Biomarkers to Diagnose Early Arthritis in Patients With Psoriasis

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ABSTRACT

Background: Psoriatic arthritis is a potentially destructive, inflammatory joint disease that affects 20% to 30% of patients with psoriasis. Psoriasis precedes the onset of joint inflammation by approximately 10 years, providing a unique opportunity to intervene and prevent or delay onset of musculoskeletal manifestations. The emergence of sensitive imaging modalities and cellular biomarkers may facilitate early identification of patients with psoriasis who have subclinical joint disease and might help stratify patients with an early onset of arthritis.

Methods: The translational studies described herein are focused on the development of cellular biomarkers identified with flow cytometry and cell culture techniques in patients with psoriasis and psoriatic arthritis.

Results and Conclusion: The combination of power Doppler ultrasound imaging and cellular biomarkers (ie, CD16 and dendritic cell specific transmembrane protein) to diagnose early psoriatic arthritis and to stratify patients with established psoriatic arthritis provides a new opportunity to optimize treatment outcomes in this potentially disabling disease.

INTRODUCTION

Psoriatic arthritis, an inflammatory joint disease associated with psoriasis, affects approximately 520,000 American adults and is associated with increased morbidity and mortality.1-3 Joint inflammation and damage accelerate within the first 2 years of disease in 50% of patients who manifest bone erosions and joint space narrowing on plain x-rays.4 In addition, some patients develop pathologic new bone formation that appears radiographically as syndesmophytes, enthesophytes, periostitis, and ankylosis. Treatment intervention in established disease is centered on the use of anti–tumor necrosis factor (TNF) agents, effective in only 55% to 60% of patients5 and on methotrexate, the most commonly used therapeutic agent in established psoriatic arthritis. Methotrexate significantly relieves psoriasis but not joint inflammation, according to the results of a recent randomized trial.6 Another approach to improve treatment outcome is an early diagnosis, with recent data indicating that treatment soon after disease onset can improve outcomes.7

The long interval (10 years on average) between the first appearance of psoriatic skin plaques and

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Disclosures
Dr. Ya-Hui Grace Chiu and Dr. Christopher T. Ritchlin have no conflicts of interest to declare.

Key words: Psoriasis, psoriatic arthritis, biomarker, osteoclast, DC-STAMP, CD16, cell fusion, signaling
development of arthritis provides an unparalleled opportunity to halt joint inflammation and damage.8 Effective treatment of psoriatic arthritis in the preclinical or initial stages, however, remains an elusive goal because of diverse clinical phenotypes, a poor understanding of the mechanisms that underlie the transition from psoriasis to psoriatic arthritis, and the lack of disease-specific biomarkers that identify patients who have preclinical arthritic disease.9 To address these critical knowledge gaps, we focused our research efforts on key pathophysiologic events in the transition from psoriasis to arthritis, to develop biomarkers that facilitate early diagnosis and reveal new treatment targets. In this article, we briefly review current scientific progress in the search for arthritis biomarkers. The majority of the research discussed herein was funded by Discovery and Translational Research Awards from the National Psoriasis Foundation.

THE LINKS BETWEEN SKIN AND JOINT INFLAMMATION IN PSORIATIC ARTHRITIS

In addition to the finding that psoriasis usually precedes arthritis, several other lines of evidence support a link between psoriasis and inflammatory joint disease (Table 1). First, several genetic and environmental risk factors are shared between the two diseases.10,11 Second, patients with these disorders suffer from the same metabolic and cardiovascular comorbidities at considerably higher rates than the general population.12,13 Third, the histopathology of psoriasis and psoriatic arthritis have several common features, including the lack of an autoantigen proliferation of resident cells (ie, keratinocytes, synoviocytes) in response to an immune response by T-helper (Th) cell types 1 and 17 (ie, Th-1 and Th-17) perpetuated by infiltrating immunocytes and granulocytes in the inflammatory tissues.14-16 Moreover, the response of these two diseases to biologic therapies and cyclosporine is also quite similar.17,18 Interestingly, targeted knockout in the keratinocytes of the transcription factors c-Jun and jun-B in a murine model resulted in marked phenotypic alterations characterized by psoriasiform skin lesions and inflammatory arthritis with features remarkably similar to psoriatic arthritis.19

