-inflammatory and pro-adhesive fibroblast phenotype [98]. We have recently found that fibroblasts stimulated with AFA positive but not with AFA negative and control IgG showed an increased capacity to digest collagen matrix and produce metalloproteinase-1 (MMP-1) while their production of total collagen, type I collagen, and tissue inhibitor of metalloproteinase-1 (TIMP-1) was unaffected [99]. In addition, AFA-positive IgG induced in human fibroblasts the preferential transcription and protein production of chemokines with pro-fibrotic and pro-angiogenic potential including CCL2/MCP-1 and CXCL8/IL-8. AFA binding to fibroblasts resulted in concomitant activation of ERK 1/2, c-Jun, and NF-κB. CCL2 production was sensitive to both proteasome and JNK inhibition, while CXCL8 was only sensitive to proteasome inhibition [100]. In the quest of the antigenic target recognized by AFA, we have taken the approach of testing the capacity of AFA to activate murine embryonal fibroblast genetically modified in order to express or not several members of the Toll-like receptor (TLR) family. AFA induced MCP-1/CCL2 mRNA in wild type, TLR-$2^{-/-}$, and TLR-$6^{-/-}$ but not in TLR-$4^{-/-}$ fibroblasts. The response to TNF used as positive control was similar in all fibroblasts. In addition, we performed experiments using blocking monoclonal antibodies (mAb) directed against human TLR4 and TLR2 [101,102]. In human dermal fibroblasts the production of MCP-1/CCL-2 in response to AFA was consistently reduced by 40% when using anti-TLR4 but not anti-TLR2 mAb, nor a control antibody, nor an LPS antagonist. These data are in agreement with those obtained using TLR$4^{-/-}$ fibroblasts and indicate that indeed AFA-positive IgG activate fibroblasts, at least in part, via TLR4. TLR4 senses LPS and LPS contamination of IgG preparations may explain our findings. This is however unlikely for several reasons. First, wild type fibroblasts cultured in the presence of polymyxin B, which binds LPS hindering its interaction with the receptor, responded normally to AFA-positive IgG. Second, LPS antagonist blocked fibroblast responses to LPS to the same extent as anti-TLR4 mAb but did not affect responses to AFA. All together our results indicate that TLR4 expression is required and mediates, at least in part, fibroblast responses to AFA-positive IgG, with no role for LPS. It should be noted that the TLR-deficient fibroblasts we used were of murine origin and that the extracellular domain of TLR4 is polymorphic between mice and humans [103]. In addition, anti-TLR4 mAb blocked the response induced by AFA in human fibroblasts by only 40%. Thus, several, not mutually exclusive, hypotheses may be raised to explain our findings. First, it may be possible that AFA recognize a cross-reactive TLR4 epitope shared between mice and humans. Second, AFA may induce an endogenous ligand that secondarily binds to TLR4 and activate fibroblasts. In this respect, the importance of endogenous ligands that interact with TLRs is increasingly recognized in the development of autoimmune diseases [104,105]. For instance, a recent paper has identified hyaluronan as an endogenous ligand binding to TLR4 and involved in the development of fibrosis and immunological abnormalities in bleomycin-induced mouse scleroderma [106]. Third, TLR4 may be one target of the targets of AFA, which may explain the partial inhibitory activity of blocking anti-TLR4 mAb. Finally, it should be added that beside our description of AFA + IgG interacting with TLR4 in

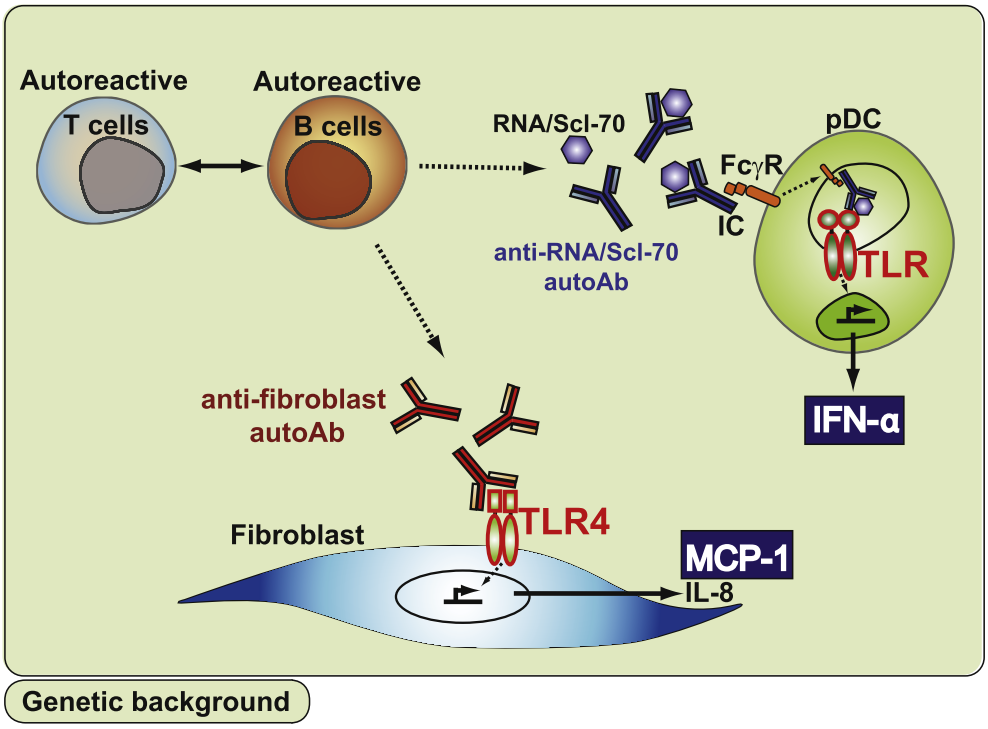

