Neutrophil activation and B-cell stimulation in the pathogenesis of Felty’s syndrome

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INTRODUCTION

The pathogenesis of Felty’s syndrome (FS) is complex. To treat and manage patients with FS, a better understanding of the disease is needed. An important first step in understanding autoimmune disease in general is to identify relevant self-antigen(s). Certain autoantibodies have diagnostic importance: anti-DNA and anticyclic citrullinated peptide enzyme-linked immunosorbent assays (ELISA) are important tests to diagnose systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), respectively. Studies of autoantibodies also reveal the mechanism involved in the expression of autoantigens and their interaction with the immune system. Genetic analysis of autoantibodies has revealed that junctional diversity and somatic mutations make essential contribution to the recognition of autoantigens.1 Affinity maturation of anti-self B-cell receptors together with clonal expansion of B cells bearing such receptors is similar to that seen against foreign antigens.2 These studies have allowed a significant progress in identifying the autoimmune pathogenesis of some diseases. For SLE, RA, and FS, the role of autoantibodies in the disease process is not entirely clear, yet a plethora of associated self-specificities provide clues about the disease process. Because FS is characterized by the inflammation-driven, progressive destruction of neutrophils, we hypothesized that autoantigens generated during neutrophil activation might play a role in this disorder. In this review, we summarize studies that have contributed to our present knowledge of FS and discuss different mechanisms that have been proposed to explain its pathogenesis.

Felty’s syndrome: an “extreme” rheumatoid disease

In 1924, Dr. A.R. Felty reported 5 patients with chronic arthritis, splenomegaly, and leucopenia, thus defining a syndrome that bears his name.2 FS is estimated to occur in about 1% to 3% of RA patients after an average of 10 to 15 years of arthritis.3,4 FS differs from RA by more severe arthritis and extra-articular manifestations.4,5,7 Serologically, FS exhibits higher titters of rheumatoid factor (RF),6,7,9 antinuclear antibodies,5,10 and antihistone antibodies.11 The characteristic autoimmune response, coupled with neutropenia, crippling
arthritis, and frequent infections, prompted Sienknecht et al. to refer to FS as a "super rheumatoid" disease. Intriguingly, FS combines features of RA and SLE and displays typical clinical findings, supporting the notion that different pathogenic mechanisms may contribute to RA and FS pathogenesis.

The most critical manifestation of FS is neutropenia, resulting in a higher incidence of bacterial infection when neutrophil counts drop below 0.5 × 10^9/l. In addition, the neutrophils from FS patients are deficient in bacterial phagocytosis due to decreased expression of Fc receptors. Other factors that contribute to a higher incidence of infection in FS patients include hypocomplementemia, elevated levels of circulating immune complexes, impaired chemotaxis, and decreased superoxide production. In conjunction with neutropenia, FS patients may also present with anemia and thrombocytopenia.

Large granular lymphocytic leukemia: a close relative of Felty’s syndrome  Most patients with large granular lymphocyte (LGL) leukemia exhibit a constellation of symptoms including fatigue, weight loss, and night sweats, characteristic of leukemic disorders, but they also present with arthritis, neutropenia, and splenomegaly. The peripheral expansion of lymphocyte populations in LGL leukemia, in particular those that are derived from CD3⁺ CD8⁺ CD57⁺ T-cells, occurs in about 30% to 40% of FS patients. Moreover, about 20% to 30% of patients with T-LGL expansion have RA. Because of the shared clinical features, it has been proposed that FS and T-LGL leukemia represent 2 diseases at the opposite ends of a clinical spectrum reflecting a common mechanism.

In support of this argument, it was found that patients with FS and LGL leukemia with arthritis also share a strong association with class-II alleles, in particular with HLA-DRB1*0401. Interestingly, no such association was found in patients with LGL leukemia without arthritis, suggesting that at least 1 subset of the LGL syndrome is unrelated to FS. Furthermore, arthritis and extra-articular manifestations are usually more severe in FS than in LGL leukemia with arthritis. Notably, hepatomegaly of FS is characterized with nodular regenerative hyperplasia, portal fibrosis, and sinusoidal lymphocytosis indicative of underlying FS vasculopathy, whereas in LGL only mild sinusoidal cytotoxic T-lymphocyte (CTL) infiltration without hepatocyte necrosis is found. Each of these observations suggests that the pathogenic mechanisms of FS and T-LGL leukemia may be parallel but not mechanistically identical.

Neutropenia in FS is thought to arise from a survival defect of mature neutrophils in the periphery, which induces a compensatory myeloid hyperplasia in the bone marrow and an increase of immature granulocytes in the circulation. In contrast, neutropenia in LGL leukemia without RA is thought to reflect impaired proliferation of neutrophils in the bone marrow, because the accumulating LGL cells may displace other cells in the bone marrow and thus directly inhibit the proliferation and differentiation of granulocyte precursors. Initially, investigators believed that FS and LGL leukemia could be distinguished by determining the clonality of lymphocytes: clonally restricted lymphocytes were assumed to be a characteristic of LGL leukemia, whereas a clonally diverse T-cell pool would be more indicative of FS. Subsequent studies found clonal expansion in patients with FS, as is often the case in autoimmune diseases. On the other hand, the expansion of lymphocytes in LGL leukemia is not always restricted to a single clone, although, as disease progresses, 1 clone usually predominates. Activation of multiple T-cell clones by a chronic viral infection or an endogenous autoantigen may drive T-LGL clonal expansion. The recent finding of “clonal drift” in LGL patients supports the view that a dysregulation of the CTL repertoire homeostasis, along with a sustained immunologic stimulus, rather than a genetic defect, underlies the expansion of T-LGL leukemia cells.