Studies showing abnormal imaging findings in joints and surrounding tissues of psoriasis patients who have no evidence of arthritis support the concept that psoriatic arthritis is triggered by immune inflammatory events that arise in the skin. Marked uptake in periaricular and articular structures have been reported with scintigraphy,20 and 68% of patients who have psoriasis without arthritis showed abnormal findings on magnetic resonance imaging (eg, synovitis, osteitis, erosions).21 Recently, musculoskeletal power Doppler ultrasound studies demonstrated that 50% to 75% of patients who have psoriasis without joint involvement manifest abnormal ultrasound findings (eg, tendinopathy, increased blood flow, synovitis, or bone erosion) at much higher frequencies than healthy controls.22-24 Collectively, the studies provide compelling evidence that psoriatic joint disease is initiated by immune responses that arise in the skin. If the inflammatory cascade does originate from the skin, therapies targeted to the psoriatic plaque will have the potential to block or delay the onset of arthritis.

Ultraviolet light B (UVB) treatment can lead to psoriasis remission in 27% to 60% of patients,
whereas the ability of phototherapy to block the onset or alleviate the severity of preclinical or early arthritis has not been formally addressed.\textsuperscript{25-27} The lack of data on this topic is particularly surprising, given that UV light can block inflammation through inhibition of interferon-\(\gamma\) release and suppression of the Th-17/interleukin (IL) -23 axis in psoriatic plaques.\textsuperscript{28,29} Additional support for this treatment approach was provided by studies with extracorporeal photochemotherapy (ie, psoralen plus ultraviolet light A, or PUVA), which demonstrated alleviation of joint inflammation in half of patients with psoriatic arthritis.\textsuperscript{30,31} Certainly, a prospective study to examine if aggressive treatment of psoriasis can prevent or attenuate psoriatic arthritis is of major importance, because a positive result would shift the emphasis on arthritis prevention to the dermatologist.

**DO THE EVENTS LEADING TO THE ONSET OF PSORIATIC ARTHRITIS BEGIN IN THE SKIN?**

An early event in the evolution of the psoriatic plaque is triggered by the cathelicidin LL-37, which complexes to self DNA and promotes interferon-\(\gamma\) release by plasmacytoid dendritic cells.\textsuperscript{16} This is followed by an influx of Th-1 and Th-17 cells into the dermis along with entry of dendritic cells (DCs) and monocytes that release a wide array of cytokines, including TNF, IL-1, IL-17, IL-22, and IL-6. These cytokines stimulate hyperproliferation of resident keratinocytes and granulocyte aggregation to form focal microabscesses. In envisioning which events in the plaque could generate an inflammatory erosive response in the joint, attention turns to inflammatory cells that migrate to the dermis, including T cells, monocytes, and DCs. We propose a model to explain how psoriatic arthritis originates from a cutaneous plaque (Figure 1). Monocytoid DCs activated in the skin migrate to the local lymph node and present antigen to T cells, which induces them to differentiate into pathogenic Th-17 lymphocytes. These activated T cells travel back to the skin and also circulate to the joint and bone marrow. They promote expansion of an inflammatory immune response in the skin and joint. They also stimulate the formation of both bone-resorbing osteoclasts (OCs) and bone-forming osteoblasts from mesenchymal cells in the bone marrow. In healthy individuals, bone homeostasis is maintained by a delicate interaction between immune cells and resident bone cells (Figure 1). However, upon the onset of arthritis, this homeostasis becomes uncoupled because of the imbalance in OC and osteoblast formation caused by abnormal pathogenic T-cell activation and proliferation, resulting in marked bone erosion and joint damage. Of note, TNF overproduction is highlighted in each compartment, although other inflammatory cytokines, such as IL-1, IL-6, IL-17, and IL-22, are also critically important. The differentiation of monocytes into OCs in bone marrow and joint is a pivotal step in the evolution of the destructive psoriatic arthritis phenotype (Figure 1).
MATURE OCS ARE GENERATED BY CELL-TO-CELL FUSION OF MONOCYTES

