

The signal transducer and activator of transcription factors lodge in immunopathogenesis of rheumatoid arthritis

F. Zare¹, M. Dehghan-Manshadi^{2,3}, A. Mirshafiey¹

¹Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran;

²Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran;

³Department of Immunology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

SUMMARY

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that affects ~1-2% of the world's population and damages synovial joints. RA is characterized by inflammation, autoantibody production, cartilage and bone destruction and synovial hyperplasia. Inflammation induces systemic and articular synthesis of pro-inflammatory cytokines, such as tumor necrosis factor alpha and interleukin-6 that play essential roles in joint and other organ damage in this disease. Considering the role of signal transducer and activator of transcription factors (STATs) in signaling of these cytokines, these proteins may be involved in the pathogenesis of RA. The expression and activity of STATs can contribute to the onset, progression and severity of RA. All STAT family members (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) have been associated with autoimmune diseases, as highlighted in several studies. In this review we aim to describe the immunobiology of STATs and its family members and the role of these proteins in the immunopathogenesis of RA.

Key words: Immunopathogenes; rheumatoid arthritis; STAT proteins.

Reumatismo, 2015; 67 (4): 127-137

■ INTRODUCTION

During innate and adaptive immune responses, certain cells such as dendritic cells and T lymphocyte secrete various cytokines. Most of these cytokines mediate their biological activities *via* the Janus kinase (JAK)-signal transducer and activator of transcription factors (STATs) pathway (1). JAK-STAT pathway have been conserved during evolution and are engaged by a wide range of cytokines, interferons (IFNs), growth factors, and related molecules. Signaling of STATs regulates the initiation, duration and intensity of immune responses (2). Genetic mutations and variations in STATs are functionally associated with different types of human diseases especially cancer and immune-related conditions (3). The over expression of STATs by pro-inflammatory cytokines or growth factors contributes to the pathogenesis of several autoimmune diseases such as rheumatoid arthritis (RA). RA is an

inflammatory disease caused by systemic and articular synthesis of pro-inflammatory cytokines, and these cytokines exert their effect *via* the JAK-STAT pathway. Identifying the properties and role of these transcription factors can thus help designing new therapeutic methods for the treatment and prevention of RA in the future.

■ SIGNAL TRANSDUCER AND ACTIVATORS OF TRANSCRIPTION FAMILY

STAT family of transcription factors was first discovered in IFN signaling pathways in the early 1990s (4, 5). Seven STAT family members have been identified (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6) until now (6). The genes of these seven STATs have been localized in three chromosomal clusters (6). The genes encoding for STAT1 and 4 map to a region of mouse chromosome1 (in human chromosome 2, bands q12 to q33); STAT2 and 6

Corresponding author
Abbas Mirshafiey
Department of Immunology
School of Public Health, Tehran University of
Medical Sciences
Tehran-14155, Box: 6446, Iran
E-mail: mirshafiey@tums.ac.ir

map to a region of mouse chromosome 10 (human chromosome 17, bands q11-1 to q22); STAT3, 5a, and 5b map to mouse chromosome 11 (human chromosome 12, bands q13 to q14-1) (6). The difference between the localization of these genes reflects the properties of STAT proteins.

STAT2 and STAT6 proteins consist of approximately 750 to 800 amino acids long, whereas the other members of STATs are approximately 850 amino acids residues (7, 8). These proteins are activated in response to a large number of cytokines, growth factors, and hormones. The various cytokines have the tendency to activate a special STAT. The mechanisms by which STATs affect gene transcription were determined by using knockout mice and new technologies. STATs have numerous binding sites in the genome which can modulate the transcription of various protein-coding genes (9).

Each of the STAT proteins has several conserved domains containing a DNA binding domain and a SH2 domain (src-homology 2). The DNA binding domain is located in the central region of each STAT protein and the SH2 domain is located in the region between 600 and 700 amino acid residues (10, 11). Also a conserved tyrosine residue exists near the carboxy-terminus of the proteins. The binding of a cytokine to its receptor rapidly induces the tyrosine phosphorylation of the receptor by JAK kinases. These phosphorylated tyrosines create docking sites for STATs. Interaction of STAT protein with phosphorylated tyrosines occurs through the SH2 domain. JAK mediates phosphorylation and dimerization of STATs between the SH2 domains and the carboxy-terminal phosphotyrosine domain. Dimerized STAT translocates into the cell nucleus. In the nucleus, it binds to DNA and promotes the transcription of genes responsive to STAT. The SH2 domain has been shown to regulate the specificity of cytokine-induced STAT activation. As a result, each STAT binds to the docking site of certain cytokine receptors. For example, STAT6 binds to phosphotyrosine docking sites on the interleukin (IL)-4 receptor α chain (12), STAT1 binds to the IFN- γ receptor, STAT5a and STAT5b bind

