In vitro thrombin generation.

C A Clark-Soloninka and C A Laskin

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The Journal of Rheumatology is a monthly international serial edited by Earl D. Silverman featuring research articles on clinical subjects from scientists working in rheumatology and related fields.
Magnetic Resonance Imaging and Polymyalgia Rheumatica

To the Editor:

Dr. McGonagle and colleagues are to be congratulated on their attempt to use magnetic resonance imaging (MRI) to assess the primary lesion in polymyalgia rheumatica (PMR) and remitting seronegative symmetrical synovitis with pitting edema (RS3PE) syndrome. They support the concept of a non-synovial pathology in PMR and RS3PE with prominent changes adjacent to the joint capsule. The enthesis and joint capsule were also considered by the same authors to be the primary site of inflammation in synovial/chondroarthropathies (SpA). Therefore they suggested a close relationship between these 2 entities and proposed to include these inflammatory arthropathies in the same group. However, the demographic, clinical, and immunogenetic characteristics of PMR and RS3PE syndrome are completely different from those of SpA. PMR is primarily a disease of the elderly, while clinical manifestations of SpA usually begin in late adolescence or early adulthood.

The combination of persistent pain with pronounced morning stiffness in the neck, shoulder, and pelvic girdles is the clinical hallmark of PMR. Musculoskeletal distal manifestations occur in about half the cases. Response to glucocorticoids is rapid and complete in PMR, but not as good in the SpA.

In our longstanding personal clinical experience and in the largest series reported in medical literature there is no evidence in PMR of the typical manifestations of SpA such as inflammatory spinal pain, dactylitis, enthesis (Achilles tendinitis and plantar fasciitis), and anterior uveitis. Further, the immunogenetic background of PMR and RS3PE is completely different from that of SpA. Similar to rheumatoid arthritis (RA), PMR is associated with the HLA-DR4 epitope and not with HLA-B27. In addition, PMR patients seldom develop radiological evidence of sacroiliitis and enthesitis. Finally, PMR is associated with giant cell arteritis (GCA), SpA with panaritis and inflammatory bowel disease.

In addition to all these differences, it is difficult to find similar characteristics that argue adequately for a unitary pathological process linking them. Arthroscopic synovial biopsy studies from shoulders of PMR patients with active disease have revealed mild synovitis with predominance of CD68+ macrophages and CD4+ T cells. These features are very similar to those described in vascular lesions of GCA, but different from those reported in SpA. In a case-control study, using shoulder MRI, we demonstrated that subcapsular and subdeltoid bursitis were the predominant and most frequently observed lesions in patients with active PMR, while they were less frequent and pronounced in early elderly-onset RA. Another unpublished case-control study of our group on a larger number of untreated patients at diagnosis using both shoulder ultrasonography and MRI confirms these data. However, also the other 2 synovial structures of the shoulder (gleno-humeral synovial and biceps tenosynovial membranes), even if less impressively, were involved by the PMR inflammatory process. This prominent involvement of extracapsular synovial structures in association with artricular synovitis is a likely basis for the diffuse discomfort of shoulder girdle observed in patients with PMR. It is difficult to explain conceptually how such an impressive involvement of shoulder synovial membranes could be an epiphemomenon secondary to a primary pathological process of joint capsule/enthesis.

McGonagle, et al considered enthesis as the primary lesion in every type of peripheral manifestations of SpA. If this mechanism explains the onset of artricular synovitis, it would appear less probable to support the impressive tenosynovitis present in dactylitis. The enthesis of the flexor digitorum superficialis and profundus are very thin. In contrast, tenosynovitis of dactylitis extends along the entire course of the digital synovial sheaths and when the synovial sheaths of finger communicate with the ulnar palmar sheath of the tendon swelling also extends into the palm of the hand. In peripheral arthritis, although enthesis may be the first lesion, artricular synovitis may become chronic and destructive in some patients. In these cases artricular synovitis seems to be the predominant lesion. Our group studied Achilles enthesis using ultrasonography (US) and MRI. The involvement of retrocalcaneal bursa was found in 75% of the cases. McGonagle and his colleagues hypothesized that retrocalcaneal bursitis like artricular synovitis was secondary to the enthesis. However, this bursitis may persist chronically, causing extensive erosive damage of the supraposterior aspect of the calcaneus. In addition, Lehtinen, et al have demonstrated using US that retrocalcaneal bursitis may occur without clinical and sonographic evidence of Achilles enthesis. Therefore, although bursitis is frequently associated with peripheral enthesis, it may become autonomous. Although enthesis is the hallmark of SpA both in the axial skeleton and in the limbs, not all the peripheral manifestations are due to enthesis involvement. Although McGonagle and colleagues’ hypothesis on the pathogenesis of inflammatory arthropathies based on MRI findings in early disease is intriguing, the data are too preliminary to take a definitive position. Further, concepts of disease pathogenesis need to incorporate findings from varied approaches, including studies on the epidemiological, clinical, pathological, and immunogenetic aspects as well as radiologic aspects.

