

Chapter II.

Clinical Immunology

Principal Author:

Betty A. Diamond, MD

Professor, Department of Microbiology and Immunology

Albert Einstein College of Medicine

Bronx, New York

Table of Contents

I.	Introduction to the Immune System	II - 1
	Antigen Stimulation: Role of MHC	II - 2
	Production of Antibodies by B Cells	II - 3
	Autoimmunity	II - 3
II.	Studies on the Immune Response to Silicone in Animals	II - 4
III.	Analytic Approach to Studies in Humans	II - 5
IV.	Studies of the Innate Immune Response	II - 8
	Cytokines as an Indicator of Inflammation	II - 8
	Natural Killer Cell Function	II - 9
	Superantigen Activity of SBI	II - 10
V.	Studies of the Adaptive Immune Response	II -11
	HLA in Symptomatic Women with SBI	II -11
	T Cell Activation in Women with SBI	II -13
	Anti-Nuclear Antibodies	II -16
	Patient Selection	II -19
	Control Population	II -20
	Methodology for ANA Determination	II -20
	Multiple Samples	II -20
	Prospective Studies	II -20
	Summary of ANA Results	II -20
	Specific Autoantibodies	II -21
	Anti-collagen Antibodies	II -21
	Anti-microsomal Antibodies	II -23
	Anti-silicone Antibodies	II -23
	Anti-polymer Antibodies	II -24
VI.	Monoclonal Gammopathy & Multiple Myeloma	II -25
VII.	Summary	II -27
	References	II -28
	Table 1	II -39

Chapter II

Clinical Immunology

Concerns have been raised by both physicians and women with silicone breast implants (SBI) that SBI may be causing rheumatic disease in women. Initial concerns focused on the possibility of an increased incidence of defined rheumatic disease; subsequently it has been suggested that SBI might cause a constellation of symptoms often found in women with rheumatic diseases that includes muscle and joint pain, but differs from previously defined diseases. Because most rheumatic diseases are associated with an inflammatory component and many are associated with autoantibodies or autoreactive T cells (see below), it was logical to look in women with SBI for activation of the immune system. It has been hypothesized that an immune response to self-tissue or to silicone itself might occur in these women. Thus, several studies have looked for silicone-reactive cells or antibody, or autoantibodies. The following review summarizes the clinical evidence for silicone-induced immune alterations in women with SBI.

I. Introduction to the Immune System

The immune response is dependent on a variety of inter-related cells and secreted molecules. A normal immune response consists of two phases, an early innate response that represents a nonspecific inflammatory reaction to a foreign substance (antigen) and a later adaptive response that is specific for the particular foreign substance or antigen that has entered the host. The innate (nonspecific) inflammatory immune response is a function of components of the immune system that are available immediately upon antigen exposure. Activation of these components is not antigen-specific and is independent of prior contact with antigen. There are both soluble and cellular components of the innate immune response. Soluble components include circulating "natural" antibodies (circulating proteins that bind to antigens), and the complement system (circulating proteins that bind antigen alone or antigen-antibody complexes and help mediate the destruction or removal of antigen). Cellular components include leukocytes, macrophages, and natural killer (NK) cells, which are circulating or tissue-bound activators of inflammation. In addition, the innate immune response includes products secreted by these cells such as cytokines, chemokines, and other proinflammatory molecules. Activation of the innate immune response leads to the secretion of cytokines and other molecules that help initiate adaptive or antigen-

specific immune responses. Defects in innate immunity most often lead to immunosuppression; however, some genetic defects in the complement system increase susceptibility to autoimmune disease. In general, however, activation of the innate immune response, or inflammation, cannot be considered evidence of an autoimmune disease.

Adaptive immune response (antigen-specific) refers to the activation of those components of the immune system that expand and mature as a result of exposure to specific antigen.

Components of the adaptive immune response include antigen-presenting cells (APC) and antigen-specific lymphocytes, T and B cells, which circulate and become sequestered at sites of antigen presentation, and release their secreted products, cytokines and antibodies, upon contact with the specific antigen that initiated the response.

Antigen Stimulation: Role of MHC

To trigger an adaptive immune response, an ordered sequence of events must occur. First, antigen-presenting cells must encounter antigen and become activated. The precise molecular mechanisms for the activation of antigen-presenting cells are not always known; however, activation can be enhanced in vivo by the use of adjuvants, compounds which potentiate immune responses when given with antigen. Following activation of antigen-presenting cells, antigen is engulfed and processed internally. Fragments of antigen are then returned to the cell surface nestled in the groove of Class I or Class II molecules encoded by genes in the major histocompatibility complex (MHC). The MHC, also called HLA (human leukocyte antigen) in humans, consists of a series of linked polymorphic genes such that in a population there are large numbers of HLA haplotypes or combinations of HLA genes. Each individual inherits two of these combinations. Molecules of each HLA haplotype are capable of associating with a distinct subset of antigenic fragments. The antigen inside the MHC groove exists almost always as a peptide. Recently, it has been shown that carbohydrate or otherwise modified peptides and even lipids can be bound by MHC. The MHC/antigen complex is recognized by T cells and, provided that a second set of signals is also delivered to the T cell by the APC, activation ensues. Once the T cell is activated, proliferation and cytokine secretion occur. The T cells become memory T cells which can more easily mount a response upon a second encounter with antigen.

Production of Antibodies by B Cells

B cells can recognize a variety of different antigens including proteins, carbohydrates, and nucleic acids. B cells in the preimmune state make IgM antibodies. For B cell activation to occur, the B cell surface receptor, i.e., membrane immunoglobulin, must bind antigen or an antigenic complex. In addition, B cells must receive help from T cells that recognize a component of the same antigen. Once B cells are activated, they proliferate, secrete antibodies that are specific for the inducing antigen, and generate memory B cells that can respond more quickly on subsequent encounters with antigen. The antibodies produced in a memory response bind more specifically and more strongly to the antigen. These antigen-induced antibodies can be identified by the presence of amino acid substitutions that result in increased affinity for antigen and by switching of the antibody isotype from IgM to IgG.