to related but distinct docking sites on the IL-2 receptor β chain (IL-2R β) and IL-7 receptor α chain (IL-7R α) (13).

STAT1

STAT1 was the first member of the STAT family identified, as a principal target of both type I and type II IFN activation (14, 15). Most physiological functions of STAT1, although not all of them, are closely related to the biological function of IFNs (16). The physiological functions of the STATs have been studied in multiple cell lines and knockout mice (5). By using knockout mice for STAT1, it has been demonstrated that they show no developmental abnormalities but display a complete deficiency of responsiveness to either IFN α or IFN γ , leading to increased susceptibility to viral infections and other microbial pathogens (17). Gain-of function mutations of STAT1 cause exaggerated IFN- γ signaling. Increased IFN- γ signaling inhibits IL-17 transcription, resulting in susceptibility to fungal infections and development of autoimmune disease (18). These data indicate that STAT1 has a role in suppressing certain autoimmune disorders. Conversely, STAT1 mediates anti-proliferative and pro-apoptotic effects of IFNs, suggesting that STAT1 has also the potential for inhibiting inflammation (19).

Increased rates of tumor formation and non-lymphoid tumor types have been observed when the STAT1 deficiency was placed on a p53 null background. It is believed that such increase might be associated with loss of tumor surveillance, possibly through the loss of the ability of IFNs to promote antigen processing and presentation by MHC class I and II (5). Despite the role of STATs in promoting oncogenesis, substantial studies demonstrated a protective role of STATs in cancer and the role of STATs in apoptosis (20, 21). Interferons have a critical role in recognizing and eliminating transformed malignant cells (21). Several types of human cancer including lung cancer, melanoma, prostate cancer, and breast cancer are caused by the weakness in ability of the signal through IFN receptors (22).

Taken together, most of IFN functions are mediated by STAT1. In physiological conditions, STAT1 controls cell growth, proliferation, apoptosis, and functions of the immune system. Polymorphisms of STAT1 have been associated with an increased risk of malignancy (23). In human with autosomal-recessive of STAT1, this deficiency causes development of a primary immunodeficiency syndrome characterized by susceptibility to viral infections and bacterial pathogens (24).

STAT2

STAT2 plays an essential role in type I, but not type 2, IFN signaling and IFN-mediated antiviral and anti proliferative functions (25). Type 1 IFNs activate STAT1, STAT2, and IRF9 as a complex of transcription factors while IFN- γ primarily activates STAT1, resulting in a STAT1-STAT1 homodimer that translocates to the nucleus. STAT2 deficient mice exhibit increased susceptibility to viral infections and host immune response is compromised (26). The role of STAT2 in IFN signaling and response to viral infection is supported by the study of Hambleton *et al.*, in which a child with lack of STAT2 had a history of disseminated vaccine-strain measles because measles virus V protein blocks IFN- α/β but not IFN- γ signaling through inhibiting STAT1 and STAT2 phosphorylation (24).

STAT3

It is difficult to identify the function of STAT3 because STAT3 is essential for the early development of mouse embryos. Unlike other STAT family knockout mice, STAT3 deficient mice are lethal embryonic by rapid degeneration (27). In order to dissect the specific functions of STAT3 in various cell lines, tissue-specific targeting of the STAT3 locus has been used (28). STAT3 as a pleiotropic transcription factor can play a role in the signal of several cytokines including IL-6, IL-10 and other gp130 cytokines. In addition it could be involved in immune and somatic cell abnormalities (9, 19). Granulopoiesis negatively regulates bone marrow progenitor cells by STAT3. Abnormalities in myeloid cells

caused by STAT3 deletion during hematopoiesis lead to over activation of innate immune responses and cause inflammatory bowel disease-like pathogenesis (29, 30). These findings indicate the immune-suppressive and anti-inflammatory function of STAT3 in the myeloid lineage.