OCs are bone-resorbing cells that play a pivotal role in bone remodeling. They arise by osteoclastogenesis, a process in which monocytes fuse and differentiate into multinucleated polarized cells in response to the cytokines, receptor activator of nuclear factor κB ligand (RANKL) and macrophage colony-stimulating factor.32 Cell-to-cell fusion is a fundamental biological event essential for a variety of developmental processes, including formation of the OC polykaryon.33 During this process, a cell with OC-forming potential extends numerous pseudopods, which elongate and approach a proximal fusion partner (Figure 2). Thus, formation of the multinucleated OC results from fusion of many monocytes. In the terminal differentiation phase, the OC develops a ruffled border to bind with the bony surface and releases numerous proteases (eg, cathepsin K) and hydrogen ions required to degrade the inorganic matrix of bone.34

CIRCULATING OC PRECURSORS (OCPs) ARE ELEVATED IN PSORIATIC ARTHRITIS AND IN A SUBSET OF PATIENTS WITH PSORIASIS

We previously showed that RANKL, a molecule that promotes differentiation of monocytes into multinucleated bone-resorbing OCs, was expressed at a very high levels by synovial fibroblastoid cells in the psoriatic knee and hip.35 RANK, the receptor for RANKL, was expressed on the surface of OCPs that emerged from vessels in the deep layers of synovium and extended in a gradient to the inflamed lining layer, where they fused to form numerous large OCs.35 On the basis of our observation of RANK-positive OCPs emerging from blood vessels in the joint, we postulated that these cells arise from committed precursors in the circulation. Intriguingly, we demonstrated that OCPs were dramatically increased in psoriatic arthritis patients compared with controls, particularly for those who had bone damage on x-ray. Of note, the number of these circulating OCPs dropped rapidly after treatment with anti-TNF agents.36 An unexpected finding was that 25% of patients who had psoriasis without arthritis had elevated OCPs, and half of the patients with the highest levels developed psoriatic arthritis within 3 years.37 Collectively, these studies revealed a highly osteoclastogenic environment in the psoriatic joint, which was coupled with and achieved by a polarized differentiation of circulating monocytes toward OC development. Our results support the notion that the frequency of OCPs in peripheral blood provides an important clue to disease pathogenesis and may serve as an arthritis biomarker in psoriasis or as a severity biomarker in psoriatic arthritis. Unfortunately, the use of cell-based assays with cell culture involved is usually problematic, especially when carried out on a large scale, because it is extremely expensive, time consuming, and unreliable when multiple operators are involved. To address this important gap, we searched for surface markers of OCPs that could be recognized easily by a monoclonal antibody (mAb) to identify OCPs by flow cytometry instead of by cell culture.

Figure 2. Cell-to-cell fusion is essential for generating multinucleated osteoclasts. It is initiated by cell-to-cell contact through pseudopod extension of osteoclast precursors. Monocytes with osteoclast-forming potential will grow pseudopods during cell culture in osteoclast-promoting conditions (ie, with receptor activator of nuclear factor κB ligand and monocyte colony-stimulating factor). To achieve cell-to-cell fusion, pseudopods need to elongate and approach their fusion partners, as shown here. Dendritic cell specific transmembrane protein and its unknown ligand are involved in this process.33,51
**CELL SURFACE MARKERS: CD16 AND DC SPECIFIC TRANSMEMBRANE PROTEIN (DC-STAMP)**

**CD16**

CD16 is the low-affinity immunoglobulin G Fcγ receptor IIIa. We chose CD16 as a biomarker candidate because of its cell surface expression and elevation of a circulating proinflammatory CD14+/CD16+ cell population in many disease conditions.38–40 These cells exhibit several unique properties, with characteristics of an OCP population. The CD16+ monocyte subset is rare in healthy controls, but is preferentially expanded two- to four-fold during infection or inflammation39–44; thus, cells in this subset are recognized as nonclassical inflammatory monocytes. We found that CD14+/CD16+ monocytes are significantly elevated in the peripheral blood of patients with psoriatic arthritis.45 On the basis of the observation that CD16 was upregulated in cells cultured in OC-promoting (ie, macrophage colony-stimulating factor and RANKL) conditions but not in DC-promoting (ie, granulocyte-macrophage colony-stimulating factor and IL-4) conditions, we examined whether CD16 upregulation also occurs in vivo and identified cell subsets that expressed intermediate and high levels of CD16 in psoriatic arthritis. Intriguingly, we found that OCs arise from circulating CD16+ monocytes in psoriatic arthritis. Finally, we showed a positive correlation between the level of CD16 cell surface expression and the extent of bone resorption, which provides strong evidence that CD16+ monocytes are the major reservoir of OCPs in psoriatic arthritis.