Autoimmunity

Because there are effective mechanisms to prevent an immune response against one's own tissue, most immune responses to foreign substances will not result in the activation of a sustained or pathogenic autoimmune response. In some individuals, however, these mechanisms fail and autoimmune disease may arise. Autoimmune diseases are the result of immune reactivity to one's own tissues or molecules and represent an aberrant activation of adaptive immunity. It is currently believed that there are two main features that contribute to the development of autoimmune disease. First, there are genetic risk factors. Often these include HLA haplotype. Since autoimmune diseases are characterized by the activation of T or B lymphocytes specific for autoantigens and T cell activation depends on T cell receptor recognition of a complex of antigenic peptide and an HLA molecule, many autoimmune diseases occur more frequently in individuals with a particular class I or class II HLA haplotype or allele. There are additional, non-HLA, susceptibility genes. Some of these may be shared among individuals with different autoimmune diseases, but others appear to be disease-specific. Second, there are environmental risk factors. Exposure to environmental triggers often appears necessary to precipitate an autoimmune disease in a genetically susceptible individual. In most cases, the nature of these triggers remains unknown. As initial case reports of rheumatic-like disease in women with SBI came out, it was therefore reasonable to question whether SBI might be such a trigger in susceptible individuals.

II. Studies on the Immune Response to Silicone in Animals

While there are species-specific aspects of the immune system, in general it is standard to gain insight into potential human immune responses by studying the immune system of an animal, especially the rat or mouse. The animal studies of the effect of silicone gel or other components of silicone breast implants (SBI) on the immune system are clearly more numerous and comprehensive than the human studies. They have resulted in several observations regarding components of SBI and the immune system. As reviewed in Chapter 1 of this report, silicone gel may function as a weak adjuvant potentiating antigen-specific immune responses. When antigen emulsified in silicone gel was administered to mice or rats, an antibody response developed which was greater than the response that occurred when antigen alone is given (Hill et al., 1996; Naim et al., 1995, 1997). Silicone gel was a less strong adjuvant than complete Freund's adjuvant (CFA), the adjuvant commonly used experimentally to induce immune responses in animals (Chang, 1993). It is important to note that the enhancement of immune responses occurred only when silicone and antigen were given together as an emulsion. Administration of antigen to one site and silicone gel to another did not lead to potentiation of an antigen-specific response (Klykken and White, 1996).

A second observation from animal studies was that administration of silicone gel to animals appears to cause a time-dependent reduction of NK cell activity when tested in vivo assays (Bradley et al., 1994; Wilson and Munson, 1996). NK cells are a component of the innate immune response and are believed to play a role in the early response to infection and in control of tumor growth. It is important to note that the reduction of NK activity in animals after silicone exposure did not correlate with enhanced tumor growth. Thus, the physiologic importance of the observation remains unclear.

Finally, silicone gel alone induced disease in two animal models. (1) It caused arthritis when injected intra-articularly in Dark Agouti rats (Yoshino, 1994). Since it did not induce arthritis when injected distal to the joints, and did not induce arthritis in joints that were not injected, disease induction appears to be a consequence of local rather than systemic inflammation. This finding is analogous to what is seen with silicone joint prostheses. (2) It induced B cell tumors, plasmacytomas, in BALB/c mice when injected intraperitoneally (Potter et al., 1996). In each of these situations, the animal was genetically predisposed to develop disease and other triggers for disease induction have been identified. Several other studies in animal models of autoimmune

disease have demonstrated that silicone neither triggers disease nor exacerbates the clinical course of ongoing disease (see Chapter 1). Nevertheless, the data showing an effect of silicone on the immune system in some strains of animals have led to a concern that silicone might have deleterious effects in humans or in a subset of humans.

III. Analytic Approach to Studies in Humans

It has been suggested that exposure to SBI may cause an autoimmune disease, although there has yet to be an agreed-upon definition of the symptom complex present in this putative disease. In general, autoimmune diseases have the following characteristics (Rose and Bona, 1993; Shoeneld and Isenberg, 1989):

1. A greater than expected frequency of a particular HLA antigen in the affected population. This HLA association reflects the importance of the HLA-antigen complex in activating T cells.
2. Evidence of activation of cells of the immune system. Most often this is demonstrated by the presence of an inflammatory infiltrate or deposition of antibodies in affected tissues. It has also been possible in recent years to demonstrate an unusually high concentration of cytokines present in the serum of some individuals experiencing ongoing immune activation. Nevertheless, inflammation alone is not sufficient to indicate the presence of an autoimmune disease.
3. Reactivity to self-antigens. This antiself- reactivity requires that specific autoantibodies be present or that T cells can be shown to become activated upon exposure to self-antigen and antigen-presenting cells.
4. Transfer of disease in animal models by transfer of lymphoid cells or antibodies. This criterion cannot be met by human studies, although it has been possible on occasion to transfer disease to immunodeficient mice using human cells or serum.

In assessing whether an immunologic disease develops upon exposure to SBI, it might also be asked whether there is evidence for an immune response to material present in SBI, either antibodies or T cells, or whether there is evidence for a clonal expansion of particular subsets of B or T cells following exposure to silicone.

Thus, in considering whether SBI cause an autoimmune disease or cause systemic activation of the immune system, it is possible to ask whether there is evidence for the conditions outlined above. In the review of the literature that follows, we address these questions and ask broadly whether there is evidence of any immunologic abnormality as well as evidence for autoreactivity or a silicone-specific immune response.

Two questions need to be kept in mind when analyzing the studies that have been performed in humans to determine whether there is either an immunologic abnormality in all women with SBI or an abnormality in symptomatic women with SBI.

1. Is there an immune abnormality caused by SBI? To address this question, it is important to compare immune function in healthy individuals without SBI to function in healthy individuals with SBI.
2. Is there a specific immune abnormality associated with clinical symptoms in women with SBI? To address this question, it is necessary to compare healthy women with SBI to symptomatic women with SBI, and symptomatic women with SBI to symptomatic women without SBI.

Addressing these questions alone cannot determine whether SBI cause disease. If all women with SBI (healthy and unwell) have an abnormal immunologic profile, then one must ask whether these immunologic abnormalities might reasonably be expected to cause disease. If only unwell women with SBI have an abnormal immunologic profile, one must ask whether the immunologic abnormality is present in symptomatic women without SBI. If this profile is seen in unwell women without SBI, then the question must be asked whether women with SBI have a greater incidence of particular diseases or of "unwellness" than the female population generally. This question is addressed in epidemiologic studies on connective tissue diseases and SBI. It is also important when asking these questions, to ascertain whether the women with SBI with an abnormal immune response are those women who received SBI as part of reconstructive surgery following

mastectomy for breast cancer, because cancer itself may cause certain alterations in the immune system (Kavanaugh and Carbone, 1996). Finally, if there is no abnormal immunologic profile that can be reliably and reproducibly identified in the available studies in either healthy or "unwell" women with SBI, it is still possible that a small number of women at risk for an SBI- induced immunologic disturbance elude detection in those studies that have been performed, or that the induced immunologic disturbance has not yet been identified.

