Alteration of Fibrous Capsule Formation by Use of Immunomodulation

Archibald S. Miller III, MS, MD; Sharon Kaye Tarpley, RN, CNOR, CPSN; Virgil V. Willard, MD; and Gary D. Reynolds, MD, PhD

The formation of symptomatic capsular contractures remains both a mystery to the plastic surgeon and a significant clinical challenge. Ninety Sprague-Dawley rats had 2 ml dimethylpolysiloxane gel-filled implants placed in two dorsal pockets. The animals were randomly assigned to three groups. One group received daily cyclosporine injections, the second group received the saline control, and the third an active human cytokine injection. One month after surgery, the intact implants and surrounding capsules were harvested. Subjective gross examination of the capsules by three different researchers showed the cyclosporine-treated capsules to be uniformly softer, looser, and thinner than those in the other groups. The cyclosporine-treated group showed a marked decrease in absolute capsular thickness (72.4 μm) as compared with both the saline solution—(97.3 μm) and the cytokine-treated (112.4 μm) control subjects. Perivascular cuffing, lymphocytic infiltrates, and collagen organization all were also significantly affected. In this article we demonstrate how alteration of T-cell function significantly affects the cellular population and collagen organization of a typical capsule.

The occurrence of symptomatic fibrous capsular contracture after cosmetic augmentation mammoplasty or breast reconstruction is well known to plastic surgeons.1,2 Capsule formation itself is not a surgical complication. Rather, the problem arises when there is a deformation of an initially cosmetically pleasing result because the capsule has contracted, creating distortion and pain. A number of investigations have attempted to explain why unilateral contractures occur at times, or not at all.3-5 On the basis of observing large numbers of inflammatory cells and lymphocytes in capsules, some investigators have suggested subclinical infections as an explanation for why breast implant contractures occurred.6 Along with others, we believe that symptomatic capsule formation is more likely an acceleration of a foreign body response.7 Insertion of the dimethylpolysiloxane gel implant in the surgical pocket activates polymorphonuclear leukocyte (PMN) incursion and macrophage invasion and initiates a continually cascading cellular event known as a foreign body reaction (Figure 1). Contracture of the fibrous capsule in some individuals may represent the culmination of this cascading response.

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The custom-made Surgitek® implants used in this study were donated by the manufacturer, Medical Engineering Corp., Racine, WI.

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This article attempts to demonstrate that alteration of intracapsular cellular contents is now possible with immunosuppressants and immunodulators. Alterations of the afferent and central limbs of cell-mediated immunity were accomplished by use of cyclosporine and a partially purified human cytokine fraction. Specifically, cyclosporine is a fungal macrolide whose principal action is to suppress cytokine production by T-helper cells, and this is the initial response, or afferent limb of cell-mediated immunity. Cyclosporine secondarily may reduce the expression for production of interleukin-2 receptors on lymphocytes that are undergoing activation. This is a central expression of activity. Cytokines are a large group of molecules involved in extracellular communications during immune responses. Their production is a response to stimulation, either specifically or nonspecifically. This is a “central” blockage effect if production is prevented with an immunosuppressive or bypassed with the addition of an externally produced fraction such as the macrophage activation factor–rich cytokine fractions used in this study.

Each of the 90 Sprague-Dawley rats had two smooth dimethylpolysiloxane gel–implants placed in generous dorsal subpinnacellular carnosum pockets. In each group, individual implants and capsules were compared to each other, then to the cyclosporine- and cytokine-treated groups. Significant cellular differences and capsular characteristics were noted among the three groups and are now reported.

Material and Methods

Ninety male Sprague-Dawley rats were anesthetized, each with pentobarbital sodium 20 mg/kg. A 30-minute preoperative dose of cefazolin 25 mg/kg intramuscularly was given. The dorsal surgical sites were then shaved, scrubbed with Betadine®, and draped in a sterile fashion. Two incisions were made over the midlateral dorsum parallel to the long axis of the body, and two generous pockets were created by blunt dissection beneath the panniculus carnosus. Two-milliliter, sterile, hemispherical, custom-made Surgitek® implants (donated by Medical Engineering Corp., Racine, WI) were then inserted into each pocket, and the incisions were closed with interrupted 4-0 nylon sutures (Figure 2). The rats were randomly assigned to three groups for daily intraperitoneal injections of (1) cyclosporine (1.25 mg in 0.1 ml), (2) saline solution control (0.1 ml), or (3) active human cytokine fraction (0.4 mg in 0.1 ml). The active human cytokine fraction is partially purified from activated T-lymphocytes. Its purification and efficacy are well demonstrated in the literature.8

The animals were anesthetized on day 30 by the method used on day 1. Blood was drawn to measure serum levels of cyclosporine. The capsules and overlying skin were...
then removed (Figure 3), and the animals were sacrificed. The capsules, identified only by code numbers, were prepared by the following methods: (1) The specimens were fixed in 10% buffered formalin, and a single full-thickness section through each capsule and overlying skin was processed and embedded in paraffin by routine histologic methods. (2) Adjacent 6-μm sections from each block were stained with hematoxylin and eosin (H&E) and Masson’s trichrome stains by standard techniques. All stained sections were examined by the same pathologist without knowledge of the treatment protocols. (3) Small blocks of formalin-fixed tissue were fixed secondarily with 2.5% glutaraldehyde and processed for electron microscopy in a routine fashion.