STAT3 deficient mice have significantly reduced responses to IL-6 and TCR/IL-2 stimulation in T cells (5). STAT3 is also required for the differentiation of the CD4 (+) T lymphocyte subset termed T helper17 (Th17) cells. Th17 cells produce IL-17 that can recruit neutrophils to the sites of inflammation and especially is a main part of host defense against bacterial and fungal infections (31). Also failure to produce IL-17 contributes to the immunopathogenesis of HIES (autosomal dominant hyper immunoglobulin E syndrome). Dominant-negative mutations in STAT3 gene result in the classical multi-system HIES (32, 33). Another cytokine that activates STAT3 is IL-22, which is important for epithelial barrier function (34). Impaired barrier function responsible for staphylococcal skin abscesses, atopic dermatitis and mucocutaneous candidiasis develops in the HIES (35). Recent studies reported an important role for STAT3 in inducing and maintaining a procarcinogenic inflammatory microenvironment, and therefore STAT3 is believed to contribute to promoting oncogenesis (27). In general, STAT3 has a complex role, because STAT3 can have various effects on different cells, depending on the cell type and activation status.

STAT4

In contrast to other STATs, that are expressed on a wide range of cell types, STAT4 is predominantly expressed by immune cells and the testis (36). STAT4 is activated by IL-12 and IL-23 in mice and is activated by type I IFN in humans through interaction with STAT2 (37, 38). STAT4 is an essential transcription factor for the biological function of several immune cells such as macrophages, mast cells, natural killer cells, dendritic cells, T helper (Th) cells, regulatory T cells, follicular helper T cells, CD8+T cell and B cells (39-42). STAT4 can affect the functions of these cells that play a role in

the pathogenesis of autoimmune diseases such as systemic lupus erythematosus, RA, inflammatory bowel disease, multiple sclerosis, type 1 diabetes, systemic sclerosis, psoriasis and experimental autoimmune myocarditis (39).

By activating IL-12, STAT4 has an essential role in the generation and proliferation of Th1 cells and is necessary for the development of Th17 cells (43, 44). STAT4-null mice have an impaired role in Th1 differentiation, IFN- γ production and cell-mediated immune responses (45, 46). STAT4 knockout mice are resistant to Th1-associated autoimmune diseases.

STAT5

STAT5 is activated by several hormones and a wide range of cytokines, including growth hormone, prolactin, epidermal growth factor, platelet-derived growth factor, the hematopoietic cytokines IL-3, granulocyte/macrophage colony stimulating factor, IL-5, erythropoietin and cytokines with common gamma (γ c) receptor chain (IL-2, IL-4, IL-7, IL-9, and IL-15) (19, 47). There are two STAT5 genes, STAT5a and STAT5B (7). Both of these genes are located on the same chromosome in both mice and humans which have 96% sequence similarity and seem to have overlapping functions (48, 49).

Mice with deficiency in STAT5a/5b show and impairment in cell growth regulating processes (47, 50, 51). Germline deletion of STAT5a and STAT5b as STAT3 deficiency, is embryonic lethal (52).

Mammary gland development is greatly impaired in STAT5a deficient mice but not in STAT5b null animals (19). T cells in these mice have decreased proliferation due to reduced expression of the IL-2R α chain (53).

Autosomal recessive mutations of STAT5b result in complex syndrome characterized by growth failure, immunodeficiency, and autoimmunity (3). These clinical disorders indicate the role of STAT5 in growth hormone signaling. Also according to some reports, deletion of STAT5b results in a phenotype similar to that seen in mice with growth hormone deficiency. This pheno-

type is further prominent in the STAT5a/b deficient mice (5). Lack of STAT5b alone also impacts on immune cells, especially T and NK cells (7).

STAT5 is an essential regulator of lymphoid development and peripheral tolerance. STAT5b is important for the generation of regulatory T cells and is required for the expression of the transcription factor FOXP3 (54). This suggests that STAT5 signaling plays an essential role in the development of T regulatory cells, which may be important in preventing autoimmunity (55). STAT5 is also important for effector and memory cell viability and has a role in myeloid cell mobilization and survival (19, 56).