It is possible to look for disturbances in both the innate and the adaptive immune response. Furthermore, within the adaptive immune response, it is possible to look for silicone-specific T or B cell activation or activation of autoreactive lymphoid cells. The studies that have been performed in humans address the following questions:

1. Is there an alteration in innate immunity?
 - a. Is there evidence for monocyte or T cell activation as determined by assays of cytokine levels?
 - b. Is there evidence that material derived from SBI can act as a T cell superantigen, a molecule that activates large numbers of T cells that have different antigenic specificities but share some structural feature of the T cell receptor?
 - c. Is there evidence for an effect on NK cell activity?

2. Is there an alteration in adaptive immunity?
 - a. Is there a particular HLA haplotype present in women with SBI and symptoms?
 - b. Is there evidence for activation of silicone-specific T cells in women with SBI?
 - c. Is there activation of silicone-specific B cells in women with SBI?
 - d. Is there evidence of T or B cell autoreactivity in women with SBI?

Case reports are not used because they include no control group and abstracts are not included because it is not possible to analyze the methodology used. Full-length articles that were not subject to peer review are included. Because of the dearth of studies, certain potential exclusion criteria for substandard methodologies have not been applied. For example, studies which use historical controls were included, but are identified. Studies in which the control group was not age-matched or sex-matched are also included, but identified. In general, both age and

sex affect parameters of the immune response. There is no discussion of the source of funding and no exclusion based on this.

IV. Studies of the Innate Immune Response

Cytokines as an Indicator of Inflammation

Cytokines are secreted by activated cells, whether activation is antigen-specific or not. Cytokines are made by activated monocytes and macrophages and so are a measure of their activation, but are also made by other cell types, (e.g., NK, B cells, T cells, and fibroblasts) and so may also be a measure of their activation. The presence of increased cytokine levels suggests ongoing inflammation but not necessarily an antigen-specific response. The studies reviewed here address whether there are increased levels of cytokines in women with SBI. A finding of increased cytokine levels might help identify a population of activated cells (e.g., NK cells, monocytes, T cells) but would not indicate an autoimmune response, nor even an antigen-specific response. While cytokine elevations are found in some patients with autoimmune disease, they are not specific to the autoimmune state. It is also important to note when considering negative data that, in general, cytokines function locally at the site of inflammation. It is possible to have an ongoing local immunologic reaction without detectable elevations of cytokines in peripheral blood. Almost all the studies that have analyzed cytokine levels in women with SBI and a comparative control group have assayed serum or plasma levels, rather than tissue concentrations.

Furthermore, only a limited number of cytokines have been analyzed. Most studies of cytokines in breast tissue are case reports without surgical control tissue. The exception is a study by Mena et al. (1994) in which soluble mediators of inflammation were measured in capsular tissue from women with SBI undergoing explantation, in skin scar tissue from women undergoing reverse augmentation mammoplasty, and in synovial tissue of individuals with unspecified forms of arthritis. Tissue was cultured for 24 hours in vitro after which time supernatants were harvested and levels of IL-2, IL-6, TNF α , and PGE2 were measured. There was no significant difference in cytokine production between capsular tissue and control tissues. Thus, cytokine production from breast tissue exposed to SBI and from skin tissue exposed to previous surgery is not distinguishable. Furthermore, among women undergoing explantation, there was no relationship between systemic symptoms and cytokine production in capsular tissue. No studies have been published on cytokine levels in joints of women with SBI or any tissue potentially affected by SBI

but distal from the implant.

Ojo-Amaize et al. (1994) studied IL-1 β , a soluble mediator of inflammation, and IL-1 receptor antagonist, a soluble IL-1 receptor that is also elevated in inflammation, in the blood of women with SBI (well and unwell). While differences were found between women with SBI and healthy age-matched controls, no conclusions can be drawn regarding the relationship between cytokine production and SBI, because of the lack of crucial control groups. There is no comparison of symptomatic women with SBI and symptomatic women without SBI to determine if certain symptoms are always associated with altered cytokine levels, independent of the presence of SBI. There is also no analysis of symptomatic women with SBI compared to well women with SBI to determine whether SBI routinely cause an increase in these cytokines.

In other studies, no differences in cytokine levels were found. Zazgornik et al. (1996) studied two cytokines, TNF α , and IL-6, as well as soluble TNF receptor in a very small number of women with SBI compared to an age- and sex-matched group of surgical patients and found no significant differences. Garland et al. (1996) studied IL-6 levels in women with SBI (health status not reported) and age-matched women without SBI. There was no difference in the level of IL-6. Blackburn, Gotting, and Everson (1997) studied IL-6, IL-8, TNF α , and soluble IL-2 receptor in symptomatic women with SBI compared to healthy age-matched controls. Levels for all cytokines assayed were below the threshold of sensitivity for the assays, so no conclusions regarding differences between these groups can be drawn. Of interest, these investigators were able to demonstrate elevated levels of these cytokines in the blood of patients with active rheumatoid arthritis.

Overall, these studies have failed to identify a SBI-induced increase in the level of any cytokine studied in serum or plasma of women with SBI. Thus, while these studies cannot be considered comprehensive and conclusive, they do not provide evidence for systemic immune activation in women with SBI.

Natural Killer Cell Function

Studies reviewed here address whether SBI alter NK cell activity, a component of the innate immune response. NK cells are non-B, non-T lymphoid cells thought to play a critical role in innate immunity to infection and in tumor surveillance. They can be distinguished from other cells of the immune system by morphologic criteria and by expression of particular surface markers.

Some reports indicate there may be decreased NK activity in certain autoimmune diseases (Blaszczyk et al., 1987; Sibbitt and Bankhurst, 1985; Struyf et al., 1990) but there are no mechanistic data to link this decrease to the development of autoimmune disease.

The initial suggestion that NK cells might be altered by silicone gel exposure came from animal toxicology studies (see Chapter 1). Vojdani, Brauther, and Campbell studied NK activity in women with SBI (Campbell et al., 1994). In this study, peripheral blood mononuclear cells from 40 symptomatic women with SBI were assayed for NK cell function prior to and following removal of the SBI. NK activity increased in 50% of the women following explantation, but decreased in 26% and was unchanged in 24%. These results are difficult to interpret. Since only a single determination of NK activity was made prior to or post explantation, the degree to which alterations in NK activity represent variations in the assay or normal fluxes within an individual was not ascertained. Furthermore, the absence of control groups of asymptomatic women without SBI, asymptomatic women with SBI, or symptomatic women without SBI leaves unresolved the relationship of the decreased NK activity to silicone exposure and the relationship of decreased NK activity to a constellation of symptoms. In another study (Granchi et al., 1995), NK activity appears to be enhanced, not diminished, in a group of women with SBI and severe local contracture. Thus, there is no reproducible observation on the effect of SBI on NK cell activity in women. In addition, there is no evidence that the reported alterations in NK activity would have functional significance. Finally, it has been well documented that NK activity is altered by stress, sleep deprivation, and medications (cortisone) even among healthy individuals (Irwin et al., 1994, 1996; Pedersen and Ullum, 1994; Pedersen et al., 1986; Shepard and Shek, 1996; van Ierssel et al., 1996). Thus, controls for stress and medication would be appropriate.