Fig. 2. Interplay between innate and acquired immune responses within the framework of SSc. The bidirectional arrows indicate probable interactions between cells. Red autoAb interacting with TLR4 and inducing the production of IL-8 and MCP-1 by fibroblasts. Blue autoAb forming immune-complexes with RNA/Scl-70-topoisomerase-1 are captured by plasmacytoid dendritic cells (pDC) via the Fcgamma receptor, interact with endovesicular TLR and induce the production of IFN-α. Dashed area: predisposing genetic background.

SSc, autoAb targeting receptors for pathogens (PRR) belonging to the innate immune system have been identified in other autoimmune pathologies including the anti-phospholipid syndrome (APS) and celiac disease [107–110]. Thus, PRR may be preferential targets of autoAb possibly because their intrinsic association with the delivery of danger signals to the adaptive immune system.

SSc and the interferon signature. Using microarrays, several studies have investigated the transcript profiles of SSc peripheral blood (PB) cells or their subpopulations. Tan et al. first reported that, compared with PB cells from controls, PB cells from patients with early SSc have a distinct transcript pattern that includes dysregulation of IFN-inducible genes [111]. This observation was replicated by other investigators in PBMC [112], monocytes, and CD4 + T cells [113] and PB cells [114] from SSc patients. In addition, it has been recently found that SSc and SLE share a substantial number of hyper-expressed genes most of which are induced by IFN [115]. In particular, a subset of SSc patients shows a "lupus-like" high IFN-inducible gene expression pattern that correlates with the presence of anti-Scl70 and anti–U1 RNP antibodies. Consistently with these findings, it was previously shown that autoAb subsets in SSc sera differentially induced IFN-α [116]. IFN-α was produced by plasmacytoid dendritic cells and required uptake of immune complexes through FcγRII, endosomal transport, and the presence of RNA, presumably for interaction with TLR7 [116]. While a definitive proof of the involvement of TLR7 is lacking, these data highlight an important function of autoAb directed against ubiquitous antigens (i.e. Scl70) in activating cells belonging to the innate immune system. They provide additional evidence that the humoral adaptive response may play a pathogenic role in SSc, particularly since higher IFN-α induction was observed in the sera from patients with diffuse SSc than in those with limited SSc thus suggesting that IFN-α may contribute to tissue injury [116].

In conclusion, it is important to stress that receptors belonging to the innate immune system and devoted to the recognition of pathogen-associated molecular patterns (PAMP) appear to be involved in the pathogenesis of SSc at several levels. TLR sensing DNA or RNA concentrated in immune complexes in the endosomal compartment participate to the induction of IFN-α. Furthermore, antibodies directed against the surface of fibroblasts and recognizing surface expressed TLR4 induce fibroblasts to produce chemokines with roles in fibrosis development (Fig. 2). These findings emphasize the interplay between the innate and adaptive immune responses in SSc and provide new perspectives in identifying targets for therapeutic interventions.

Take-home messages

- T cells with preferential type 2 cytokine production are present early in SSc course and enhance extracellular matrix deposition
- Fibroblasts produce profibrotic chemokines when activated by Th2 cells and autoantibodies directed against fibroblast surface
- TLR4 is a target of antifibroblast antibodies (AFA)
- PBMC from SSc individuals express genes induced by interferon-alpha

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References

[1] Mutsaers SE, Bishop JE, McGrouther G, Laurent GJ. Mechanisms of tissue repair: from wound healing to fibrosis. Int J Biochem Cell Biol 1997;29:5–17.