Neutropenia in Felty’s syndrome  Recurrent infections due to neutropenia are the major challenge in treating FS patients. Therefore, an understanding of the causes of neutropenia and their relationship to the overall clinical picture of FS is important. Both cellular and humoral mechanisms are thought to contribute to FS neutropenia. About 77% of FS patients have antineutrophil cytoplasmic antibodies (ANCA) that do not match other typical ANCA specificities. A part of ANCA reactivity may be due to immune complexes of RF autoantibodies, as it was found that RF immune complexes have the ability to activate neutrophils. Due to the biophysical properties of the immune complexes, stronger activation was induced by precipitated rather than by soluble immune complexes or noncomplexed immunoglobulin G (IgG). Earlier studies also suggested that immune complexes have the potential to induce apoptosis of neutrophils, an effect that was dependent on reactive oxygen species, particularly hydrogen peroxide. Autoantibodies against the eukaryotic elongation factor-1A-1 that is expressed in myeloid cell precursors are present in about 66% of FS patients but not in control individuals, suggesting a possible role of autoantibodies in directly inhibiting granulopoiesis or enhancing the clearance of neutrophils. Thus, it is possible that an autoimmune process which leads to the production of RF autoantibodies, or other autoantibodies that activate neutrophils, over time contributes to FS neutropenia as a consequence of immune complex-driven neutrophil depletion.

An inherent tendency of FS sera for the activation of neutrophils is consistent with the increased activation and endothelium adherence of normal donor neutrophils that were incubated with FS sera. Similarly, mice infused with
the sera or isolated immune complexes from FS patients, showed an immediate drop of neutrophil counts, perhaps due to the sequestration of neutrophils in the spleen following their enhanced activation and increased adherence to the endothelium. Thus, the spleen may be an important organ that, following neutrophil activation in the periphery, performs multiple FS-specific roles, including neutrophil sequestration and autoantibody induction and selection. Accordingly, the splenic red pulp of FS patients expands, the sinus proliferates, macrophages infiltrate, and germinal centers develop. The role of the spleen in FS pathogenesis can also be judged from the fact that nearly 100% of the patients with FS show an improvement in their neutrophil counts within 48 hours following splenectomy. Yet, the lack of long-term benefits of splenectomy, the inconsistent correlation between splenomegaly and neutropenia, and the common clinical features and outcomes between FS and RA patients with neutropenia but without splenomegaly led to calls for dropping splenomegaly as a required criterion for diagnosing FS.

Neutrophil activation: at the center of Felty’s syndrome pathogenesis The predominance of neutrophilia in FS patients clearly points toward a connection between increased neutrophil cell death and arthritis. It remains to be established how decreased numbers of neutrophils stimulate an autoimmune response leading to arthritis. Neutropenia was proposed to be a consequence of splenic sequestration, but no evidence for neutrophil phagocytosis in the spleen of 27 FS patients could be obtained. Recently, a new form of neutrophil death, NETosis, has been discovered. It is possible that NETosis will provide some useful insights into the pathogenesis of FS. The process of NETosis receives its name from the formation of neutrophil extracellular traps (NETs), the release of nuclear chromatin from neutrophils following their enhanced activation, as may occur in sepsis. Deimination of proteins is also implicated in breaking of tolerance and promoting autoimmunity, particularly in RA. Antibodies to deiminated flaggrin, previously known as antikeratin antibodies, are strongly associated with RA. Several other deiminated proteins, including fibrin, vimentin, and collagen are recognized by autoantibodies in RA patients, and they have been identified in the synovial joints of RA patients. The relationship between RA and autoantibodies to deiminated proteins is so strong that detection of antideiminated protein antibodies forms the basis of a highly sensitive and specific RA diagnostic test. Detection of antibodies to deiminated proteins in RA patients precedes the onset of disease manifestations and their presence predicts severe erosive arthritis. All these observations point to an important pathophysiological role for deimination in the etiology of RA. Consistent with this, it was found that PAD4 mRNA is 4- to 5-fold more stable in RA-susceptible haplotypes than in nonsusceptible haplotypes.