Recent studies showed a dual role for STAT5b, both pro- and anti-inflammatory. On the one hand, it contributes to the development and homeostasis of lymphoid cells, on the other hand, in conditions of immunocompetency, limits T cell hyperactivity. The activated STAT5 can relatively increase the proliferation, survival and invasion of tumor cells. Therefore, it can have a role in the pathogenesis of hematologic and solid-organ tumor (57).

STAT6

STAT6 is activated by IL-4 and IL-13 that are essential for the functional responses of T helper 2 (Th2) lymphocyte (58). Activated STAT6 modulates the expression of Th2 chemokines (59). Some human genes including immunoglobulin heavy chain ϵ , CD23, GATA-3 and MHC class II are targeted by STAT6.

STAT6 deficiency causes impaired Th2 responses, with reduced Th2 differentiation, reduced proliferative responses to IL-4 and diminished IgE production (60, 61). STAT6 gene polymorphisms are associated with asthma and allergy in various populations (62). The elevated level of IL-4 and IL-13 cytokines found in human asthma and clinical trials provide a rationale for the selective target the IL-4/IL-13/STAT6 pathway (63). As expected, STAT6 null mice are impaired in most of the physiological functions related to IL-4 and could be protected from allergic pulmonary manifestations (60, 64).

Thus, STAT6 is a critical transcription factor in the Th2 signaling pathway and is a potential genetic factor contributing to asthma.

■ RHEUMATOID ARTHRITIS AND SIGNAL TRANSDUCER AND ACTIVATORS OF TRANSCRIPTION

RA is characterized by inflammation, autoantibody production, cartilage and bone destruction and synovial hyperplasia (65, 66). Inflammation caused by systemic and articular synthesis of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF- α), IL-6, and IL-17 play an essential role in joint and other organ damage in this disease (67). These proinflammatory cytokines are abundant in RA tissue in the majority of the patients (68, 69). By engaging their receptors, these cytokines cause homodimerization and activation of their receptors, resulting in the activation of associated JAK. The activated JAKs recruit and phosphorylate STATs proteins (70, 71). The phosphorylated STAT protein dimerizes and translocates into the nucleus. In the here, it binds to promoter of genes and regulates gene expression. Therefore, STATs activation can play an essential role in inflammation and autoimmune diseases (72). The first report of STAT activation in RA patients described that activated STAT3 can be found in synovial fluid (SF) cells and that soluble factors in the SF of RA patients can induce STAT3 activation in monocytes (73-75). It has been shown that STAT3 contributes to promoting cell survival and growth in many cell types through the induction of pim-1, c-myc, cyclin-D, and Bcl-X (76, 77). STAT3 is important for the survival and expansion of growth factor-dependent SF cells that are important in the pathogenesis of RA (76). The activated STAT3 increases tumor cell proliferation, survival and invasion while suppressing anti-tumor immunity. The persistent activation of STAT3 also mediates tumor-promoting inflammation. STAT3 has dual role in tumor inflammation and immunity by promoting pro-oncogenic

inflammatory pathways, including nuclear factor- κ B (NF- κ B) and IL-6-GP130-JAK pathways, and by opposing STAT1 and NF- κ B-mediated T helper 1 anti-tumor immune responses (57). Studies revealed that IL-6 produced in SF of RA patients can function as an autocrine growth factor (75). The study of Takeda et al., demonstrates that Stat3 activation is involved in IL-6-dependent T cell proliferation through prevention of apoptosis independently of Bcl-2 because in STAT3 deficient T cells and IL-6-induced proliferation was severely impaired. IL-6 did not enhance cell cycle progression, but prevented apoptosis of normal T cells. In contrast, IL-6 did not prevent apoptosis in Stat3-deficient T cells. Antiapoptotic protein, Bcl-2, was normally up-regulated in response to IL-6 even in STAT3-deficient T cells (28).

This observation is consisted with the protective effect of the anti-IL-6 receptor monoclonal antibody against T cell-mediated colitis and inflammatory arthritis models (28, 78).

IL-6 and IL-23 activate STAT3 that plays a critical role in generating Th17 cells (79). Activated Th17 cells release IL-17A and other pro-inflammatory cytokines (80). IL-17A modulates immune response through its cell-surface receptor expressed on epithelial and endothelial cells, fibroblasts and leukocytes by promoting neutrophil recruitment and releasing further pro-inflammatory mediators.