Superantigen Activity of SBI

One hypothesis that has been proposed is that silicone is a T cell superantigen. Superantigens are molecules that bind to the T cell receptor. They do not bind at the conventional T cell receptor antigen binding site, but rather bind to all T cells expressing a particular structural component of the T cell receptor. Superantigens, therefore, activate many more T cells than are activated by conventional antigen and lead to a massive secretion of cytokines that can cause vascular collapse, as in toxic shock syndrome. One study has examined whether silica, rather than silicone which is the major constituent of SBI, might be a superantigen (Ueki et al., 1994). Chrysotile was

incubated with peripheral blood T cells of 3 healthy individuals. In two individuals, there was reported augmentation of V β 5.3 expressing T cells and in one of V β 6.7 expressing T cells. Proliferation of T cells expressing particular V β chains is consistent with superantigenic stimulation. Only three individuals were studied. The authors concluded that two individuals showed a similar pattern of T cell activation, although the data shown do not clearly demonstrate this. Furthermore, there are internal inconsistencies in the data that make it difficult to be confident of the methodology. For example, the number of V β 5.2 and V β 5.3 T cells present in cell cultures is far less than the number of V β 5.3 cells alone. Thus, the specificity of the antibody staining is questionable. A study by O'Hanlon et al. (1996) on 20 capsular tissue samples used a different technique, polymerase chain reaction detection of T cell receptor mRNA, to look for a restriction of T cell receptors in T cells activated at the site of SBI. In 14 samples, T cell receptor mRNA was detected. There was great variability in T cell receptor gene expression, an observation that suggests SBI do not contain a T cell superantigen. Thus, there is no evidence at this point for superantigen stimulation of T cells by SBI materials.

V. Studies of the Adaptive Immune Response

HLA in Symptomatic Women with SBI

An investigation of an adaptive immune response to silicone begins with a search for HLA haplotypes that might mediate the antigen-specific response. Studies of susceptibility genes for immunologically mediated disease are still in their infancy in humans; however, one genetic locus that has been well studied in human autoimmune disease and has been shown to constitute a risk factor in many diseases is HLA. Since the structure of an HLA molecule will affect the nature of the peptide antigen it is able to bind and present to T cells, it is logical that HLA may constitute a significant risk factor or protective factor for autoimmune disease. There are multiple different HLA haplotypes in humans. Definite HLA associations have been described for a number of human autoimmune diseases, of which the best known are ankylosing spondylitis, juvenile diabetes, and rheumatoid arthritis. It must be noted that the presence of a disease-associated HLA molecule does not ensure disease. Even among individuals possessing two copies of the allele that confers the greatest risk of rheumatoid arthritis, only one in seven will develop disease. In contrast, one in 600 individuals without a disease susceptibility allele will develop disease. Thus, presence of the susceptibility allele does not guarantee disease, but indicates that disease might be

more easily triggered in that individual than in an individual lacking the susceptibility allele. Conversely, absence of a susceptibility allele does not ensure against disease.

Since antigen-specific T cells are activated by an antigen/HLA complex, it is reasonable to ask whether symptomatic patients with SBI share particular MHC alleles and whether these are the same as or different from those found in women with a similar symptom complex but without SBI. It is important in these types of studies that the populations are matched for ethnicity since a susceptibility allele may be disease-associated in one ethnic population but not in another due to the presence or absence of other shared genes. For example, the HLA DR4 alleles that mediate susceptibility to rheumatoid arthritis in individuals of Northern European ancestry do not associate with susceptibility in Black or Hispanic populations (McDaniel et al., 1995; Teller et al., 1996).

Only two studies have focused on identifying an HLA haplotype that associates with symptomatology in women with SBI. Morse et al. (1995) assessed whether women with SBI and scleroderma-like symptoms share an HLA DQ motif with women with scleroderma but without SBI. The control population of scleroderma patients without SBI was historical. There was a second control population of healthy individuals without SBI. The authors assessed whether women with SBI and scleroderma-like symptoms had a lower frequency of leucine at residue 26 of the DQ β chain than the healthy control group, similar to what had previously been found in scleroderma patients without SBI. A decreased frequency of leucine at residue 26 was found, suggesting that women with SBI and scleroderma are similar to other women with scleroderma. The only conclusion that can be drawn from this study is that women with scleroderma with SBI and women with scleroderma without SBI share a similar genetic marker, and that SBI are not associated with scleroderma-like symptoms in a novel, genetically distinct population. The study cannot be used to conclude that scleroderma is caused by SBI, nor does it prove that scleroderma is not triggered by SBI. Rather, the study shows that scleroderma may arise in a particular susceptible population, whether or not the individuals have SBI.

A second well-designed study by Young et al. (1996) analyzed whether particular HLA Class I and Class II molecules were present in symptomatic women with SBI. Four groups were analyzed: group 1 included 77 women with SBI and debilitating (fibromyalgia-like) symptoms; group 2 included 37 women with SBI but few symptoms; group 3 included 54 healthy women without SBI; and group 4 included 31 women without SBI but with fibromyalgia, a rheumatic

disease characterized primarily by muscle pain and sleep disturbance. There were no differences in HLA Class I (A, B, or C) alleles among the groups; however, groups 1 and 4 had an increased frequency of Class II DR53 and DR7, suggesting that symptomatic women with SBI share HLA DR alleles with women with fibromyalgia. This study cannot be interpreted to show that SBI cause disease. Rather, women with fibromyalgia, whether or not they have SBI, have a particular HLA profile. It is important to note that this study and one other (Burda et al., 1986) demonstrated increased DR4 in fibromyalgia, while two additional studies found no HLA association with fibromyalgia (Biasi et al., 1994; Horven et al., 1992). Furthermore, it is important to note that there is no evidence to date that fibromyalgia is an autoimmune disease. There is no clear HLA association, no documented autoreactivity, and no evidence of tissue damage. Thus, any similarity found between some women with SBI and women with fibromyalgia cannot be construed to suggest those women with SBI have an autoimmune disease.