[2] Chizzolini C. T lymphocyte and fibroblast interactions: the case of skin involvement in systemic sclerosis and other examples. Springer Semin Immunopathol 1999;21:431–50.
[3] Chizzolini C. Update on pathophysiology of scleroderma with special reference to immunoinflammatory events. Ann Med 2007;39:42–53.
[4] Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. J Clin Invest 2007;117:557–67.

[5] Jimenez SA, Derk CT. Following the molecular pathways toward an understanding of the pathogenesis of systemic sclerosis. Ann Intern Med 2004;140:37–50.
[6] Tan FK, Arnett FC. Genetic factors in the etiology of systemic sclerosis and Raynaud phenomenon. Curr Opin Rheumatol 2000;12:511–9.
[7] Mayes MD, Trojanowska M. Genetic factors in systemic sclerosis. Arthritis Res Ther 2007;9(Suppl 2):S5.
[8] Trojanowska M, LeRoy EC, Eckes B, Krieg T. Pathogenesis of fibrosis: type 1 collagen and the skin. J Mol Med 1998;76:266–74.
[9] Shi-Wen X, Denton CP, McWhirter A, Bou-Gharios G, Abraham DJ, du Bois RM, et al. Scleroderma lung fibroblasts exhibit elevated and dysregulated type I collagen biosynthesis. Arthritis Rheum 1997;40:1237–44.
[10] Shi-Wen X, Denton CP, Dashwood MR, Holmes AM, Bou-Gharios G, Pearson JD, et al. Fibroblast matrix gene expression and connective tissue remodeling: role of endothelin-1. J Invest Dermatol 2001;116:417–25.
[11] Eckes B, Hunzelmann N, Ziegler-Heitbrock HW, Urbanski A, Luger T, Krieg T, et al. Interleukin-6 expression by fibroblasts grown in three-dimensional gel cultures. FEBS Lett 1992;298:229–32.
[12] Yamamoto T, Eckes B, Krieg T. High expression and autoinduction of monocyte chemoattractant protein-1 in scleroderma fibroblasts. Eur J Immunol 2001;31: 2936–41.
[13] Kessler-Becker D, Smola S, Krieg T, Eckes B. High plasminogen activator inhibitor type 2 expression is a hallmark of scleroderma fibroblasts in vitro. Exp Dermatol 2004;13:708–14.
[14] Igarashi A, Nashiro K, Kikuchi K, Sato S, Ihn H, Grotendorst GR, et al. Significant correlation between connective tissue growth factor gene expression and skin sclerosis in tissue sections from patients with systemic sclerosis. J Invest Dermatol 1995;105:280–4.
[15] Chizzolini C, Rezzonico R, Ribbens C, Burger D, Wollheim FA, Dayer JM. Inhibition of type I collagen production by dermal fibroblasts upon contact with activated T cells: different sensitivity to inhibition between systemic sclerosis and control fibroblasts. Arthritis Rheum 1998;41:2039–47.
[16] Chizzolini C, Parel Y, De Luca C, Tyndall A, Akesson A, Scheja A, et al. Systemic sclerosis Th2 cells inhibit collagen production by dermal fibroblasts via membrane-associated tumor necrosis factor alpha. Arthritis Rheum 2003;48: 2593–604.
[17] Mori Y, Ishida W, Bhattacharyya S, Li Y, Platanias LC, Varga J. Selective inhibition of activin receptor-like kinase 5 signaling blocks profibrotic transforming growth factor beta responses in skin fibroblasts. Arthritis Rheum 2004;50:4008–21.
[18] Pannu J, Gore-Hyer E, Yamanaka M, Smith EA, Rubinchik S, Dong JY, et al. An increased transforming growth factor beta receptor type I:type II ratio contributes to elevated collagen protein synthesis that is resistant to inhibition via a kinase-deficient transforming growth factor beta receptor type II in scleroderma. Arthritis Rheum 2004;50:1566–77.
[19] Varga J. Scleroderma and Smads: dysfunctional Smad family dynamics culminating in fibrosis. Arthritis Rheum 2002;46:1703–13.
[20] Mori Y, Chen SJ, Varga J. Expression and regulation of intracellular SMAD signaling in scleroderma skin fibroblasts. Arthritis Rheum 2003;48:1964–78.
[21] Asano Y, Ihn H, Yamane K, Kubo M, Tamaki K. Impaired Smad7-Smurf-mediated negative regulation of TGF-beta signaling in scleroderma fibroblasts. J Clin Invest 2004;113:253–64.
[22] Pannu J, Asano Y, Nakerakanti S, Smith E, Jablonska S, Blaszczyk M, et al. Smad1 pathway is activated in systemic sclerosis fibroblasts and is targeted by imatinib mesylate. Arthritis Rheum 2008;58:2528–37.