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In Vitro Thrombin Generation

To the Editor:

We read with interest the article by Hanly and Smith1. We were concerned about their use of detergent in both the wash and diluents used throughout their anticoagulant assay. The NCCLS draft guidelines (1999) for anticoagulant testing specifically recommend against such an inclusion. In addition, the assay does not incorporate any standard control serum (Harris Standards, for example), which are readily available commercially. These standards would enable the use of not only a standard curve but also the GPL/MPL units used by most investigators.

The inhibition of in vitro thrombin generation by 56% of the aPL-positive plasma was an interesting observation, but even more intriguing were the 12% of plasma samples that actually accelerated thrombin generation. Could Dr. Hanly speculate how, or if, these latter samples differed from those causing thrombin inhibition? Was there an antibody or isotype specificity or clinical profile associated with the accelerated reaction?

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REFERENCE


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REFERENCES


In Vitro Thrombin Generation

To the Editor:

We thank Dr. Salvareni for commentary on the classification of arthritis and in particular on how patients with polymyalgia rheumatica (PMR) should be classified. We would clarify some of the issues raised. First, we did not state that PMR and spondyloarthropathy (SpA) were identical enthesitis-associated pathologies. Rather, we have suggested that both these entities may have a primary non-synovial-based pathology. In the case of SpA, this is intimately associated with enthesitis, but in PMR it may relate to the capsule and extra-articular synovial tissues.

We agree that the demographic, clinical, and immunogenetic features of PMR are completely different from SpA. We also agree that the authors' extensive personal experience of PMR does not support the idea of an enthesitis and HLA-B27 associated pathology. However, from a clinical viewpoint both reactive arthritis (an example of SpA) and PMR are heralded by an abrupt onset, high acute phase response at onset, and ultimately a good prognosis in the majority of cases, suggesting that both are in some way distinct from the majority of patients with rheumatoid arthritis (RA). Salvareni, et al argue that this difference relates to tenosynovitis and bursitis, both being common in PMR compared to RA. They also cite histological data supporting a primary synovial based pathology in PMR. However, while a unitary pathology remains controversial we have noted in 2 different regions of the body, namely the shoulder and the hand, that inflammatory changes in the extra-articular soft tissues adjacent to the joint capsules are common in PMR but not RA. Further, in our experience, bursitis is not more common than in RA.

These extra-articular tissue changes in PMR are reminiscent of the extra-articular changes noted by ourselves and others in SpA. Based on these observations we have suggested that a capsule based or at least a non-synovial based pathology could explain the findings in PMR that provides some kind of anatomical link with SpA. Indeed the excellent clinical studies by Salvareni and colleagues have shown extra-articular swelling of joints in PMR that supports our assertion of an extra-articular based disease process.

We argue that it is these diffuse extra-articular changes that may determine the symptomatology in PMR rather than the bursitis or tenosynovitis. However, we accept that the inflammatory reaction associated with severe synovitis could extend outside the joints in a non-specific way. This latter scenario may explain some of the symptomatology in PMR and may also explain why some patients with RA have a polymyalgic-like illness.

Salvareni and colleagues then address the issue of the relationship of enthesitis to dactylitis. We note their comments, but further high resolution MRI images using fat suppression are needed to address the issue of dactylitis in SpA. The issue of the relationship between enthesitis and bone erosions in retrocalcaneal bursitis is also addressed. While we appreciate that the erosions occur proximally to the enthesis insertion, they do nevertheless occur adjacent to the peritendinous fat-capsule (a region that like the enthesis itself is subject to considerable stressing during normal foot dorsiflexion). In relation to this we have pointed out that the enthesitis pathology is more diffuse than is generally considered.

Overall we agree that the data on enthesitis are too preliminary to state that every diseased joint and site in SpA is secondary to enthesitis. However, enthesitis and the associated osteitis is a very common theme that links all sites of involvement in SpA. The concept that inflammatory synovitis may be secondary to primary disease in other sites such as the enthesis in SpA and the capsule in PMR needs to be tested in longitudinal studies using more sensitive imaging techniques.
Dr. Hanly and Ms. Smith reply

To the Editor:

We thank these authors for their thoughtful comments on our work. We are familiar with the literature on the use of detergent in anti-carcioldin ELISA assays. When we addressed this in the context of our own laboratory assay we found that inclusion of detergent in both wash and diluent buffers provides a more reproducible and sensitive assay. However, as described in our paper, detergent is not included in the step to block nonspecific binding after the ELISA plate has been coated with cardinalin. We agree with their comment on the use of standard control sera and since the time of the initial publication we have modified our assay to include them.