Th17 cell and Th17-related cytokines play a central role in the development, onset and chronicization of establishing various types of autoimmune diseases including RA (81). Blockade of the IL-6-STAT3 pathway in CD4(+) T cells resulted in defective differentiation of Th17 cells *ex vivo* and *in vitro*, and confirmed the role of the IL-6-STAT3 pathway in the generation of Th17 cells, indicating that it could represent a suitable target for the treatment of autoimmune diseases (82, 83).

De Hooge et al. showed that STAT3 are activated in inflamed synovium during the course of the disease and may contribute to the chronicity of inflammation in a murine zymosan-induced arthritis model (74).

Krause et al. reported the role of STAT3 in mediating the abnormal growth and survival properties of RA synoviocytes (76). A dominant negative mutant of STAT3, termed Stat3-YF, generated by using gene transfer with retroviral vectors in cell cultures showed that deletion of STAT3 function causes conversion of epidermal growth factor (required for the survival of rheumatoid synoviocytes) to a death factor by apoptosis. Further studies on synovial fibroblasts reported the role of STAT3 in suppressing the proapoptotic effects of STAT1 (20). Studies in STAT1 deficient mice showed that STAT1 has a primary role in IFN- γ signaling (84). STAT1 plays an important role in the both inflammatory and antiviral function of IFN- γ as well as their ant proliferative and pro-apoptotic effects (85).

Up regulation of STAT1 in rheumatoid tissue has been shown, but its role and extent of activation remain uncertain. De Hooge et al. reported that zymosan-induced arthritis in STAT1-deficient mice is associated with increased joint inflammation and formation of granuloma (74). Yoshida et al. using microarray analysis in 16 patients (11 patients with RA and 9 with osteoarthritis (OA) suggested that the expression levels of STAT1 were significantly higher in RA synovium compared to OA (86). Recently Qihui Zhou et al., reported mir-146a induced proinflammatory phenotype of T regulatory cells through increased STAT1 activation, indicating a possible pathogenic role in RA (87).

Kasperkovitz et al. demonstrated that expression of STAT1 in RA patients is increased compared to OA and reactive arthritis. STAT1 was particularly abundant in T and B lymphocytes in focal inflammatory infiltrates and in fibroblast-like synoviocytes in the intimal lining layer. Some studies showed the expression of STAT1 is raised in synovial tissue (ST) in patients with RA and the result of Kasperkovitz et al. study demonstrate the activation of the STAT1 pathway in RA synovium by raised STAT1 protein expression and concomitantly increased tyrosine (701) and serine (727) phosphorylation (88). Xiaoyu Hu et

al. showed that subthreshold concentrations of IFN- γ , which did not activate macrophages, increased their sensitivity to subsequent IFN- γ stimulation; this resulted in increased STAT1 activation and increased IFN- γ -dependent gene activation. Stimulation of IFN- γ signaling was mediated by the induction of STAT1 expression by low doses of IFN- γ that did not effectively induce feedback inhibition. IFN- γ signaling was sensitized *in vivo* after IFN- γ injection, and STAT1 expression was increased after injection of lipopolysaccharide in RA synovial cells. These results identify a mechanism that sensitizes macrophages to low concentrations of IFN- γ and regulates IFN- γ responses in acute and chronic inflammation (89).

STAT1 can also induce a number of others genes such as caspase-1, IP-10, IRF-1, GBP1, and ICSBP that are selectively up-regulated in RA. STAT1 could thus represent an important factor for inflammatory cell infiltration (90).

The function of STAT4 and STAT6 in inflammatory arthritis is less known. However, both of these STATs play an important role in inflammation (91). STAT4 acts in signaling pathway of IL-12, which mediates differentiation of Th1 cells and their primary function (92). In contrast, Th2 cell differentiation is induced by IL-4 with signals through STAT6 (61). Finnegan et al. reported that arthritis severity in mice appeared to be regulated by IL-4 through a STAT6 dependent mechanism (93). In IL-4 or STAT6 deficient mice, they observed a more severe and progressive arthritis suggesting IL-4 mediates anti-inflammatory reaction through STAT6 (93). Walker et al. was reported the expression of STAT6 is heterogeneous in Synovial tissue biopsy specimens in patients with RA and STAT6 might have a possible homeostatic role in addition to anti-inflammatory effects (94). These effects were associated with elevated levels of the pro-inflammatory cytokines such as IL-12, TNF- α , and IFN- γ . IL-12 which can regulate the expression of IFN- γ through STAT4-dependent pathway (93).