[23] Bhattacharyya S, Ghosh AK, Pannu J, Mori Y, Takagawa S, Chen G, et al. Fibroblast expression of the coactivator p300 governs the intensity of profibrotic response to transforming growth factor beta. Arthritis Rheum 2005;52:1248–58.
[24] Mimura Y, Ihn H, Jinnin M, Asano Y, Yamane K, Tamaki K. Constitutive thrombospondin-1 overexpression contributes to autocrine transforming growth factor-beta signaling in cultured scleroderma fibroblasts. Am J Pathol 2005;166:1451–63.
[25] Uitto J, Kouba D. Cytokine modulation of extracellular matrix gene expression: relevance to fibrotic skin diseases. J Dermatol Sci 2000;24(Suppl 1):S60–9.
[26] Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. Faseb J 2004;18:816–27.
[27] Leask A, Denton CP, Abraham DJ. Insights into the molecular mechanism of chronic fibrosis: the role of connective tissue growth factor in scleroderma. J Invest Dermatol 2004;122:1–6.
[28] Hardie WD, Davidson C, Ikegami M, Leikauf GD, Le Cras TD, Prestridge A, et al. EGF receptor tyrosine kinase inhibitors diminish transforming growth factor-alpha-induced pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2008;294: L1217–1225.
[29] Mauviel A, Heino J, Kahari VM, Hartmann DJ, Loyau G, Pujol JP, et al. Comparative effects of interleukin-1 and tumor necrosis factor-alpha on collagen production and corresponding procollagen mRNA levels in human dermal fibroblasts. J Invest Dermatol 1991;96:243–9.
[30] Solis-Herruzo JA, Brenner DA, Chojkier M. Tumor necrosis factor alpha inhibits collagen gene transcription and collagen synthesis in cultured human fibroblasts. J Biol Chem 1988;263:5841–5.
[31] Verrecchia F, Pessah M, Atfi A, Mauviel A. Tumor necrosis factor-alpha inhibits transforming growth factor-beta /Smad signaling in human dermal fibroblasts via AP-1 activation. J Biol Chem 2000;275:30226–31.
[32] Yamane K, Ihn H, Asano Y, Jinnin M, Tamaki K. Antagonistic effects of TNF-alpha on TGF-beta signaling through down-regulation of TGF-beta receptor type II in human dermal fibroblasts. J Immunol 2003;171:3855–62.
[33] Rosenbloom J, Feldman G, Freundlich B, Jimenez SA. Inhibition of excessive scleroderma fibroblast collagen production by recombinant gamma-interferon. Association with a coordinate decrease in types I and III procollagen messenger RNA levels. Arthritis Rheum 1986;29:851–6.
[34] Postlethwaite AE, Holness MA, Katai H, Raghow R. Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin 4. J Clin Invest 1992;90:1479–85.
[35] Serpier H, Gillery P, Salmon-Ehr V, Garnotel R, Georges N, Kalis B, et al. Antagonistic effects of interferon-gamma and interleukin-4 on fibroblast cultures. J Invest Dermatol 1997;109:158–62.
[36] Oriente A, Fedarko NS, Pacocha SE, Huang SK, Lichtenstein LM, Essayan DM. Interleukin-13 modulates collagen homeostasis in human skin and keloid fibroblasts. J Pharmacol Exp Ther 2000;292:988–94.
[37] Prescott RJ, Freemont AJ, Jones CJ, Hoyland J, Fielding P. Sequential dermal microvascular and perivascular changes in the development of scleroderma. J Pathol 1992;166:255–63.
[38] Kahari VM, Sandberg M, Kalimo H, Vuorio T, Vuorio E. Identification of fibroblasts responsible for increased collagen production in localized scleroderma by in situ hybridization. J Invest Dermatol 1988;90:664–70.
[39] Scharffetter K, Lankat-Buttgereit B, Krieg T. Localization of collagen mRNA in normal and scleroderma skin by in-situ hybridization. Eur J Clin Invest 1988;18:9–17.
[40] Hoffmann KF, McCarty TC, Segal DH, Chiaramonte M, Hesse M, Davis EM, et al. Disease fingerprinting with cDNA microarrays reveals distinct gene expression profiles in lethal type 1 and type 2 cytokine-mediated inflammatory reactions. Faseb J 2001;15:2545–7.
[41] Sandler NG, Mentink-Kane MM, Cheever AW, Wynn TA. Global gene expression profiles during acute pathogen-induced pulmonary inflammation reveal divergent roles for Th1 and Th2 responses in tissue repair. J Immunol 2003;171:3655–67.
[42] Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. Nat Rev Immunol 2004;4:583–94.
[43] Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2008;214: 199–210.
