Complement Determinations in the Synovial Fluid and Serum of a Patient with Erythema Nodosum Leprosum

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Erythema nodosum leprosum (ENL) is an acute reactional complication of lepromatous leprosy manifested by fever and multiple erythematous tender skin nodules. Occasionally, patients with ENL may be afflicted with iritis, neuritis, glomerulonephritis and arthritis.

The arthritis associated with ENL has been likened to rheumatoid arthritis; acute and occasionally symmetrical, involving the hands and large limb joints (6). Yet the arthritis may regress spontaneously while other components of the ENL persist (8). Furthermore, in seropositive rheumatoid arthritis, complement levels in synovial fluid are depressed, suggesting increased degradation by the immune complexes within the joint tissue and fluid (10).

This report describes complement component levels within synovial fluid and serum in a patient with acute arthritis and ENL.

Case report. As previously reported (7), a 47 year old Mexican native taking unknown medications was admitted with one week of fever, facial rash and oligoarthritis. Examination verified fever, erythematous nodular lesions over the face, arm and abdomen, lymphadenopathy, tenderness of the hands and wrists and effusion in the left ankle.

Synovial fluid demonstrated inflammatory characteristics, including a poor mucin clot, protein 4 gm%, sugar 69 mg%. In addition, multiple lepra bacilli were found free and within polymorphonuclear leukocytes and histiocytic lepra cells (Fig. 1). Buffy coat smears of peripheral blood revealed a 1+ bacteremia. Total serum protein was 6.5 gm per 100 ml (albumin 2.9 gm). VDRL, ANA and latex fixation tests were negative.

MATERIALS AND METHODS

Serum and synovial fluid were fresh frozen at -70°C. Within four weeks, C1 (10), C4 (14), and C3 activator (Behring Diagnostics) were determined by stoichiometric, hemolytic inhibition, and hemolytic techniques. C3 (β,C globulin) protein was quantitated by end point immunodiffusion using M-Partigen paks obtained from Behring Diagnostics, Sommerville, New Jersey. Immunoelectrophoresis was performed in 1% agarose using barbital buffered saline pH 8.6 in .01M EDTA (ethylene diamine tetra acetic acid) (9) against rabbit anti C3/C3c (β,C/β,A) and anti C3 activator (Behring Diagnostics).

RESULTS

The serum hemolytic activities of CH50, C1, C4, C2, C3 activator and the protein concentration of C3 were within the normal limits (Table I-A). The simultaneous synovial fluid complement activities were greater than 40% of the serum activities for all components. Synovial fluid of patients studied by Ruddy and Austen (13) with seronegative rheumatoid arthritis have similar CH50, C1, and C4 activity as found in fluids of patients with degenerative arthritis. C2 in contrast was lower in seronegative rheumatoid arthritis.
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Table 1. Complement studies in ENL.

<table>
<thead>
<tr>
<th></th>
<th>CH50</th>
<th>C1</th>
<th>C4</th>
<th>C2</th>
<th>C3</th>
<th>C1 INH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Normal serum</td>
<td>120-</td>
<td>40,000</td>
<td>30,000</td>
<td>400-</td>
<td>80-</td>
<td>27,000</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>±15,000</td>
<td>±12,000</td>
<td>600</td>
<td>140</td>
<td>±10,000</td>
</tr>
<tr>
<td>ENL serum</td>
<td>242</td>
<td>25,000</td>
<td>44,000</td>
<td>459</td>
<td>144</td>
<td>26,600</td>
</tr>
<tr>
<td>B. ENL SF</td>
<td>100</td>
<td>13,500</td>
<td>22,000</td>
<td>197</td>
<td>70</td>
<td>23,400</td>
</tr>
<tr>
<td>ENL SF units/gm prot. +</td>
<td>25</td>
<td>3,380b</td>
<td>5,500</td>
<td>49</td>
<td>17</td>
<td>5,850</td>
</tr>
<tr>
<td>RA SF seroneg. (14)*d</td>
<td>27.7</td>
<td>10,000</td>
<td>±4,000</td>
<td>±500</td>
<td>±19</td>
<td>±2</td>
</tr>
<tr>
<td>DJD SF (14)*d</td>
<td>24.3</td>
<td>7,000</td>
<td>7,800</td>
<td>130</td>
<td>18.5</td>
<td>—</td>
</tr>
<tr>
<td>FA SF seropos. (14)*d</td>
<td>11.6</td>
<td>5,000</td>
<td>700</td>
<td>25</td>
<td>14</td>
<td>—</td>
</tr>
</tbody>
</table>