**DC-STAMP**

We chose DC-STAMP as a potential OCP because of its cell surface location on monocytes, its critical role in OC fusion, and its expression in the endoplasmic reticulum and not the cell membrane in monocytoid DCs.33,46,48,49 DC-STAMP is a seven-pass transmembrane protein required for cell-to-cell fusion of monocytes to form OCs and giant cells.46–49 Mice lacking DC-STAMP exhibit a mild increase in bone mass as a result of impaired bone resorption (ie, few tartrate resistant acid phosphatase–positive multinucleated OCs) and increased osteoblastic bone formation.50 At the time, only a polyclonal murine antibody was available, and this, together with the lack of knowledge regarding the authentic ligand of DC-STAMP, greatly hindered our understanding of DC-STAMP function on human cells.

To address this gap, we generated a mAb, 1A2, to an epitope shared by murine and human DC-STAMP. We showed that mAb 1A2 blocked OC formation in vitro, distinguished human peripheral-blood mononuclear cells (PBMCs) with a high potential to form OCs (ie, level of surface DC-STAMP correlated with in vitro osteoclastogenesis), and tracked DC-STAMP surface expression, which declined steadily during OC differentiation.51 We identified four major patterns of DC-STAMP in human PBMCs (Figure 3). Intriguingly, patients with psoriatic arthritis have more DC-STAMP–positive cells than do healthy controls; many patients with positive cells belong to DC-STAMP pattern IV, one with very high DC-STAMP expression. The unequal distribution of DC-STAMP patterns between patients with psoriatic arthritis and healthy controls supports the concept that DC-STAMP can serve as a marker to distinguish patients with psoriatic arthritis from healthy controls. In addition, we revealed an immunoreceptor tyrosine inhibitory motif (ITIM) on its cytoplasmic tail (Figure 4). ITIMs often pair with stimulatory receptor motifs (ie, immunoreceptor tyrosine-based activation motifs) to regulate signaling events in immune and bone cells. Indeed, we also found that DC-STAMP is physically associated with CD16.
which suggests that these two molecules interact to regulate signaling during OC differentiation. A model for this interaction is shown in Figure 5.

**A UNIQUE DC-STAMP-POSITIVE T-CELL SUBSET IS EXPANDED IN PSORIATIC ARTHRITIS**

Th-17 cells are a unique T-cell subset developmentally and functionally distinct from classical Th-1 and Th-2 lineages. They are characterized by the production of IL-17 and IL-22 cytokines. Most importantly, Th-17 cells are the major pathogenic T cells that contribute to many human autoimmune diseases. Three lines of evidence support the direct involvement of Th-17 in psoriatic diseases. First, Th-17 cells are present and increased in psoriatic plaques. Second, an antibody that neutralizes the IL-12 p40 chain—thereby neutralizing both IL-23 and IL-12—has potent effects in treating psoriasis and is also effective against psoriatic arthritis. Third, Th-17 cells are elevated in the circulation of patients with psoriatic arthritis. We recently found a subset of CD4+ cells that also express DC-STAMP. The function of DC-STAMP on this population is likely related to cell signaling and not to fusion, given that ITIMs are centrally involved in T-cell activation. Of great relevance, from a translational perspective, was the finding that these cells have a Th-17 profile and are selectively increased in patients with psoriatic arthritis compared with patients who have psoriasis and with controls. This recent finding raises the possibility that the DC-STAMP-positive T cell belongs to an arthritogenic subset. Additional studies are underway to understand the importance of this cell population in psoriatic arthritis.