Felty’s syndrome: new insights and unanswered questions Because FS is associated with an inflammation-driven, progressive destruction of neutrophils, and deiminated histones are quite clearly the most abundant PAD4 substrates in neutrophils exposed to inflammatory stimuli, we hypothesized that FS autoantibodies would specifically bind to deiminated histones. Thus, we tested the idea by preparing deiminated histones in vitro and assaying FS sera for autoantibodies to deiminated histones. Indeed, we found that a majority of FS sera bind deiminated histones with preference over nondeiminated histones in ELISA. In contrast, such a preference was rare in the sera of patients with RA and SLE. On western blots, we could identify the target of FS IgG antibodies as deiminated histone H3, although binding to deiminated core histones H2A and H4 and to linker histone H1 was also observed. The identification of autoantibodies to deiminated histones in FS suggests that neutrophil activation provides autoantigens that drive the immune response in this disorder. Because autoantibodies to deiminated histones are rare in RA, we concluded that they represent a population of autoantibodies that may serve to distinguish FS from RA. Moreover, it is tempting to conclude that such autoantibodies identify FS-specific immune activation mechanisms. If so, then autoantibodies to deiminated histones could serve a useful purpose in identifying and monitoring progression of FS. In addition, these results implicate NETosis in the immune stimulation of B cells in FS. How and when tolerance to deiminated histones is compromised are yet to be investigated, though our studies clearly suggest a role

Deimination of histones plays an important role in decondensation of chromatin during NET formation and identification of deiminated histones in circulation is proposed as a marker of neutrophil activation, as may occur in sepsis. Deimination of proteins is also implicated in breaking of tolerance and promoting autoimmunity, particularly in RA. Antibodies to deiminated flaggrin, previously known as antikeratin antibodies, are strongly associated with RA. Several other deiminated proteins, including fibrin, vimentin, and collagen are recognized by autoantibodies in RA patients, and they have been identified in the synovial joints of RA patients. The relationship between RA and autoantibodies to deiminated proteins is so strong that detection of antideiminated protein antibodies forms the basis of a highly sensitive and specific RA diagnostic test. Detection of antibodies to deiminated proteins in RA patients precedes the onset of disease manifestations and their presence predicts severe erosive arthritis. All these observations point to an important pathophysiological role for deimination in the etiology of RA. Consistent with this, it was found that PAD4 mRNA is 4- to 5-fold more stable in RA-susceptible haplotypes than in nonsusceptible haplotypes.
for inflammation-induced neutrophil activation and NETs in FS pathogenesis.

Our results further suggest that tolerance of deaminated histones is compromised only after a prolonged period of immune stimulation. It is not surprising that tolerance of deaminated histones is difficult to overcome as deiminated histones may have severe consequences, as implied by a comparison between RA and FS.

We also tested sera from patients with Wegener’s granulomatosis, Churg-Strauss syndrome, and patients with microscopic polyangiitis, group of vasculitis disorders characterized by the presence of ANCA, known to activate neutrophils. Notably, all 3 groups of ANCA vasculitis sera showed little-to-no binding to histones. When histone binding was observed, the preference was invariably towards nondeaminated histones rather than deaminated histones.

NETs are increasingly implicated in SLE pathogenesis and, as discussed above, they may be equally important in FS. When autoantibodies to deaminated histones from FS patients were incubated with neutrophils from nonautoimmune individuals, neutrophils were activated and deimination of histones was observed. Thus, FS sera contain substances that strongly activate mature neutrophils, induce histone deimination and NETosis, thus generating an abundant and continuous supply of autoantigens. It remains to be tested whether the “netting” neutrophils are cleared and, as discussed above, they may be equally important in FS.

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ARTYKUŁ POGLĄDOWY

Rola aktywacji neutrofili i stymulacja limfocytów B w patogenezie zespołu Felty’ego

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STRESZCZENIE
Zespół Felty’ego (Felty’s syndrome – FS) jest ciężką chorobą zapalną stawów, która charakteryzuje się przewlekłą aktywacją neutrofili oraz prowadzi do neutropenii i skłonności do nieustannych zakażeń. Zagrażające życiu objawy kliniczne FS skłaniają badaczy do ciągłych poszukiwań nowych i skutecznych metod jego leczenia. W tym artykule przeglądowym omówiono przełomowe dokonania w badaniach nad FS i skoncentrowano się na wynikach ostatnio przeprowadzonych badań dotyczących swoistości antygenowej przeciwciał występujących w surowicy pacjentów z tą chorobą. Niedawno opublikowane dane wskazują, że immunoglobuliny G (IgG) pochodzące z surowicy pacjentów z FS wybiórczo i swoistoe wiążą się z histonami poddanymi deiminacji i zewnątrzkomórkowymi sieciami chromatynowymi neutrofilów (neutrophil extracellular traps – NETs). Deiminacja jest procesem przemiany określonych reszt argininy w cytrulinę za pomocą enzymu deiminazy peptydoligina 4. Wcześniejsze obserwacje wskazują, że IgG pacjentów z FS silnie wiążą się cytrulinowanymi peptydami. Na podstawie tych obserwacji można przypuszczać, że NET-ova, rodzaj śmierci komórki, która polega na uwolnieniu sieci NET, dostarcza antygenów, które stymulują odpowiedź limfocytów B w tej grupie pacjentów. Te spostrzeżenia pozostają w zgodzie z wynikami niedawno przeprowadzonych badań nad innymi chorobami i wspiera paradygmat, że NEToza pełni wiodącą rolę w patogenezie reakcji autoimmunologicznych.

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