[44] Ferrarini M, Steen V, Medsger Jr TA, Whiteside TL. Functional and phenotypic analysis of T lymphocytes cloned from the skin of patients with systemic sclerosis. Clin Exp Immunol 1990;79:346–52.
[45] Gruschwitz MS, Vieth G. Up-regulation of class II major histocompatibility complex and intercellular adhesion molecule 1 expression on scleroderma fibroblasts and endothelial cells by interferon-gamma and tumor necrosis factor alpha in the early disease stage. Arthritis Rheum 1997;40:540–50.
[46] Mavalia C, Scaletti C, Romagnani P, Carossino AM, Pignone A, Emmi L, et al. Type 2 helper T-cell predominance and high CD30 expression in systemic sclerosis. Am J Pathol 1997;151:1751–8.
[47] Salmon-Ehr V, Serpier H, Nawrocki B, Gillery P, Clavel C, Kalis B, et al. Expression of interleukin-4 in scleroderma skin specimens and scleroderma fibroblast cultures. Potential role in fibrosis. Arch Dermatol 1996;132:802–6.
[48] Scaletti C, Vultaggio A, Bonifacio S, Emmi L, Torricelli F, Maggi E, et al. Th2-oriented profile of male offspring T cells present in women with systemic sclerosis and reactive with maternal major histocompatibility complex antigens. Arthritis Rheum 2002;46:445–50.
[49] Atamas SP, Yurovsky VV, Wise R, Wigley FM, Goter Robinson CJ, Henry P, et al. Production of type 2 cytokines by CD8+ lung cells is associated with greater decline in pulmonary function in patients with systemic sclerosis. Arthritis Rheum 1999;42:1168–78.
[50] Luzina IG, Atamas SP, Wise R, Wigley FM, Xiao HQ, White B. Gene expression in bronchoalveolar lavage cells from scleroderma patients. Am J Respir Cell Mol Biol 2002;26:549–57.
[51] Parel Y, Aurrand-Lions M, Scheja A, Dayer JM, Roosnek E, Chizzolini C. Presence of CD4+CD8+ double-positive T cells with very high interleukin-4 production potential in lesional skin of patients with systemic sclerosis. Arthritis Rheum 2007;56:3459–67.
[52] Boin F, De Fanis U, Bartlett SJ, Wigley FM, Rosen A, Casolaro V. T cell polarization identifies distinct clinical phenotypes in scleroderma lung disease. Arthritis Rheum 2008;58:1165–74.
[53] Del Galdo F, Jimenez SA. T cells expressing allograft inflammatory factor 1 display increased chemotaxis and induce a profibrotic phenotype in normal fibroblasts in vitro. Arthritis Rheum 2007;56:3478–88.
[54] Yurovsky VV, Wigley FM, Wise RA, White B. Skewing of the CD8+ T-cell repertoire in the lungs of patients with systemic sclerosis. Hum Immunol 1996;48:84–97.
[55] Sakkas LI, Xu B, Artlett CM, Lu S, Jimenez SA, Platsoucas CD. Oligoclonal T cell expansion in the skin of patients with systemic sclerosis. J Immunol 2002;168:3649–59.
[56] Farge D, Henegar C, Carmagnat M, Daneshpouy M, Marjanovic Z, Rabian C, et al. Analysis of immune reconstitution after autologous bone marrow transplantation in systemic sclerosis. Arthritis Rheum 2005;52:1555–63.
[57] Kurasawa K, Hirose K, Sano H, Endo H, Shinkai H, Nawata Y, et al. Increased interleukin-17 production in patients with systemic sclerosis. Arthritis Rheum 2000;43:2455–63.
[58] Murata M, Fujimoto M, Matsushita T, Hamaguchi Y, Hasegawa M, Takehara K, et al. Clinical association of serum interleukin-17 levels in systemic sclerosis: is systemic sclerosis a Th17 disease? J Dermatol Sci 2008;50:240–2.
[59] Deleuran B, Abraham DJ. Possible implication of the effector CD4+ T-cell subpopulation TH17 in the pathogenesis of systemic scleroderma. Nat Clin Pract Rheumatol 2007;3:682–3.
[60] Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, et al. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med 1996;183:2593–603.
[61] Radstake TR, van Bon L, Broen J, Hussiani A, Hesselstrand R, Wuttge DM, et al. The pronounced Th17 profile in systemic sclerosis (SSc) together with intracellular

expression of TGFbeta and IFNgamma distinguishes SSc phenotypes. PLoS ONE 2009;4:e5903.
[62] Fuschiotti P, Medsger Jr TA, Morel PA. Effector CD8+ T cells in systemic sclerosis patients produce abnormally high levels of interleukin-13 associated with increased skin fibrosis. Arthritis Rheum 2009;60:1119–28.