We were also intrigued by the acceleration in in vitro thrombin generation by 12% of our plasma samples. As this represented only a subgroup of 7 patients, the analysis of clinical-serologic correlations is limited. As reported the most significant clinical-serologic correlations were found to be with inhibition rather than acceleration of in vitro thrombin generation. Within the group of 7 patients only 2 had a history of venous thrombosis and there did not appear to be any association with antibody specificities. We have also observed that accelerated in vitro thrombin generation can be induced by some normal plasma samples. Therefore at this stage the significance, if any, of this observation is unclear.

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John G. Hanly, MD; Stephanie A. Smith, MS.

REFERENCE

Fibromyalgia and Hypermobility

To the Editor:

Authors and editorials speculate about the association of fibromyalgia (FM) and hypermobility. After recognizing this association for over 15 years and using it clinically in caring for people with FM, I find it a very effective tool in management (Goldman, unpublished data and). A few points need to be made: (1) FM symptoms and signs are not consistent from visit to visit. On some days patients will have many tender points and on other days very few. At present, for studies we are shakelocked to the criteria, but in clinical practice we know better. FM includes a cornucopia of problems. We are aware of the complexities of this condition and can also use a lot of other portions of the examination in their therapy. FM is just not one single condition. Remember when we referred to it as fibrositis? Are we ahead? (2) The comments about hypermobility changing with age are important, and we should also consider comorbid conditions that can interfere with the diagnosis of hypermobility, like arthritis or old injuries. I also agree that localized versus generalized could make a difference in how one makes the diagnosis. The revised and old Brighton criteria will be other points in time as we search for a better way to classify hypermobility. The problem is more in the development of studies than in the practical clinical setting, where one can weigh all these factors without rigid restrictions. We need more data to define what hypermobility is, but our best data present are in the clinical findings. (3) One of the most important maneuvers a clinician can do is to point out the hypermobility to a patient with FM, explain the association, and use it as leverage to encourage an appropriate exercise program. Those who exercise do better. There is meaning in this finding. It is muscle tone, muscle mass, joint protection, and exercise “endorphins.”

We still have a lot to learn about both FM and hypermobility, but the two are interconnected.

Atlanta, Georgia, USA.

John A. Goldman, MD.

REFERENCES

Dr. Fitzcharles replies

To the Editor:

We thank Dr. Goldman for his response regarding fibromyalgia (FM) and hypermobility. His comments reflect both the wisdom and observations of an astute clinician.

The multiplicity and variability of symptoms occurring in FM are indeed difficult to understand. It is clearly easier for physicians and patients alike to accept illnesses that have objective abnormalities on physical examination or laboratory testing. We wholeheartedly agree that criteria for FM are restrictive to the clinician when addressing an individual patient, and should not be applied in this context. They are, however, a “best attempt” when defining homogeneous patient populations for the purpose of study.

We agree that there is still much to be learned regarding classification and clinical associations of hypermobility. There is, however, increasing evidence that hypermobility may be a factor in at least some patients with FM and we appreciate the input of a physician with extensive clinical experience.

Muscle deconditioning is likely a complicating factor in many patients with FM, including those with normobility. Until our understanding of the nature of this condition is improved, we too believe that exercise is to be encouraged.

Hôpital Général de Montréal, Mary-Ann Fitzcharles, MB, CHB, FRCP(C). Montréal, Canada.

Dr. Baum replies

To the Editor:

Dr. Goldman makes some interesting points but I would like to clarify from my point of view some of his statements. (1) Fibromyalgia (FM) is a puzzling condition, but most patients have a fairly consistent pattern. Criteria are set up so when we do studies the investigators have some consistency in the identification of the patients they treat. The many signs and symptoms they show does not prove that we are not dealing with a single condition. The establishment of the criteria did put us ahead. (2) Hypermobility does change with age, but in large scale studies of populations the effects of comorbid conditions are minimized. The syndrome of hypermobility can be basically put as clinically symptomatic joint pain even in a single joint if that joint is hypermobile. This is as unrestrictive as one can get while still based on the physical examination and listening to patients’ complaints. Another problem is that hypermobility decreases with age and FM increases with age. There is some overlap of these groups but they do represent “out of sync” populations. (3) I have trouble pointing out anything to
most of my patients with FM! However, I do agree with Dr. Goldman that getting them to exercise does seem to be of value. However, if it's a single joint that is hypermobile that isn't much help in the patient who has widespread pain, a major feature of FM.

I must respectfully disagree with Dr. Goldman, who has studied this relationship, but I do not believe the two are interconnected.

Allergy/Immunology and Rheumatology Unit, John Baum, MD. Rochester, New York, USA.