If there were a unique HLA haplotype found in symptomatic women with SBI, it would be suggestive of SBI causing a new disease. To date there is no unique HLA haplotype that has been found for symptomatic women with SBI. The conclusion that can be tentatively drawn from the two studies discussed above is that women with particular sets of symptoms share HLA haplotypes regardless of whether they have SBI. This does not constitute evidence that the disease or symptom complex is causally related to SBI. Possibly, SBI trigger disease in a susceptible population; possibly, SBI are unrelated to the development of disease. Information obtained from epidemiologic studies that address potentially susceptible individuals, such as those with a particular HLA haplotype, are needed.

All other reports on HLA in women with SBI are case reports or abstracts.

T Cell Activation in Women with SBI

T cells responsive to antigen will proliferate in the presence of that antigen together with antigen-presenting cells. The studies reviewed below analyze T cell proliferation in response to silicone, silica, or to connective tissue autoantigens. It is important to note, however, that T cells proliferate in response to mitogens as well as antigens. Mitogens are substances that cause polyclonal nonantigen-specific T cell proliferation as they do not engage the antigen-binding site of a T cell receptor. Mitogens do not induce T cell memory, and there is no model of mitogen-activated T cells causing disease.

To provide evidence for an autoimmune disease, one might look for T cell responses to autoantigens. No specific autoantigen has been identified in women with SBI. In general, the studies to identify a population of activated T cells in women with SBI have focused on the presence or absence of silicone-specific T cells, that is, T cells that proliferate in the presence of silicone.

Most T cells recognize protein antigens. It is not clear in the studies of silicone-reactive T cells whether silicone is assumed to bind to proteins present in culture media and modify peptide antigens, or whether there is recognition of silicone alone through a set of unidentified T cell receptors. While T cell activation can be measured by antigen-induced cytokine release, specific T cell activation has been assayed almost exclusively by proliferation in response to "antigen" challenge. In general, there are significant methodologic problems with the studies, ranging from uncertainty regarding the state of the silicone used for stimulation, to the composition of control populations, to concerns regarding the analysis of data. Furthermore, most studies are not designed to distinguish whether silicone or some material from SBI is recognized as an antigen by a T cell receptor, or is a T cell mitogen.

Ojo-Amaize et al. (1994) reported an enhanced T cell response to silicon dioxide, silicon, or silicone gel in symptomatic women with SBI. The state of antigen in the antigen preparations is not clear in this study as the silicone gel was extracted before use. Furthermore, it appears there are two different sets of control subjects for these studies, one for silicon dioxide, another for silicon and silicone gel. Finally, the analysis of the data is unconventional. An individual is considered to have a positive response if there is an elevated response to any of the three antigens at any of three concentrations. Only one patient is shown with an enhanced response to an antigen at all three concentrations. In more conventional analyses, this individual might be considered the only responder. Furthermore, the results would differ if each antigen were analyzed separately, as the number of responders to any one antigen was small.

In a study by Smalley et al. (1995), T cell response to silicon dioxide (silica) was examined in symptomatic women with SBI. The control group in this study was neither sex- nor age-matched to the group with SBI. There was also no control group of symptomatic women without SBI. While no raw data are shown, the patients appear to have higher T cell stimulation indices than controls. This observation has not been confirmed. In fact, it appears, that a second cohort of women without SBI showed a much higher stimulation index than the first control population

(Young, 1996). Had these women also been in the control population, there would have been little to no difference between women with SBI and the control group. Thus, the reproducibility of the findings is not clear.

Ellis et al. (1997), looking for autoreactive T cells, studied T cell reactivity to connective tissue components as well as reactivity to implant material. Twenty-six symptomatic women with SBI were compared to 23 age-matched healthy controls without SBI. It is not stated whether the control population included only women or both men and women. While the women with SBI had increased T cell proliferation to collagen I, collagen III, fibrin, and fibronectin, they had no increased reactivity to myelin basic protein, transferrin, bovine serum albumin, tetanus, or silica D4 or silicone gel. Although these are interesting results, without a symptomatic control group without SBI it is not possible to conclude the responses are related to SBI.

Ciapetti et al. (1995) compared the T cell response to silicone gel in women with SBI to that of women without SBI who were, on average, over 15 years younger. They conclude that lymphocytes from women receiving SBI for augmentation show increased T cell proliferation to gel. A standard stimulation index was not calculated; instead stimulation in the women with SBI was compared to stimulation in the control group. The difference in stimulation between groups was less than twofold and the proliferation in the presence of gel extract was less than in its absence. This is, therefore, a highly unorthodox presentation of data. In general, if proliferation is lower in the presence of antigen than in its absence, it cannot be concluded that there is an antigen-specific response.

T cells express either the CD4 or the CD8 molecule on their surface. In general, the ratio of CD4/CD8 cells is fairly constant among individuals. Vojdani et al. (1992) report an increased CD4/CD8 ratio in women with SBI. The women with SBI were symptomatic while the control group was healthy. Because of the absence of a control group of symptomatic women without SBI or asymptomatic women with SBI, it is not possible to determine whether the altered ratio reflects the symptomatic status of the women with SBI or the presence of implants. Furthermore, there is no literature on individuals with an enhanced CD4/CD8 ratio that can be used to understand the significance of such an observation, should it characterize women with SBI.

A study by Katzin et al. (1996) compared T cells from breast tissue and peripheral blood of women with SBI obtained at the time of implant removal to peripheral blood T cells of control individuals (not sex- or age-matched). In this study, the T cells obtained from breast tissue

displayed increased expression of HLA-DR antigen, an activation marker, compared to peripheral blood cells. There was also a decrease in the number of CD4+CD45+ RO+ T cells (helper T cell phenotype) in implant-associated T cells of patients with SBI compared to peripheral blood T cells of patients. The comparison of peripheral blood cells of patients and controls showed only a slight decrease in CD4+CD45+ RO+ cells in patients. As the control population was not age- or sex-matched, this difference is difficult to interpret. Finally, the cells of patients with SBI were sometimes stained after freezing and thawing; it is not clear that control cells were similarly handled. Thus, differences in cell phenotype may reflect differences in processing.

A study by Granchi et al. (1995) analyzed expression of surface molecules on peripheral blood T cell and mononuclear cell (NK cell) cytotoxic activity in 22 women with SBI and Baker II or Baker III and IV contractures of whom 16 had received implants following mastectomy. The control group consisted of 10 nonage-matched healthy women. The absence of a control group with cancer but without SBI is a critical deficiency in this study. No phenotypic difference in T cells was observed among the groups by flow cytometry. Increased cytotoxicity to the tumor cell line was, however, observed in the women with SBI and Baker III or IV contractures. This is in contrast to the study of Campbell et al. (1994) suggesting diminished NK cell activity in women with SBI.