**FUTURE DIRECTIONS**

The results discussed here indicate that OCPs are upregulated in psoriatic arthritis patients and in a subset of patients who have psoriasis without musculoskeletal symptoms. Both CD16 and DC-STAMP are expressed on the surface of OCPs. They physically interact and possibly regulate signaling through activation and inhibition motifs during osteoclastogenesis. DC-STAMP is essential for cell fusion, a pivotal process during OC differentiation. Moreover, DC-STAMP is present on the surface of a subset of T cells that bears a Th-17 signature. Collectively, these findings support an augmented immune response that emanates from the skin, but they do not provide direct evidence to support this model.

**Figure 4.** The immunoreceptor tyrosine-based inhibitory motif (ITIM) is present in the cytoplasmic tail of mouse and human dendritic cell specific transmembrane protein (DC-STAMP). Mouse and human DC-STAMP share a high homology at the protein level and, thus, can be recognized by the anti–DC-STAMP monoclonal antibody 1A2. Both molecules have seven-pass transmembrane domains (orange labels) and six-amino-acid-long ITIMs (pink box) with one amino acid variation (human with serine and mouse with lysine on amino acid position 411).

**Figure 5.** A model of signaling cascades in the regulation of osteoclastogenesis. A dual signal is required for initiating osteoclast precursor (OCP) differentiation into mature osteoclasts (OCs). Signal 1 is from receptor activator of nuclear factor κB ligand (RANKL) engagement with receptor activator of nuclear factor κB; signal 2 is from immunoreceptor tyrosine-based activation motif (ITAM)–bearing and/or immunoreceptor tyrosine-based inhibitory motif (ITIM)–bearing receptors. Dendritic cell specific transmembrane protein (DC-STAMP) and TREM2, OSCAR, or CD16 interactions are thought to be involved in signal 2. A detailed explanation of this model can be found in the text of this article and in our previous publication.51
To formally test this hypothesis, we are examining skin biopsies from patients who have psoriasis with and without arthritis to determine if specific cell subsets (e.g., DC-STAMP–positive T cells, OCPs) or molecules (e.g., RANKL, IL-17) are selectively upregulated in skin of patients with psoriatic arthritis. The finding that RANKL is upregulated in keratinocytes of patients with psoriasis compared with patients who have systemic lupus erythematosus and in healthy controls provides preliminary evidence for an osteoclastogenic stimulus in the psoriatic plaque.60 We also are exploring the possibility that OCPs and Th-17 cells are expanded in the bone marrow of patients with psoriatic arthritis by performing bone marrow aspirates and by comparing the phenotypes of these cell populations in bone marrow with those in PBMCs. These studies will help determine if inflammatory T cells, activated by an inflammatory cutaneous response, promote arthritis by interacting with monocytoïd populations in the bone marrow.

The current challenge for clinicians is whether we can combine the imaging modalities with cellular biomarkers to optimize both patient care and treatment outcomes. The following procedures are likely to apply the strength of our current translational research to the unmet requirements for clinical diagnosis: Patients with psoriasis will be screened for musculoskeletal inflammation first by a questionnaire in the dermatology office,61 followed by testing with power Doppler ultrasound imaging and cellular biomarkers. On the basis of clinical evaluation results, imaging studies, and laboratory tests, patients at risk for arthritis may be targeted to receive more aggressive therapy (e.g., UVB phototherapy or systemic agents) to prevent or delay the onset of arthritis. Before we can recommend such a strategy, however, it is critical to understand if abnormal imaging signals or cellular biomarkers, such as DC-STAMP expression on monocytes and T cells, are predictive of subsequent arthritis.

To determine if levels of DC-STAMP on T cells or monocytes are predictive of new-onset psoriatic arthritis, we continue to collect and analyze the data on DC-STAMP expression from patients with psoriasis who are being observed on the longitudinal International Psoriatic Arthritis Research Team registry. Additionally, use of power Doppler ultrasound imaging and cellular biomarkers in early psoriatic arthritis may help distinguish patients with more aggressive disease (e.g., early erosions, widespread enthesitis) —who may benefit from anti-TNF therapies—from those who have focal mild synovitis with no erosions—who may benefit from nonsteroidal anti-inflammatory drugs or disease-modifying antirheumatic drugs. Most important, we hope to reveal novel therapeutic targets that will provide treatment options with greater efficacy and safety for patients with psoriatic arthritis.

REFERENCES