REFERENCES


Letters

A Distinct Syndrome Including Features of Systemic Sclerosis, Erosive Rheumatoid Arthritis, Anti-Topoisomerase Antibody, and Rheumatoid Factor

To the Editor:

While musculoskeletal symptoms are commonly encountered in patients with systemic sclerosis (SSc)1,2, an inflammatory deforming arthropathy resembling rheumatoid arthritis (RA) is rare3. We identified 4 female patients, mean age 52.5 years, who developed prominent symmetrical small joint arthritis on a background of established SSc. Clinical, radiological, and serological features are summarized in Table 1. All had pulmonary fibrosis and diffuse skin involvement. Nodules and erosive changes were seen in 3 of the 4 patients. All were rheumatoid factor (RF) positive, 3 were positive for antinuclear antibodies, 3 were anti-topoisomerase-1 antibody positive, and none was anti-centromere antibody (ACA) positive. HLA profiles in 3 of the 4 patients did not show homogeneity. Both the clinical presentation of the polyarthritis and the pattern of radiographic change seen in these patients, including erosions in the metacarpophalangeal joints and carpal bones with associated joint space narrowing and bony destruction, are more typical of rheumatoid arthritis (RA) than normally seen in SSc4. The 4 patients had autoantibodies typical of RA and diffuse SSc, with positive RF and anti-topoisomerase-1 antibodies4.

Table 1. Clinical, radiological, and serological features of the 4 patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at Diagnosis (scleroderma), yrs</th>
<th>Raynaud’s Phenomenon</th>
<th>Pulmonary Fibrosis</th>
<th>Erosive Polyarthritis</th>
<th>Rheumatoid Nodules</th>
<th>RF</th>
<th>ANA</th>
<th>Anti-Centromere Antibody</th>
<th>Anti-topoisomerase Antibody</th>
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<td>1:200</td>
<td>—</td>
<td>1:1280</td>
</tr>
</tbody>
</table>

Table 2. HLA typing of 3 patients with SSc and features of RA.

<table>
<thead>
<tr>
<th>Patient</th>
<th>HLA Type</th>
<th>DQB1</th>
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<tbody>
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<td>4, 8, 53</td>
<td>04, 0302</td>
</tr>
<tr>
<td>3</td>
<td>1, 17, 52</td>
<td>04, 0601</td>
</tr>
<tr>
<td>4</td>
<td>1, 15, 51</td>
<td>05, 0602</td>
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HLA class II profiles are shown in Table 2. Either HLA-DR4 or DR1, both frequently associated with RA, was present in the 3 patients we tested5. The DR3 and DR11 genotypes that are associated with scleroderma were not found in any patient in this report, nor in those overlap patients previously reported6. The number of patients is too small, however, to comment on the significance of the absence of these genotypes.

The pattern of synovial histology in SSc in previous studies has varied from an inflammatory synovitis to moderate or dense fibrosis of the synovium without inflammatory change7. The synovitis, when present, has been characterized by lymphocytic infiltration of the subsynovial layer, but with an absence of lymphoid follicles, little proliferation of synovial cells, and minimal pannus formation. The immunohistological features of synovium from patients with SSc and articular manifestations, however, have not previously been reported. Synovial membrane was obtained by arthroscopic biopsy from one of our study patients who developed prominent knee synovitis (Patient 2). Immunohistologic analysis revealed lining layer hyperplasia and an intense, focal, CD3 and CD4 positive mononuclear cell infiltrate (Figure 1). This pattern of immunological staining is typical of that seen in RA.

The coexistence of RA and SSc in individual patients has been controversial due both to the frequency of articular manifestations and RF seropositivity, which can occur in up to 25% of patients with SSc8. The 4 patients we describe had diagnostic features of SSc with Raynaud’s phenomenon, proximal scleroderma, and pulmonary fibrosis. All later developed a RF positive erosive arthropathy consistent with RA, suggesting the presence of an overlap syndrome. A number of the previously reported cases with coexisting SSc and RA have similar features, including the development of seropositive arthritis after onset of SSc, presence of pulmonary fibrosis, and positive anti-topoisomerase-1 antibodies in some9.

More recently, a contrasting subset of patients with an overlap of RA and limited cutaneous SSc (lcSSc) has been reported10. In this report, 3 patients developed lcSSc on a background of longstanding RA. A similar association between limited skin involvement, positive ACA, and a deforming arthropathy consistent with RA has also been described by Mihara, et al.11. These patients had marked differences in both clinical and serologic characteristics compared to those described in our report: arthritis preceded onset of SSc, skin involvement was limited rather than diffuse, pulmonary fibrosis was not present, ACA were present, and anti-topoisomerase-1 antibodies were absent. The authors suggested that an epitope spreading mechanism might be the cause of the lcSSc immune response. It is possible that a similar mechanism may operate for the group described in this study, although this hypothesis has not been formally tested.
The coexistence of these 2 diseases poses questions about pathogenesis, diagnosis, and appropriate management. The first is whether the erosive deforming arthropathy is part of the spectrum of scleroderma. This seems unlikely, due to the clinical, radiological, and immunohistochemical features typical of RA seen in these patients. Further, within this group, 2 distinct subsets of patients with overlapping features of SSc and RA with very different clinical and serologic patterns are described. Optimum management also differs for these subsets — 3 of the 4 patients described were treated as for RA with methotrexate, with a consequent improvement in symptoms and signs of joint inflammation and a fall in markers of inflammation. However, in the other subset of patients, the lcSSc component of the disease appears to be mild and does not require aggressive therapy.