[63] Granel B, Allanore Y, Chevillard C, Arnaud V, Marquet S, Weiller PJ, et al. IL13RA2 gene polymorphisms are associated with systemic sclerosis. J Rheumatol 2006;33:2015–9.
[64] Wood N, Whitters MJ, Jacobson BA, Witek J, Sypek JP, Kasaian M, et al. Enhanced interleukin (IL)-13 responses in mice lacking IL-13 receptor alpha 2. J Exp Med 2003;197:703–9.
[65] Matsushita T, Hasegawa M, Hamaguchi Y, Takehara K, Sato S. Longitudinal analysis of serum cytokine concentrations in systemic sclerosis: association of interleukin 12 elevation with spontaneous regression of skin sclerosis. J Rheumatol 2006;33:275–84.
[66] Valentini G, Baroni A, Esposito K, Naclerio C, Buommino E, Farzati A, et al. Peripheral blood T lymphocytes from systemic sclerosis patients show both Th1 and Th2 activation. J Clin Immunol 2001;21:210–7.
[67] Chizzolini C, Parel Y, Scheja A, Dayer JM. Polarized subsets of human T helper cells induce distinct patterns of chemokine production by normal and systemic sclerosis dermal fibroblasts (Abstract). Clin Exp Rheumatol 2005;23:739.
[68] Keane MP, Arenberg DA, Lynch III JP, Whyte RI, Iannettoni MD, Burdick MD, et al. The CXC chemokines, IL-8 and IP-10, regulate angiogenic activity in idiopathic pulmonary fibrosis. J Immunol 1997;159:1437–43.
[69] Yamamoto T, Eckes B, Mauch C, Hartmann K, Krieg T. Monocyte chemoattractant protein-1 enhances gene expression and synthesis of matrix metalloproteinase-1 in human fibroblasts by an autocrine IL-1 alpha loop. J Immunol 2000;164:6174–9.
[70] Yamamoto T. Chemokines and chemokine receptors in scleroderma. Int Arch Allergy Immunol 2006;140:345–56.
[71] Distler JHW, Jüngel A, Caretto D, Kowal-Bielecka O, Gay RE, Michel BA, et al. MCP-1 stimulates the release of the profibrotic cytokine IL-4 by inducing Th2 differentiation in Th0 cells (Abstract). Ann Rheum Dis 2005;64:SIII110.
[72] Yamamoto T, Eckes B, Hartmann K, Krieg T. Expression of monocyte chemoattractant protein-1 in the lesional skin of systemic sclerosis. J Dermatol Sci 2001;26:133–9.
[73] Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. Nature 2000;404:407–11.
[74] Hogaboam CM, Bone-Larson CL, Lipinski S, Lukacs NW, Chensue SW, Strieter RM, et al. Differential monocyte chemoattractant protein-1 and chemokine receptor 2 expression by murine lung fibroblasts derived from Th1- and Th2-type pulmonary granuloma models. J Immunol 1999;163:2193–201.
[75] Zhu Z, Ma B, Zheng T, Homer RJ, Lee CG, Charo IF, et al. IL-13-induced chemokine responses in the lung: role of CCR2 in the pathogenesis of IL-13-induced inflammation and remodeling. J Immunol 2002;168:2953–62.
[76] Gharaee-Kermani M, McCullumsmith RE, Charo IF, Kunkel SL, Phan SH. CC-chemokine receptor 2 required for bleomycin-induced pulmonary fibrosis. Cytokine 2003;24:266–76.
[77] Rizou C, Ioannidis JP, Panou-Pomonis E, Sakarellos-Daitsiotis M, Sakarellos C, Moutsopoulos HM, et al. B-Cell epitope mapping of DNA topoisomerase I defines epitopes strongly associated with pulmonary fibrosis in systemic sclerosis. Am J Respir Cell Mol Biol 2000;22:344–51.
[78] Hu PQ, Fertig N, Medsger Jr TA, Wright TM. Correlation of serum anti-DNA topoisomerase I antibody levels with disease severity and activity in systemic sclerosis. Arthritis Rheum 2003;48:1363–73.
[79] Sato S, Hayakawa I, Hasegawa M, Fujimoto M, Takehara K. Function blocking autoantibodies against matrix metalloproteinase-1 in patients with systemic sclerosis. J Invest Dermatol 2003;120:542–7.
[80] Nishijima C, Hayakawa I, Matsushita T, Komura K, Hasegawa M, Takehara K, et al. Autoantibody against matrix metalloproteinase-3 in patients with systemic sclerosis. Clin Exp Immunol 2004;138:357–63.