In general, in these studies the lack of appropriate control groups, the poor stimulation indices, and the absence of raw data to analyze make interpretation difficult. There are no reproducible, convincing data to show that silicone or any component of SBI is a T cell antigen or that any connective tissue component has become a T cell target autoantigen in women with SBI. There are, furthermore, no data that reproducibly show an altered phenotype of peripheral blood T cells in women with SBI.

Anti-Nuclear Antibodies

Studies reviewed here examine whether SBI cause activation of B cells, specifically whether SBI lead to production of anti-nuclear antibodies (ANA). ANA are found in the serum of affected individuals in several defined rheumatic diseases. The commonest assay for ANA is the fluorescent ANA (FANA) which uses fluoresceinated secondary antibody to detect serum ANA and the human epithelial cell line Hep-2 as antigen. Each laboratory defines the dilution of serum it uses to determine positive reactivity (e.g., 1:20, 1:40, 1:64, 1:80, 1:160) and the degree of

fluorescence, graded as 1+ to 4+, used to define positive reactivity (e.g., 1+ or 2+) (vonMuhlen and Tan, 1995). Furthermore, each laboratory may use different reagents for the test and may select a different control population. Thus, there is significant interlaboratory variation in ANA testing. Furthermore, a recent study by Tan et al. (1997) demonstrates significant interlaboratory variability even when the same definition of a positive assay is used (coefficient of variance of 50%). This variability is, in part, a function of the subjective determination of fluorescence positivity and, in part, a consequence of the use of different reagents in the assay.

In each laboratory, the serum dilution and the degree of fluorescence used to define positive serum is determined based on achieving 5% positivity in a control population. The control group may, however, vary from laboratory to laboratory; therefore, subsets of healthy individuals may show greater than 5% positivity. For example, when De Vlam et al. (1993) studied ANA positivity in healthy blood donors in Belgium, they found 62 of 485 individuals were ANA positive by the laboratory's standard. Twenty individuals (5%) however, had a titer higher than 1:80 and 23 had IgG ANA. The recent study by Tan et al. (1997) also demonstrates how much the frequency of ANA positivity depends on the dilution of serum being assayed; 3.3% of healthy controls were positive at a 1:320 dilution of serum; 5% at a 1:160 dilution; 13.3% at a 1:80 dilution; and 31.7% at a 1:40 dilution. Thus, data for ANAs in women with SBI must be compared to data on sera from control populations assayed in the same laboratory at the same time.

Some characteristics of the control population are known to affect the incidence of ANA positivity. ANA are more commonly positive in women than in men (Fritzler et al., 1985; Thomas and Robinson, 1993), so sex-matched controls are critical. Several studies also suggest that ANA positivity increases with age, so age-matched controls are also critical (Ruffatti et al., 1990; Slater et al., 1996; Xavier et al., 1995). Xavier et al. in a study of healthy Japanese people, found that 11.4% of those over 65 showed ANA positivity, compared to 3.8% of a younger group. Fritzler et al. (1985) screened healthy female blood donors and found that more than 10% of women over 45 had ANA titers greater than 1:80. Similar findings have been reported by a number of other investigators (Ruffatti et al., 1990; Slater et al., 1996). It is important to note, however, that not all investigators found an increase in ANA positivity in the healthy elderly (Juby et al., 1994). Finally, there is some literature on the incidence of ANAs in individuals with a malignancy (Klajman et al., 1983; Turnbull et al., 1978). Few studies address specifically the incidence of a

positive ANA in women with breast cancer, but the available data would suggest that approximately 15% may have a positive ANA. As this number comes from a study of Japanese women, it is not clear it can be extrapolated to the studies described below (Imai et al., 1979). Nonetheless, there is some evidence that autoantibody production is part of a paraneoplastic syndrome in a number of malignancies.

It is also important to note that many drugs cause positive ANAs (von Muhlen and Tan, 1985). One that is rarely mentioned but that may be important in this analysis is estrogen. Clearly, in animals, estrogen can induce positive ANAs and even anti-DNA antibodies (Ahmed et al., 1989; Blank et al., 1990). Whether this is true in humans is unknown.

There is a small but critical literature discussing the positive predictive value of an ANA for the development of a rheumatic disease in symptomatic and asymptomatic individuals (Aho et al., 1992; Dinerman et al., 1986; Emlen and O'Neill, 1997; Slater et al., 1996; Yadin et al., 1989). By definition, at least 5% of apparently healthy individuals have a positive ANA (Aho et al., 1992; Dinerman et al., 1986; Emlen and O'Neill, 1997; Slater et al., 1996; Yadin et al., 1989). The significance of a positive ANA in healthy individuals was examined by Shoenfeld et al. They screened 506 healthy women and found that 12% had a high titer ANA (Yadin et al., 1989). After five years of follow-up, only 12% of these had developed some symptoms but none had developed a definite connective tissue disease (CTD). In general, it would appear that only a small percentage of individuals with a positive ANA will ever develop SLE (Aho et al., 1992). It is also possible to ask whether the ANA can predict which symptomatic individuals will progress to a defined connective tissue disease. Several investigators have studied the progression of individuals with a positive ANA and Raynaud's phenomenon (RP) to a defined connective tissue disease. RP is characterized by decreased blood flow to the fingers. It occurs in approximately 5% of the female population (Riera et al., 1993). Only a small number of women with RP alone progressed to a defined disease. Five to 20% of individuals with both a positive ANA and RP progressed over five to six years to a defined disease, again demonstrating that ANAs are best used to exclude disease (Fitzgerald et al., 1988; Gerbracht et al., 1985; Kallenberg et al., 1988; Luggen et al., 1985; Sheiner and Small, 1987). In general, a negative ANA can be used to screen out autoimmune rheumatic disease (SLE, scleroderma, myositis, MCTD, Sjögren's syndrome), but a positive ANA is only 10–15% predictive of an autoimmune rheumatic disease even in symptomatic individuals (Emlen and O'Neill, 1997; Homburger, 1995; Slater et al., 1996). Thus, a

positive ANA is not evidence of an autoimmune rheumatic disease. In addition, it should be noted that many individuals who describe some symptoms suggestive of connective tissue disease but who do not meet criteria for a defined connective tissue disease will fail to progress or become asymptomatic in a five-year follow-up (Alarcon et al., 1996; Clegg et al., 1991; Fitzgerald et al., 1988; Gerbracht et al., 1985; Kallenberg et al., 1988; Luggen et al., 1995; Williams et al., 1998). In a study by Williams et al. (1998) of 213 individuals with RP, undefined connective tissue disease (UCTD), or unspecified polyarthritis (UPA) followed over a five-year period, only 36 developed a classic CTD; 63 were lost to follow up, 18 went into remission, and the rest showed no progression of disease.