While it is possible that these patients may have been affected by 2 separate diseases, the presence of a consistent clinical pattern seen in these and previously reported cases (diffuse skin involvement, pulmonary fibrosis, Raynaud’s phenomenon, RF positive erosive polyarthritis, and anti-topoisomerase antibody) corroborates the evidence for the existence of this particular subset of SSc-RA overlap syndrome.

REFERENCES


Extracellular Matrix Interactions with Osteoblasts in Osteoarthritis. A Preliminary Report

To the Editor:

Osteoarthritis (OA) is the most common joint disorder worldwide, representing one of the major causes of work disability in the active population and the main condition of disability in the total population over age 65 years. Despite this striking epidemiological impact, the etiology as well as the progression mechanisms of this disease are still relatively unknown. Several studies have shown the importance of changes occurring in articular cartilage and subchondral bone in determining this disease: slow and progressive destruction and loss of cartilage occurs, in association with a series of subchondral bone changes that lead to trabecular thickening and osteophyte formation.

Interactions between articular cartilage and subchondral bone are still unclear; however, it is generally accepted that integrity of subchondral bone components (osteoblasts, OB, osteocytes, osteoclasts, and bone matrix) is required to maintain homeostasis and overriding articular cartilage integrity. In OA the normal function of subchondral OB could be altered; therefore OB may influence bone homeostasis and remodelling (through both induction of osteoclast mediated matrix resorption and enhancement of mineral deposition) and may interfere with cartilage metabolism, possibly playing an important role in OA pathogenesis. Matrix-cellular interactions in almost all tissues play a key role in ensuring tissue homeostasis through a variety of cellular membrane receptors, including bone tissue. Among these receptors, integrins link extracellular matrix (ECM) components and the cellular membrane of bone cells, mediating cell-cell interactions. They also contribute to the signalling of several stimuli (biomechanical, chemical, and mechanical!) from the extra- to the endocellular environment, and to several cellular activities such as the modulation of protein synthesis, chemotaxis, and cell differentiation. To date, there are few studies on integrin expression in human OB in normal and in pathological conditions. We evaluated integrin expression on OB isolated from both healthy subjects and patients with OA.

We isolated human OB from subchondral cancellous bone specimens...
obtained from 4 patients (3 female, 1 male, mean age 66.5 yrs, range 65–71) undergoing total knee replacement arthroplasty for OA. Before isolation, we characterized bone specimens depending on the integrity of overlying articular cartilage, identifying 2 areas of articular damage: the minimum damage area in which cartilage appeared normal, with translucent, smooth, unbroken surface, and the maximum damage area in which the cartilage surface appeared rough and eroded, yellowish, soft, fibrillated, and even completely disintegrated. Histological examination of the macroscopic maximum damage area showed clear evidence of injury, with fibrosis, erosions (ulcerations), cracking, and disruption of the cartilage surface. In the bone underlying the area of cartilage erosion trabecular thickening, sub-articular cysts (especially where overlying cartilage was absent), and focal pressure necrosis were observed. However, in the minimum damage area the articular cartilage and subchondral bone appeared histologically less affected and in some areas resembled normal cartilage and subchondral bone. We considered OB isolated from the “minimum” and “maximum” macroscopical damage areas as 2 distinct cellular populations.

For each OA patient 8 subchondral bone specimens, all from the femoral condyles, were taken: 4 from the maximum damage area and 4 from the minimum damage area. Normal human OB isolated from cancellous bone specimens of 3 healthy subjects (1 female, 2 male, mean age 35.6 yrs, range 52–45) undergoing surgery for traumatic fracture of a lower limb were used as controls. Informed consent was obtained from all subjects.