[81] Fujimoto M, Hamaguchi Y, Yazawa N, Komura K, Takehara K, Sato S. Autoantibodies to a collagen-specific molecular chaperone, heat-shock protein 47, in systemic sclerosis. Clin Exp Immunol 2004;138:534–9.
[82] Tan FK, Arnett FC, Reveille JD, Ahn C, Antohi S, Sasaki T, et al. Autoantibodies to fibrillin 1 in systemic sclerosis: ethnic differences in antigen recognition and lack of correlation with specific clinical features or HLA alleles. Arthritis Rheum 2000;43:2464–71.
[83] Tan FK, Arnett FC, Antohi S, Saito S, Mirarchi A, Spiera H, et al. Autoantibodies to the extracellular matrix microfibrillar protein, fibrillin-1, in patients with scleroderma and other connective tissue diseases. J Immunol 1999;163:1066–72.
[84] Lundberg I, Antohi S, Takeuki K, Arnett F, Steiner G, Brumeanu TD, et al. Kinetics of anti-fibrillin-1 autoantibodies in MCTD and CREST syndrome. J Autoimmun 2000;14:267–74.
[85] Morse JH, Antohi S, Kasturi K, Saito S, Fotino M, Humbert M, et al. Fine specificity of anti-fibrillin-1 autoantibodies in primary pulmonary hypertension syndrome. Scand J Immunol 2000;51:607–11.
[86] Murai C, Saito S, Kasturi KN, Bona CA. Spontaneous occurrence of anti-fibrillin-1 autoantibodies in tight-skin mice. Autoimmunity 1998;28:151–5.
[87] Zhou X, Tan FK, Milewicz DM, Guo X, Bona CA, Arnett FC. Autoantibodies to fibrillin-1 activate normal human fibroblasts in culture through the TGF-beta pathway to recapitulate the "scleroderma phenotype". J Immunol 2005;175:4555–60.
[88] Yasuoka H, Ihn H, Medsger Jr TA, Hirakata M, Kawakami Y, Ikeda Y, et al. A novel protein highly expressed in testis is overexpressed in systemic sclerosis fibroblasts and targeted by autoantibodies. J Immunol 2003;171:6883–90.
[89] Tamby MC, Humbert M, Guilpain P, Servettaz A, Dupin N, Christner JJ, et al. Antibodies to fibroblasts in idiopathic and scleroderma-associated pulmonary hypertension. Eur Respir J 2006;28:799–807.
[90] Henault J, Tremblay M, Clement I, Raymond Y, Senecal JL. Direct binding of anti-DNA topoisomerase I autoantibodies to the cell surface of fibroblasts in patients with systemic sclerosis. Arthritis Rheum 2004;50:3265–74.
[91] Henault J, Robitaille G, Senecal JL, Raymond Y. DNA topoisomerase I binding to fibroblasts induces monocyte adhesion and activation in the presence of anti-topoisomerase I autoantibodies from systemic sclerosis patients. Arthritis Rheum 2006;54:963–73.
[92] Lunardi C, Bason C, Navone R, Millo E, Damonte G, Corrocher R, et al. Systemic sclerosis immunoglobulin G autoantibodies bind the human cytomegalovirus late protein UL94 and induce apoptosis in human endothelial cells. Nat Med 2000;6:1183–6.
[93] Lunardi C, Dolcino M, Peterlana D, Bason C, Navone R, Tamassia N, et al. Antibodies against human cytomegalovirus in the pathogenesis of systemic sclerosis: a gene array approach. PLoS Med 2006;3:e2.
[94] Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, et al. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. N Engl J Med 2006;354:2667–76.
[95] Svegliati S, Olivieri A, Campelli N, Luchetti M, Poloni A, Trappolini S, et al. Stimulatory autoantibodies to PDGF receptor in patients with extensive chronic graft-versus-host disease. Blood 2007;110:237–41.
[96] Classen JF, Henrohn D, Rorsman F, Lennartsson J, Lauwerys BR, Wikstrom G, et al. Lack of evidence of stimulatory autoantibodies to platelet-derived growth factor receptor in patients with systemic sclerosis. Arthritis Rheum 2009;60:1137–44.
[97] Loizos N, Lariccia L, Weiner J, Griffith H, Boin F, Hummers L, et al. Lack of detection of agonist activity by antibodies to platelet-derived growth factor receptor alpha in a subset of normal and systemic sclerosis patient sera. Arthritis Rheum 2009;60:1145–51.
[98] Chizzolini C, Raschi E, Rezzonico R, Testoni C, Mallone R, Gabrielli A, et al. Autoantibodies to fibroblasts induce a proadhesive and proinflammatory fibroblast phenotype in patients with systemic sclerosis. Arthritis Rheum 2002;46:1602–13.