Several studies have reported a polymorphism of STAT4 which is associated with susceptibility for developing systemic lupus erythematosus, Sjögren's syndrome, RA, and Crohn's disease (9, 95). Moreover, STAT6 single-nucleotide polymorphisms are associated with asthma and allergy (62).

The result of a study by Andrey Antov et al. demonstrated that, in conventional CD4 T cells, IL-2 triggers signaling pathways that promotes the proliferation and survival by activating the STAT5 transcription factor and by increasing the expression of the antiapoptotic protein, Bcl-2. They showed that bcl-2 deficiency does not affect CD25 CD4 T reg homeostasis, and that ectopic expression of this molecule fails to rescue CD25 CD4 T reg numbers or prevent the development of autoimmunity in IL-2-deficient mice. Furthermore, transient activation of STAT5 is sufficient to increase CD25 CD4 T reg numbers in IL-2 deficient mice. Their study uncovered an essential role for STAT5 in maintaining CD25 CD4 T reg homeostasis and self-tolerance (96). Yao et al. found that STAT5 plays an essential role in regulating regulatory T cells (Tregs), so that STAT5 and STAT3 appear to have opposing roles in the regulation of Foxp3. STAT5 activates transcription of the Foxp3 gene *via* binding to the promoter and first intron of this gene (52, 97). In addition, by regulating the IL-2R α -chain, STAT5 influences the survival of Tregs (36). In conclusion, the expression and activity

of STAT1 are increased in RA synovium, especially in patients with active disease. Survival of RA synovial fibroblasts could be promoted by STAT3. In addition, murine arthritis knockout models suggest that IL12, signaling through STAT4 pathway, is required for the development of proteoglycan-induced inflammatory arthritis. Therefore, regulation of STAT1, STAT3, STAT4, and STAT6 may be contributed in arthritis severity (Table I).

Regarding, STAT has no enzymatic activity, therefore any therapeutic agent would have to block STAT expression, cytokine receptor binding, dimerization, or DNA binding (98). The first STAT inhibitor appropriate for clinical use was designed based on interfering with DNA binding and use for treatment of malignancies (9). Clinical trials for advanced malignancies are also under investigation for approaching to a newer group of small-molecule inhibitors targeting STAT3, including OPB-51062 and OPB-31121 (33). Preclinical results showed that STAT inhibitors are effective in animal models of autoimmune disease (99). Also intrabodies, which bind with high specificity to phosphorylated STAT3, represent a possible novel avenue for the development of STAT inhibitors (100).

■ CONCLUSIONS

Signaling through JAKs and STATs are fundamental drivers of cytokines for host defense, immunity, and inflammation. The

Table I - The function of signal transducer and activators of transcription in rheumatoid arthritis.

STAT1	Increased joint inflammation and formation of granuloma in STAT1 deficient mice. Induces caspase-1, IP-10, IRF-1, GBP1, and ICSBP that are selectively up-regulated in RA. An important factor for inflammatory cells infiltration.
STAT3	Role in survival and expansion of growth factor-dependent SF cells. Chronicity of inflammation in a murine zymosan-induced arthritis model. Suppressing the proapoptotic effects of STAT1.
STAT4	Important role in inflammation. Susceptibility for developing RA.
STAT5	Influences the survival of Tregs.
STAT6	Important role in inflammation. Regulating of arthritis severity in mice. Homeostatic role in addition to anti-inflammatory effects.

STAT, signal transducer and activators of transcription; RA, rheumatoid arthritis; SF, synovial fluid; Tregs, regulatory T cells.

involvement of STATs in RA pathogenesis is an emerging area of research and is reviewed elsewhere. Studies of STAT molecule are being revealing information about how these proteins participate in triggering transcription and the biologic effects of their activation. Understanding the pathological roles of STATs would certainly help in the development of new therapeutic strategies. Technologic advances have certainly facilitated a broad understanding of the function of STAT proteins. However, much remains to be learned. Since STATs might have divergent effects in the pathogenesis of RA, depending on cell type and possibly disease stage, it would be interesting to follow how the story unfolds over the next few years.

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