Cancellous subchondral bone was sampled using a bone-biopsy needle to obtain bone specimens. Each specimen was washed in sterile polysaline buffer solution to eliminate medulla, then incubated with alpha-minimum essential medium (α-MEM, Gibco, Gaithersburg, MD, USA) supplemented with antibiotics and 0.5 mg/ml collagenase (Sigma, St. Louis, MO, USA) for 1 h (37°C, 5% CO₂). After incubation and removal of collagenase, each sample was incubated (37°C, 5% CO₂) in cell culture flasks in α-MEM supplemented with antibiotics and 15% fetal calf serum (FCS) (checking for residual hematopoietic bone marrow cells at 24 h), until OB adhered to the culture flasks (range 10–20 days). We evaluated basal alkaline phosphatase activity by spectrophotometric assay (Metr BioSystem, Mountain View, CA, USA) and osteocalcin synthesis after stimulation with D₃-vitamin by chemiluminescence assay (Nichols Institute Diagnostic, San Juan Capistrano, CA, USA) to confirm osteoblastic lineage of adherent cells. The values of both osteocalcin production and alkaline phosphatase activity were corrected by evaluation of intracellular total protein content, which is directly related to a total number of metabolically active cells, as described[12]. To perform the analysis, OB were detached with 1% trypsin (Gibco), then inactivated with FCS supplemented α-MEM and resuspended in polysaline buffer solution. Immunofluorescence staining was performed using monoclonal antibodies directed towards alpha (α₆, α₅) and beta (β₃, β₅, β₄, β₆) integrin chains and CD44 on cellular membranes. The negative controls were performed with an irrelevant antibody. Both direct (α₆, T-Cell Diagnostic; CD29, Serotec, Oxford, UK; CD44 and β₃, Becton-Dickinson, all fluorescein isothiocyanate labelled) and indirect (α₅, α₆, β₃, Immunotech; β₃ and β₅, Chemicon; as a second step, phycoerythrin (PE) labelled goat anti-mouse, Serotec) immunofluorescence staining was performed depending on the Mab utilized. Each Mab was incubated in the dark for 20 min (4°C), then each was washed twice and resuspended in 250 μl of polysaline buffer solution. Subsequent acquisition was performed by cytofluorimetric analysis (FlowScan, Becton Dickinson). Percentage analysis of the number of cells expressing integrin chains showed no differences in the OB populations examined, as all cells expressed the same integrin chains. Therefore the mean intensity of fluorescence for each sample and related negative control was evaluated. Each experiment was performed in triplicate. Statistical analysis was performed using ANOVA test, with p < 0.05 as a significant value. Integrin chains α₆, α₅, α₆, and β₃, showed a higher expression in the “minimum” damage OA group compared to both the “maximum” damage and normal groups (p < 0.05).

Compared to the normal group, OB from the “maximum” damage group showed a higher expression of β₃ integrin chain (p < 0.05), whereas

**Figure 1.** Expression of integrins and CD44 on osteoblast populations from patients with OA. Data are expressed as ratio between mean intensity of fluorescence of positive samples and negative controls. MAX: maximum, MIN: minimum degree of damage to overlying cartilage. *P < 0.05 MIN vs MAX and MIN; **p < 0.05 NORM vs MAX and MIN. Error bars represent standard deviation.

Letters

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OB from the "minimum" damage group showed a higher expression of \( \alpha_1 \) (p < 0.05). The expression of \( \beta \), integrin chain (very high in all groups) and CD44 was significantly higher (p < 0.05) in OB from the normal group compared to both the "minimum" and maximum damage OA groups, as illustrated in Figure 1.

ECM-cell interactions play an important role in osteoarthritic bone tissue. In this preliminary study we have shown that many integrin chains are expressed more in OB from OA patients than from healthy donors. Indeed, there are differences in the pattern of integrin expression between osteoarthritis OB populations: integrin chains \( \alpha_1 \) and \( \beta_3 \) are expressed more in minimal damage OB than either maximum damage cells or normal cells, suggesting that interactions between collagen, laminin, and other components of the ECM with bone cells are predominant when initial changes in bone structures occur, but diminish in the late stages of OA. This could reflect a distinct cellular character with regard to cellular metabolic processes such as proliferation and protein synthesis. Increased cellular metabolism may enhance bone remodelling in the early stages of OA that, associated with changes in the overlying articular cartilage, leads to a progressive decrease in articular function. In the late stages, as the articular cartilage wears away, interactions between ECM and bone cells are reduced, leading to a reduction of bone metabolic processes and remodelling, with consequent thickening of the trabeculae and bone degeneration.

University of Foggia, Francesco P. Cantatore, MD, PhD; University of Bari, Carmine A. Acquista, MD; Bari, Italy. Addolorata Corrado, MD; Florencia Iannone, MD, PhD; Giovanni Ladapula, MD.

REFERENCES


DNA Microsatellite Markers for Estrogen Receptor-\( \beta \) Are Not Associated with Systemic Lupus Erythematosus

To the Editor:

It is widely recognized that systemic lupus erythematosus (SLE) and other autoimmune diseases are more prevalent in women than in men. SLE occurs 9 times more often in women than men. Based on several lines of evidence from both human and murine studies, it has been hypothesized that sex hormones play an important role in this bias of disease susceptibility in women. Clinically, it has been shown that both female and male patients with active lupus have abnormal sex hormone levels, and that among females, the estrogens 16a-hydroxyestrone and estriol are increased significantly, while levels of several androgens, including testosterone, are decreased. Additionally, studies in which the estradiol levels in mice were manipulated also show the negative influence this hormone has on the development of autoimmune disease in NZB/NZW mice.

Genetic polymorphisms have been described linked to the \( ERB \) gene. These polymorphisms can be used as markers in genetic studies of SLE to determine whether there are associations between disease susceptibility and polymorphic alleles linked with the \( ERB \) gene loci. The \( ERB \) gene, located on chromosome 14, contains a polymorphic dinucleotide CA repeat marker that has been shown to be highly polymorphic, with its alleles widely distributed in the Japanese population.