The following evaluations were then performed for every capsule: (1) thickness, (2) the number of lymphocytes in the capsule, (3) perivascular and lymphoid cuffing around the small vessels of the cutis in the capsule, and (4) collagen maturity. With a calibrated ocular micrometer, 10 measurements of capsular thickness were made from each H&E section, by moving around the circumference of the capsule in random increments. Before statistical analysis was started, the two largest capsular thicknesses in each treatment group were deleted to minimize the effects of possible tangential sectioning through a folded or distorted capsule. Infiltration of lymphocytes within the wall of a capsule, as seen on the H&E sections, was graded subjectively on an arbitrary scale of (0) none, (1) slight, (2) mild or scattered, (3) moderate, and (4) dense. Assessment was made on 20 separate fields for each capsule examined. Assessment of perivascular and lymphoid cuffing was made on each H&E section by use of the same grading, and 20 fields were again evaluated.

Grading of the collagen organization within the capsular wall, on the Masson’s trichrome-stained sections, was based on the number and patterns of collagen fibers: (0) none, (1) scattered, (2) scattered groups, (3) numerous groups, and (4) highly ordered, densely packed fibers. Random specimens from each group were examined by electron microscopy to correlate those grades with the ultrastructure of the collagen fibers. Statistical analyses were performed first to detect any differences between the two capsules in each animals. A chi-square test of independence was used to determine differences between the control-treated animals.

Results

The cyclosporine mean serum level in the animals receiving it was found to be 615 mg/L. Blood from control animals was tested also, and these results were negative. The level measured has been previously shown to be therapeutically significant. No animals in any group showed signs of clini-
Table 1. Capsular thickness

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (μm)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine (n = 25)</td>
<td>11.24</td>
<td>NS</td>
</tr>
<tr>
<td>Saline solution (n = 22)</td>
<td>9.73</td>
<td>.002*</td>
</tr>
<tr>
<td>Cyclosporine (n = 25)</td>
<td>7.24</td>
<td>.0005†</td>
</tr>
</tbody>
</table>

NS, Not significant.
This represents the calculated means and represents 20 measurements per capsule in each group or approximately 1000 measurements per group.
* Versus cytokine
† Versus saline group

Table 2. Lymphocytic infiltrate

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine</td>
<td>2.34</td>
<td>NS</td>
</tr>
<tr>
<td>Saline solution*</td>
<td>2.19</td>
<td>.0001</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>1.13</td>
<td>.0001†</td>
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</tbody>
</table>

NS, Not significant
The lymphocytic grading of each injection group was done on every specimen and graded as above. Scoring was 0=none, 1=dense infiltration.
*Versus cytokine.
†Versus saline solution.

Figure 4. Each group received daily injections. Representative sections.

.0001. The same trend is noted in the saline solution group. This is demonstrated in Table 3 and Figure 5. Collagen organization was found to be highly progressed in the cyclosporine group in comparison to the cytokine group, 2.00 versus 1.62 to a P value of .0006. Again, the same trend was noted in the saline solution group. This is shown in Table 4 and Figure 6. Electron micrographic studies showed no giant cell formation in the cyclosporine group. A linear, orderly arrangement of the capsule was seen in the cyclosporine group. The saline solution and cytokine controls showed disorganization, whorling patterns, and an absolute increase in vascularity. This can be seen in Figure 7. Three separate surgeons not involved in removal of the capsules with implants graded each intact capsule/implant specimen and uniformly graded the cyclosporine group capsule as being thinnest and loosest around the implant.
**Discussion**

The physiology of the immune response is a complex, staged reaction. It can be divided into two major sections: humoral and cellular. The cellular response within the foreign body reaction when a implant is placed begins at the initial surgical insult (Figure 1). At the commencement of surgery, small luminal vessels are damaged, vasoactive amines are released, and the primary inflammatory response occurs. Vascular permeability changes yield tissue edema from serum influx. Complement is activated, chemical mediators are released, and the initial invasion of PMNs occurs. T lymphocytes ingesting autogenous debris and devitalized tissue, and a further cellular cascade results. T-lymphocyte invasion occurs, as well as other monocytioc cellular elements such as macrophages. T-lymphocyte incursion into the area of injury yields initial cytokine release, causing further cellular invasion in response to the foreign body or implant, but there is not a specific antigenic recognition. Nonsensitized T cells undergo blastogenesis, yielding an amplified cellular response. This reaction will continue until the offending agent is removed. The cytokine fraction has been shown to bypass the central limb of immunity and lead to increased phagocytosis, chemotaxis, and intracellular killing.