\*Complement and complement component values were adjusted to units: gram protein and compared to adjusted synovial fluid values reported by Ruddy and Austen (14).
\*Normal serum C1 units of Ruddy and Austen (14) are two to four times higher than values recorded in our laboratory. Other hemolytic and protein values are comparable.
\*Mg protein: 100 ml.
\*Units: gram protein.

The total pattern of CH50, C1, C4, C2 in the synovial fluid of our patient (Table 1) is most consistent with that found in seronegative rheumatoid arthritis or degenerative joint disease.

When serum and synovial fluid were electrophoresed against antisera to C3 (β, C — β,a) similar patterns of C3 were found in normal sera and in the patient's sera (Fig. 2). Both were partially converted to β,a as evidenced by the slightly more anodal migration of the serum compared to the synovial fluid. The slight conversion of the sera to β,a may be a reflection of freezing, storage, and thawing of both samples. However, the synovial fluid showed native β,C mobility. The middle trough contains anti-C3 proactivator serum. Of interest is the fact that the synovial fluid C3 proactivator had cathodal migration compared to the serum. This implies that the C3 proactivator (factor B) was converted to the C3 activator (B) in the synovial fluid. Such conversion can ensue with activation of C3 with insoluble polysaccharides, endotoxin, or aggregated IgA (15). Zymosan-treated normal serum (not shown) gives similar C3 activator mobility as that noted in the synovial fluid.3

Fig. 2. Immunelectrophoresis of serum and synovial fluid in ENL: 1) Normal human serum (upper well); ENL synovial fluid (middle well) and ENL serum (lower well). Patterns were developed with rabbit anti β,C serum (Behring Diagnostics) placed in the first and third troughs. ENL synovial fluid did not show any anodal mobility of β,A conversion as suggested by normal and ENL serum. 2) The middle trough contains anti-C3 proactivator serum (Behring Diagnostics). Faintly visible is a precipitin arc in the synovial fluid showing more cathodal migration than that noted in the serum specimen. To clarify the position of the light anti-C3 proactivator lines a dotted line has been placed under these precipitin arcs.

1During the publication of this manuscript we have had the opportunity to study another synovial fluid sent to us from Drs. Ken Nies and Tom Rea at USC. Complement studies including CH50, C1 and C4 were similar, despite the chance that the fluid may have been thawed. IEP (immunelectrophoresis) again showed conversion of B to B.
DISCUSSION

The mechanisms producing the transient arthritis associated with ENL remain unclear. Recent reports suggest that complement-fixing immune complexes may be operative. Thus, circulating immune complexes have been defined by Clq precipitation (15), and granular deposits of immunoglobulins and complement have been demonstrated by immunofluorescence surrounding dermal vessels (13), dermal-epidermal junctions (2) and renal glomeruli (16).

In some arthritic conditions where immune complexes are thought to be operative, complement components are depressed. Thus, in seropositive rheumatoid arthritis, where immune complexes have been characterized in synovial fluid leukocytes (13) and tissue (1), the synovial fluid complement activity is markedly depressed. Furthermore IEP of synovial fluid against an antihuman C3 antiserum may show conversion products of C3 (βC to βA) demonstrated by an additional arc with more anodal migration than native βC (19).