The distribution of a series of 18 recently described highly polymorphic alleles linked to the \( ERB \) gene was examined among patients with SLE and healthy blood donors as controls. To reveal different \( ERB \) alleles, polymerase chain reaction (PCR) was performed as described; the distribution of \( ERB \) gene alleles is shown in Table 1. Thirteen of 18 alleles were widely distributed in the patient and control groups, demonstrating for the first time that this gene is highly polymorphic among a non-Japanese population.

Table 1. Estrogen receptor-beta allele marker distribution in patients with SLE.

<table>
<thead>
<tr>
<th>Marker</th>
<th>SLE Patients (n = 135*)</th>
<th>Healthy Controls (n = 164)</th>
<th>p</th>
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<tbody>
<tr>
<td>141</td>
<td>0.00(0)</td>
<td>0.00 (0)</td>
<td>—</td>
</tr>
<tr>
<td>143</td>
<td>0.00 (0)</td>
<td>0.00 (0)</td>
<td>—</td>
</tr>
<tr>
<td>145</td>
<td>0.00 (0)</td>
<td>0.00(0)</td>
<td>—</td>
</tr>
<tr>
<td>147</td>
<td>0.00 (0)</td>
<td>0.00 (0)</td>
<td>—</td>
</tr>
<tr>
<td>149</td>
<td>0.04 (5)</td>
<td>0.04 (7)</td>
<td>1.0000</td>
</tr>
<tr>
<td>151</td>
<td>0.05 (7)</td>
<td>0.06 (10)</td>
<td>0.8059</td>
</tr>
<tr>
<td>153</td>
<td>0.01 (2)</td>
<td>0.01 (1)</td>
<td>0.5909</td>
</tr>
<tr>
<td>155</td>
<td>0.11 (15)</td>
<td>0.07 (12)</td>
<td>0.3118</td>
</tr>
<tr>
<td>157</td>
<td>0.07 (10)</td>
<td>0.05 (9)</td>
<td>0.6349</td>
</tr>
<tr>
<td>159</td>
<td>0.10 (14)</td>
<td>0.05 (9)</td>
<td>0.1204</td>
</tr>
<tr>
<td>161</td>
<td>0.17 (23)</td>
<td>0.18 (29)</td>
<td>1.0000</td>
</tr>
<tr>
<td>163</td>
<td>0.24 (23)</td>
<td>0.31 (51)</td>
<td>0.2446</td>
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<tr>
<td>165</td>
<td>0.13 (17)</td>
<td>0.17 (28)</td>
<td>0.3307</td>
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<tr>
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<td>0.03 (5)</td>
<td>0.3873</td>
</tr>
<tr>
<td>169</td>
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<td>0.01 (1)</td>
<td>0.5909</td>
</tr>
<tr>
<td>171</td>
<td>0.00 (0)</td>
<td>0.01 (1)</td>
<td>0.1000</td>
</tr>
<tr>
<td>173</td>
<td>0.00 (0)</td>
<td>0.01 (1)</td>
<td>1.0000</td>
</tr>
<tr>
<td>175</td>
<td>0.00 (0)</td>
<td>0.00 (0)</td>
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</table>

* number of alleles; ^ frequency.
tion. The most common alleles in both patients and controls were ER8-163, 161, 167, 155, and 157, respectively. The alleles ER8-149, 151, 153, 169, 171, and 173 were uncommon among patients and controls, and the alleles ER8-141, 143, 145, 147, and 175 were not detected in any patient or control. Representative genotypes were confirmed by DNA sequencing of apparently homozygous patients and using homozygous cell lines. Data were analyzed by 2 tailed Fisher's exact test, odds ratio (OR), and 95% confidence interval; no statistically significant differences in the distribution of the alleles were detected between patients and controls.

While much research implicates a role for estrogen in susceptibility to SLE, using the polymorphic marker tested here, we were unable to find evidence for association with susceptibility to SLE linked to ERβ. We did find, similar to results in the Japanese population, that this marker is highly polymorphic and widely distributed among a North American Caucasian population. Studies identifying and examining new genetic markers linked to ERβ, estrogen receptor-alpha, and other sex hormone receptors remain warranted based upon the strong epidemiologic evidence implicating a role for gonadal hormones in SLE.1

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REFERENCES

Glucocorticoids But Not NSAID Abort Attacks in Hyper-IgD and Periodic Fever Syndrome

To the Editor:
Hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) has been included with familial Mediterranean fever and familial Hibernian fever, now called tumor necrosis factor receptor associated periodic syndrome (TRAPS), in a new group of disorders known as hereditary fevers. An abnormal inflammatory response seems to be involved in their pathogenesis and the genetic basis has been elucidated in all 3 (Table 1). Clinically, they are characterized by recurrent attacks of fever and different organ localized inflammation.