It is interesting to note that capsular thickness was related to the number of inflammatory cells present and was inversely proportional to collagen “maturity” or organization. That is, thick capsules had marked perivascular infiltrates and thinner ones did not (Figures 5 and 6).
Table 3. Perivascular cuffing

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine</td>
<td>1.60</td>
<td>NS</td>
</tr>
<tr>
<td>Saline*</td>
<td>1.22</td>
<td>.0001†</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>0.15</td>
<td>.0001†</td>
</tr>
</tbody>
</table>

NS, Not significant.
Perivascular cuffing was graded as mononuclear cell populations surrounding blood vessels in the capsule. Scaling was 0=none, 1=dense cellular cuffing.
*Versus cytokine.
†Versus saline solution.

Table 4. Collagen content

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine</td>
<td>1.62</td>
<td>NS</td>
</tr>
<tr>
<td>Saline*</td>
<td>1.36</td>
<td>.0006†</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>2.00</td>
<td>.0006†</td>
</tr>
</tbody>
</table>

NS, Not significant.
The collagen content was evaluated after staining and a significant alteration was noted between groups.
*Versus cytokine.
†Versus saline solution.

This correlation between inflammatory infiltration and capsular thickness has been reported in the past. In comparing the specimens in the electron micrographs seen in Figure 7, the cytokine capsule shows numerous inflammatory cells with loose, whorling collagen fibrils and increased vascularity. The saline solution control has fewer cells and partially polymerized collagen fibers. Finally, the cyclosporine capsule shows mainly fibroblasts in highly organized linear collagen bundles and few inflammatory cells. Cyclosporine seems to interfere with lymphoblastic metabolism, which yields a functional decrease in lymphocyte reactivity. However, this is only seen if it is given immediately before or just after an immunologic challenge. This suggests that “helper” lymphocyte replication is also inhibited. This alters helper–to–suppressor cell ratios adversely, without having an effect on nonspecific inflammatory cell reactivity.

Our results show the lymphocytic response to polysiloxane gel implants in the cyclosporine-treated rats decreased (Figures 5 and 7). Capsular thickness was also significantly less in cyclosporine-treated rats (Figure 4).

This response may have been due to suppression of the normal T-lymphocyte cascade reaction, thereby decreasing interstitial fluid content and encouraging collagen cross-linking at an earlier phase of wound healing in comparison to the cytokine group. Although it seems logical that a decrease in the cellular response would have delayed wound healing or allowed bacterial growth, there were no infections or implants dehiscence in the cyclosporine group.

Comparison of each of the groups to the other leads to some interesting questions that will need to be addressed in future studies. The positive control group or cytokine group showed a markedly increased inflammatory reaction and disorganized collagen fibrils. This, however, has been proposed to be the type of capsule that has been...
associated with minimal contractures. The cyclosporine group shows a highly organized, densely packed collagen network that has been shown to be associated with capsular contractures. The saline solution group differed only slightly from the cyclosporine group. The cyclosporine capsules were thin and diaphanous (Figure 3), whereas the saline groups were thicker.

This initial study helps formulate the following questions:

1. Should a positive control such as interleukin-2 or macrophage activation factor be included in future capsular studies to help elucidate cell populations directly involved in contracture?

2. Can cyclosporine or other immunosuppressants be used to selectively inhibit cellular population until those cells not involved in contracture can be eliminated?

3. Would it be possible to chemically bond an immunosuppressant to implant shells, effectively “blinding” the patient’s cellular immune response to the implant’s presence, and would this encourage dehiscence?

The scope of this study was to show that immunologic mechanisms and their alterations are keys to understanding capsular contractures. More definitive studies such as the measurement of intraluminal capsular pressures, specific analysis of collagen type ratios, identification of total typical capsular cellular contents such as long-term giant cell formation, and prolonged studies with greater numbers of animals will surely follow.

Conclusion

We have shown a pharmacologic manipulation of immune mechanisms involved in dimethyldiolysiloxane gel capsular formation. In vivo alteration of the afferent and central limbs of the immune response led to significant differences in capsular characteristics. Therefore further studies may allow this easily reproducible animal model to delineate specific cell populations involved in capsular contracture because it contains both a positive and negative control. Further understanding of the early mechanisms of capsule formation and the possible manipulation of those mechanisms are the next steps in definitive treatment, prevention, and understanding of this clinical dilemma.

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References