Synovial fluids in systemic lupus, almost always have depressed CH50 values. A survey of 26 synovial fluids studied by Pekin and Zvaifler (12) revealed very low CH50 in all samples tested. The highest synovial fluid activity in the patients noted above was 50% lower than that found in the ENL synovial fluid.

In our patient, measurement of multiple complement components and degradation products in synovial fluid showed only slight evidence of alteration of complement activity. C2 hemolytic activity when measured as units/gram protein in synovial fluid was lower than that reported by Ruddy and Austin (13) in either degenerative joint disease or seronegative rheumatoid arthritis. C3 protein concentration was between that noted for seronegative arthritis and degenerative joint disease. No evidence of marked conversion of C3 (βC to C3b βA) could be determined by immunoelectrophoresis. However, synovial fluid C3 proactivator migrated slightly more cathodally than the serum C3 proactivator indicating conversion of the proactivator to the C3 activator. The reason for the C3 proactivator conversion in the synovial fluid is as yet unclear, in the presence of nonconverted C3. It is possible that early conversion of the C3 proactivator occurred as a result of either mycobacterium or immune complex activation. Alternatively freezing and thawing the sample might have resulted in conversion of C3 proactivator. Studies of C3 proactivator in other pathologic synovial fluids should resolve this question.

When complement component activity was corrected for grams of protein in the synovial fluid, the values in our patient are more closely related to those reported in seronegative rheumatoid arthritis and degenerative and inflammatory joint diseases (15, 17). Complement activity is significantly higher than that reported in systemic lupus, and seropositive rheumatoid arthritis, diseases in which immune complexes have been overwhelmingly incriminated in the pathologic response. The finding of Mycobacterium leprae free in the synovial fluid and within foamy macrophages, coupled with the relatively high values of synovial fluid complement activity, signify to us that a mechanism different from that observed in seropositive rheumatoid arthritis and lupus is involved. Such a mechanism more likely resulted from a response to the mycobacterium or its pathologic sequelae than from an immune complex activation of the complement and leukotactic systems. It is likely that with persistent degradation of lepra organisms and continued production of antimicrobial antibodies, antigen antibody complexes could be deposited in vascular endothelia. Such complexes might fix complement and perpetuate the synovial inflammation. Nonetheless, in our patient, we were not able to document evidence of marked complement component depletion.

Certainly, static measurement of complement components cannot detect minor alterations of complement synthetic and catabolic rates. Nor can a single case report provide conclusive evidence of a disease mechanism. It does provide us, however, with clues to possible mechanisms, and in ENL synovitis, this is the first report to our knowledge of complement component activity in the synovial fluid.

SUMMARY

Simultaneous serum and synovial fluid CH50, C1, C4, C2, C1 esterase inhibitor and C3 protein were determined in a patient with acute erythema nodosum leprosum. The pat-
tern of synovial fluid complement activity coupled with the demonstration of multiple lepra bacilli free and within histiocytes is more consistent with an infectious than an immune complex induced synovitis.

RESUMEN
Se determinaron en forma simultánea CH50, C1, C4, C2, inhibidor esterase de C1 y proteína de C3, en el suero y líquido sinovial de un paciente con "eritema nodosum leprosum" agudo. El patrón de actividad del complemento del líquido sinovial, junto con la demostración de múltiples bacilos de lepra libres y dentro de los histioci- tos, en más consistente con una sinovitis infecciosa que con una sinovitis por complejo inmune.

RÉSUMÉ
Chez un malade souffrant d'érythème noueux lépreux aigu, on a déterminé simultanément dans le sérum et dans le liquide synovial, le CH 50, le C1, le C4, le C2, l'inhibiteur de l'esterase C1 et la protéine C3. Le profil d'une activité complé­mentaire du liquide synovial, de même que la mise en évidence de nombreux bacilles de la lepre libre ou bien dans les histiocytes, est plus en accord avec un processus infectieux qu'avec une synovite produite par un complexe immu ne.

Acknowledgment. We wish to thank Aline Al­lenty for her competent technical assistance.

REFERENCES