Twenty-three studies of the incidence of positive ANAs in women with SBI were reviewed (Table 1) (Blackburn et al., 1997; Bridges et al., 1993, 1996; Brunner et al., 1996; Claman and Robertson, 1994, 1996; Cuellar et al., 1995a, b; Edworthy et al., 1998; Freundlich et al., 1993; Gabriel et al., 1994; Lewy and Ezrailson, 1996; Park et al., 1998; Peters et al., 1994, 1997; Press et al., 1992; Rowley et al., 1994; Silverman et al., 1996a; Solomon, 1994; Tenenbaum et al., 1997; Teuber et al., 1993; Vasey et al., 1994; Zazgornik et al., 1996). These were evaluated according to the criteria that follow.

Patient Selection

Nearly all of the studies have a selection bias. Most patients were “sick” or “concerned” or referred from attorneys or other physicians who knew that the investigators had an interest in women with SBI. Most were referred to rheumatologists and, therefore, were also from a subset of women selected for rheumatic symptoms. Nonetheless, in almost no study was an attempt made to describe the symptom complex or to subclassify patients. In some studies, women with SBI with defined connective tissue diseases were included in the study population. In the studies where the incidence of defined CTD was reported, it varied from 10–20% of “sick” patients with SBI; thus failure to analyze these patients separately is a substantial cause of error. Only a few studies examined healthy women with SBI, to determine if SBI induce ANA positivity.

Control Population

Several studies did not use age-matched controls. Since there is some evidence that ANA positivity increases with age, an age-matched control group is very important. Similarly, not all studies used sex-matched controls. Since women have a greater incidence of ANA positivity, the

use of a female control group is very important. To determine if the symptom complex of symptomatic women with SBI is, by itself, associated with a positive ANA, a control population of symptomatic patients without SBI is needed. When there was such a control group, it was usually women with fibromyalgia. It is not always clear from the studies whether the women with fibromyalgia or the other symptomatic women without SBI in fact share a symptom complex with the symptomatic women with SBI.

Methodology for ANA Determination

Knowing how ANAs were interpreted is important for the reasons described in the introduction. In most cases, the titer used to define a positive ANA was reported but not the fluorescence intensity that was used to determine positivity (1+ vs. 2+). In some cases historical controls were used; thus, it is likely that different technicians examined the sera and it is possible that different reagents were used. This would be a substantial source of error. In many studies it is not clear whether those reading the ANA were blinded to implant status. This is a potential problem, since definitions of positive or negative need to be determined before the data are analyzed and not designed around the data.

Multiple Samples

Since most of the positive ANAs were low titer, often a single (twofold) dilution away from a negative reading, it would be useful to have more than one sample to evaluate.

Prospective Studies

There are no studies of serum from women before and after SBI to help support a causal relationship between SBI and ANA positivity.

Summary of ANA Results

The results of the studies are summarized and referenced in Table 1. In five studies, healthy women with SBI were studied. In three of these, women with SBI showed increased ANA positivity; in two they did not. In one of these studies, there is no control group of healthy women without SBI, but only one of 12 healthy women with SBI had a positive ANA, similar to what is usually seen in most healthy control populations. In four of five studies in which symptomatic

women with SBI were compared to symptomatic women without SBI, there was no increased incidence of ANA present in women with SBI. Furthermore, in six of 11 studies in which healthy controls without SBI were compared to women with SBI (healthy and symptomatic), there was no difference between the groups.

Overall, there is no compelling scientific evidence that SBI are associated with positive ANAs. Asymptomatic women with SBI do not reproducibly show an increased incidence of ANA. Symptomatic women with SBI have the same incidence of ANA positivity as symptomatic women without SBI. Finally, ANA titers, when found in women with SBI without defined connective tissue disease, are low and generally nonspecific. This result may often reflect hypergammaglobulinemia rather than specific antibody formation and, as discussed above, is not predictive of developing a disease.

Specific Autoantibodies

The search for specific autoantibodies in women with SBI has, in general, failed to reveal an abnormal incidence of most autoantibodies in women with SBI. Studies examining rheumatoid factor, and antibodies to Sm, Ro, and La, other antigens targeted in SLE, do not suggest an increased incidence in women with SBI, when women meeting criteria for a defined connective tissue disease are excluded.

Anti-collagen Antibodies

The single autospecificity that has been analyzed in some detail is anti-collagen antibodies. It is important to note that all studies on this topic have been performed by a single group of investigators in a small group of patients (Bar-Meir et al., 1995; Rowley et al., 1994; Teuber et al., 1993). Presumably, anti-collagen antibodies were sought because symptoms in women with SBI often involve the musculoskeletal system, anti-collagen antibodies have been described in some patients with rheumatoid arthritis, and anti-collagen antibodies appear to play a role in some mouse models of arthritis.

Teuber et al. (1993) initially compared anti-collagen reactivity in 46 women with SBI, including two women with a defined connective tissue disease, 38 symptomatic women, and six asymptomatic women, to age-matched healthy women. Collagen reactivity was considered positive when an ELISA reading was greater than three standard deviations above the mean of the

controls. Thirty-five percent of the women with SBI (16/46) displayed anti-collagen reactivity (26% to collagen I and 15% to collagen II) compared to 9% of healthy controls. The raw data are not given, so it is not clear how strongly any of the sera bound collagen (Teuber et al., 1993).

Rowley et al. (1994) studied anti-collagen reactivity in 70 women with SBI (many of whom were included in the above study) of whom 65 were symptomatic, 82 women with SLE, 94 women with rheumatoid arthritis, 43 healthy women, and 90 healthy individuals not age- or sex-matched. Forty-one percent of symptomatic women with SBI (29/65) showed anti-collagen reactivity by ELISA, compared to 29% of SLE patients, 48% of RA patients, and 6% of controls. Reactivity to both native and denatured collagen was measured for women with SBI and RA, but only reactivity to native collagen for women with SLE. If one examines the data and compares reactivity to native collagen only, there is no difference between women with SBI and women with SLE. Women with RA had less reactivity to native type I collagen and more reactivity to native type II collagen. Reactivity to denatured collagen was the same for patients with RA and women with SBI. Reactivity to collagen I and II was studied also by Western blotting using peptide fragments of collagen displayed on a nitrocellulose filter. The authors suggest that reactivity to collagen I is distinct in women with SBI. Nine women with SBI showed reactivity to collagen I peptides. These patients appear to display individual patterns of reactivity. There was no consistent peptide specificity seen. Furthermore, Western blotting was performed with bovine collagen peptides, not human collagen. There is no control group studied for reactivity to bovine collagen.