[99] Fineschi S, Cozzi F, Burger D, Dayer JM, Meroni PL, Chizzolini C. Anti-fibroblast antibodies detected by cell-based ELISA in systemic sclerosis enhance the collagenolytic activity and matrix metalloproteinase-1 production in dermal fibroblasts. Rheumatology (Oxford) 2007;46:1776–85.
[100] Fineschi S, Goffin L, Rezzonico R, Cozzi F, Dayer JM, Meroni PL, et al. Antifibroblast antibodies in systemic sclerosis induce fibroblasts to produce profibrotic chemokines, with partial exploitation of toll-like receptor 4. Arthritis Rheum 2008;58:3913–23.
[101] Elson G, Dunn-Siegrist I, Daubeuf B, Pugin J. Contribution of Toll-like receptors to the innate immune response to Gram-negative and Gram-positive bacteria. Blood 2007;109:1574–83.
[102] Daubeuf B, Mathison J, Spiller S, Hugues S, Herren S, Ferlin W, et al. TLR4/MD-2 monoclonal antibody therapy affords protection in experimental models of septic shock. J Immunol 2007;179:6107–14.
[103] Smirnova I, Poltorak A, Chan EK, McBride C, Beutler B. Phylogenetic variation and polymorphism at the toll-like receptor 4 locus (TLR4). Genome Biol 2000;1 RESEARCH 002.001-002.010.
[104] Marshak-Rothstein A, Rifkin IR. Immunologically active autoantigens: the role of toll-like receptors in the development of chronic inflammatory disease. Annu Rev Immunol 2007;25:419–41.
[105] Marshak-Rothstein A. Toll-like receptors in systemic autoimmune disease. Nat Rev Immunol 2006;6:823–35.
[106] Yoshizaki A, Iwata Y, Komura K, Ogawa F, Hara T, Muroi E, et al. CD19 regulates skin and lung fibrosis via toll-like receptor signaling in a model of bleomycin-induced scleroderma. Am J Pathol 2008.
[107] Raschi E, Testoni C, Bosisio D, Borghi MO, Koike T, Mantovani A, et al. Role of the MyD88 transduction signaling pathway in endothelial activation by antiphospholipid antibodies. Blood 2003;101:3495–500.
[108] Pierangeli SS, Vega-Ostertag ME, Raschi E, Liu X, Romay-Penabad Z, De Micheli V, et al. Toll-like receptor and antiphospholipid mediated thrombosis: in vivo studies. Ann Rheum Dis 2007;66:1327–33.
[109] Satta N, Dunoyer-Geindre S, Reber G, Fish RJ, Boehlen F, Kruithof EK, et al. The role of TLR2 in the inflammatory activation of mouse fibroblasts by human antiphospholipid antibodies. Blood 2007;109:1507–14.
[110] Zanoni G, Navone R, Lunardi C, Tridente G, Bason C, Sivori S, et al. In celiac disease, a subset of autoantibodies against transglutaminase binds toll-like receptor 4 and induces activation of monocytes. PLoS Med 2006;3:e358.
[111] Tan FK, Zhou X, Mayes MD, Gourh P, Guo X, Marcum C, et al. Signatures of differentially regulated interferon gene expression and vasculotrophism in the peripheral blood cells of systemic sclerosis patients. Rheumatology (Oxford) 2006;45:694–702.
[112] York MR, Nagai T, Mangini AJ, Lemaire R, van Seventer JM, Lafyatis R. A macrophage marker, Siglec-1, is increased on circulating monocytes in patients with systemic sclerosis and induced by type I interferons and toll-like receptor agonists. Arthritis Rheum 2007;56:1010–20.
[113] Duan H, Fleming J, Pritchard DK, Amon LM, Xue J, Arnett HA, et al. Combined analysis of monocyte and lymphocyte messenger RNA expression with serum protein profiles in patients with scleroderma. Arthritis Rheum 2008;58:1465–74.
[114] Bos CL, van Baarsen LG, Timmer TC, Overbeek MJ, Basoski NM, Rustenburg F, et al. Molecular subtypes of systemic sclerosis in association with anti-centromere antibodies and digital ulcers. Genes Immun 2009;10:210–8.
[115] Assassi S, Mayes MD, Arnett FC, Gourh P, Agarwal SK, McNearney TA, et al. Systemic sclerosis and lupus: points in an interferon-mediated continuum. Arthritis Rheum 2010;62:589–98.
[116] Kim D, Peck A, Santer D, Patole P, Schwartz SM, Molitor JA, et al. Induction of interferon-alpha by scleroderma sera containing autoantibodies to topoisomerase I: association of higher interferon-alpha activity with lung fibrosis. Arthritis Rheum 2008;58:2163–73.