The fever in HIDS is often incapacitating and is associated with lymphadenopathy, abdominal pain, arthritis or arthralgia, and skin lesions. During attacks acute phase markers and serum proinflammatory cytokines [interleukin 6 (IL-6), tumor necrosis factor-a (TNF-a), and interferon-y] and some of their inhibitors (IL-1 alpha, TNF receptor 55, and TNF receptor 75) are elevated. Mononuclear cells in whom show high production of these cytokines, which persists during remission. However, the hallmark biochemical finding that permits diagnosis and differentiation from other periodic fever syndromes is a polycy whole increase in serum IgD. IgD seems to have an immunoregulatory function, but its pathogenetic role in HIDS is controversial because of the lack of correlation between serum IgD levels and onset or frequency of attacks or their severity. Moreover, no alteration in lymphocyte subpopulations or proliferative response has been reported.

Table 1. Genetic basis of the hereditary fever syndromes.

<table>
<thead>
<tr>
<th>Mode of inheritance</th>
<th>PMF</th>
<th>HIDS</th>
<th>TRAPS</th>
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</thead>
<tbody>
<tr>
<td>Chromosomal locus</td>
<td>Autoosomal</td>
<td>Autoosomal</td>
<td>Autoosomal</td>
</tr>
<tr>
<td>Mutant gene</td>
<td>MEQV</td>
<td>MVK</td>
<td>TNFR1</td>
</tr>
<tr>
<td>Encoded protein</td>
<td>Marenostatin</td>
<td>Mevalonate</td>
<td>Type 1 TNP</td>
</tr>
<tr>
<td></td>
<td>Pyrin</td>
<td>kinase</td>
<td>receptor</td>
</tr>
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</table>


We describe a 25-year-old woman of Spanish origin who had recurrent fever since age 5 years. Attacks consisted of abrupt onset fever to 39-40°C in association with sore throat, painful cervical adenopathy, headache, marked arthralgia, and rash. Symptoms relapsed every 3 to 6 weeks and lasted 3 days on average, regardless of administration of antibiotics or antipyretics. Her 22-year-old brother showed a similar but not clinical picture with 3 or 4 attacks every year. Exhaustive investigations for infectious, immunological, and tumoral causes of fever in our patient were negative. Her parents and 4 sisters were asymptomatic. Successive empirical treatments with aspirin (1 g tid for 6 mo), indomethacin (50 mg bid for 3 mo), and colchicine (1 mg daily for 3 mo) were prescribed. None of these drugs prevented or shortened the fever recurrences. A diagnosis of familial Mediterranean fever was unlikely as colchicine therapy was ineffective and soroastitis was absent. On the other hand, the apparent inheritance pattern does not exclude the probability of TRAPS. Serum IgD levels were found to increase (above 94.7 U/ml, 95th percentile of healthy controls in our laboratory) in both the woman and her brother (175 and 247 U/ml, respectively, average of 2 determinations). This is consistent with the diagnosis of HIDS. These cases were included in the International HIDS Registry (Nijmegen, Netherlands); a search for mutations in the mevalonate kinase gene is in progress. Given the severity of the attacks in our patient, she was treated empirically with 30 mg prednisone in the morning for 3 days followed by rapid tapering and withdrawal within one week. Six to 8 hours after the first dose of prednisone, fever disappeared and there was a dramatic clinical improvement of the remaining symptoms until a new attack occurred.

The prognosis of HIDS is good, and is not associated with an increased risk of infection, neoplasia, or amyloidosis. However, it has no effective treatment and the patients often have symptoms throughout life. Occasional reports have described good clinical responses to colchicine or glucocorticoid treatment. In the present case, both colchicine and nonsteroidal anti-inflammatory drugs (NSAID) failed to prevent relapses and to attenuate the clinical symptoms. In contrast, prednisone was highly and rapidly effective in aborting the attacks.

The molecular mechanisms of the anti-inflammatory effects of NSAID and glucocorticoids are different. Recently, the role of some polysinepropyl phosphates (PPIP), such as presequaleine diphosphate, in neutrophil signal transduction and modulation of inflammatory responses has been described. Changes in the intracellular availability of these compounds might mediate some of the effects of autacoids on inflammation and partially explain the anti-inflammatory action of aspirin.

Mevalonate kinase, whose activity is low in HIDS, catalyzes the phosphorylation of mevalonate, one of the early steps in the biochemical pathway to synthesize isoprenoids and steroids. An insufficient PPIP production in neutrophils derived from reduced mevalonate kinase activity could justify a low response to aspirin in this case of HIDS. On the other hand, glucocorticoids could exert their effects through a non-PPIP dependent mechanism, such as the interference of nuclear factor-κB activation.

In addition to the diagnostic and therapeutic challenges posed by HIDS,
this syndrome, as well as other hereditary fevers, represents a natural model whose study may allow better understanding of the molecular mechanisms involved in the inflammatory response.

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