Subsequently, Bar-Meir et al. (1995) confirmed reactivity to collagen I and II by ELISA in women with SBI, many of whom were symptomatic, compared to age- and sex-matched controls. Sera from 14 of 116 women with SBI showed reactivity to collagen I compared to one of 134 control sera. Sera from ten of 116 women with SBI showed reactivity to collagen II compared with one of 134 control sera. Women with defined CTD were not excluded from the group with SBI. There was also no control group of symptomatic women without SBI. Thus, it is not possible to attribute the anti-collagen reactivity to SBI, rather than to defined or undefined CTD.

Overall, the above data suggest that some symptomatic women with SBI may have antibodies to collagen I and II by ELISA. The number decreases when reactivity is assayed by Western blotting and there is no consistent pattern of reactivity seen by Western blotting. There are no data on healthy women with SBI or on women with atypical complaints but without SBI.

Since the data derive from a single laboratory, it is necessary to obtain independent confirmation of these results. Furthermore, even the existence of such antibodies would not mean there is an antibody-mediated or antibody-associated disease. No study considers the relevance of these antibodies to clinical symptoms.

Anti-microsomal Antibodies

One study has examined anti-microsomal antibodies in women with SBI and found a significant increase compared apparently to a historical control group that is not described (Brunner et al., 1996). One additional study failed to confirm this observation (Gabriel et al., 1994).

Anti-silicone Antibodies

One question that can be asked to address the immunogenicity of silicone is whether individuals with SBI develop antibodies to silicone. While the question appears to be simple, an answer is difficult to obtain because there are substantial problems in developing an assay for such antibodies. Silicone is sticky and will bind nonspecifically to proteins. With a sticky substrate, high levels of plasma immunoglobulins or hypergammaglobulinemia alone will give high values on ELISA.

The first suggestion of the existence of anti-silicone antibodies came in a report from Goldblum et al. (1995) studying patients exposed to silastic shunts. The paper has both methodologic problems and internal contradictions and the findings were later retracted, since binding was found to be highly dependent on serum albumin levels.

Wolf and colleagues (1993) developed an assay for anti-silicone antibodies using bovine serum albumin (BSA) bound to polystyrene plates, followed by hydroxyl-silicone. They used this assay to examine serum from 67 healthy females, ten diabetic individuals presumably exposed to silicone syringes, 15 women with SBI without rupture, and 19 women with SBI with rupture. They concluded that the diabetic patients are indistinguishable from the control group, that anti-silicone antibodies are increased in women with SBI, and that they are highest in women with rupture. The assay raises methodologic issues. There is no confirmation that the antibody binding to ELISA plates is mediated through a conventional antigen binding site. In addition, there are no data describing whether the women with SBI were symptomatic and no control group of symptomatic women without SBI. Rose and colleagues (1996) used the Goldblum and the Wolf

assays to generate data showing that women with connective tissue disease as well as women with SBI show elevated anti-silicone reactivity. These studies did not determine whether the assays detected specific anti-silicone antibodies or hypergammaglobulinemia.

Kossovsky and colleagues (1993) developed the DetectSil assay for binding to antigens that have been modified by silicone. In this assay, microtiter plates are coated first with silicone and then with various self-antigens (fibrinogen, collagen, fibronectin) prior to applying test serum. Binding of sera to plates without antigen was often greater than binding to plates with antigen, consistent with the known nonspecific stickiness of silicone and suggesting that the assay might be detecting hypergammaglobulinemia. This study is uninterpretable as the background binding (which is subtracted from test values) is not defined, and the raw data have been lost.

Shen et al. (1996) developed an assay to detect sodium silicate attached to BSA. Plates are coated with BSA, followed by sodium silicate, followed by test serum. Forty symptomatic women with SBI, 91 asymptomatic women with SBI, 52 women with connective tissue disease, and healthy nonage-matched women were studied. There was a significant increase in ELISA reactivity in sera of symptomatic women with SBI, compared to asymptomatic women with SBI. This assay also has methodological problems. When human serum albumin rather than BSA was used as an antigen, no reactivity was detected. Furthermore, silicate is not silicone, and the exposure to silicate of women with SBI is not known.

Rosenau, Schneebaum, and Schur (1996) tried to develop an assay for anti-silicone antibodies but concluded that the assays detected hypergammaglobulinemia only.

Overall, it appears that a reproducible assay for anti-silicone antibodies has not yet been developed. There are no conclusive data demonstrating that silicone or silicone-modified protein is antigenic to the humoral immune system.

Anti-polymer Antibodies

There is one study of antibodies to partially polymerized acrylamide in women with SBI. It is not clear why such antibodies should be induced by SBI (Tenenbaum et al., 1997). The data show that anti-polymer antibodies are present in 17% of nonage-matched control women, 20% of women with SBI and defined connective tissue disease (CTD), and 10% of women with CTD without SBI. It appears that approximately 25% of women with SBI without defined CTD showed reactivity. Reactivity was reported to be higher in “sick” patients than in “well” patients.

Since the stratification of patients was unconventional, it is difficult to interpret this observation. Furthermore, the theoretical relevance of this assay to SBI is unclear, and the data do not suggest a relationship between SBI and anti-polymer specificity. Finally, the investigators show no difference between women with SBI and fibromyalgia patients.

In summary, studies of antibodies in women with SBI do not reproducibly demonstrate an increase in autoantibodies. Studies of particular anti-nuclear antibodies have not revealed a specificity elevated in symptomatic or asymptomatic women with SBI. Studies of ANA are inconsistent. Very few studies have control populations of symptomatic women without SBI or separately evaluate symptomatic and asymptomatic women with SBI. Without an analysis of such populations, it is not possible to know whether changes in serologic parameters might relate to “unwellness” or to SBI. In general, any increases in ANA positivity in women with SBI tend to disappear when age-matched or fibromyalgia controls are used.

Finally, no studies have related either autoantibodies or anti-silicone antibodies to disease in women with SBI. There is no animal model of arthritis, CNS disease, or muscle weakness induced by the nonspecific low titer ANAs that are seen in some symptomatic women with SBI. There have been no studies of “affected” tissue to demonstrate antibody-mediated tissue damage.

VI. Monoclonal Gammopathy and Multiple Myeloma

Potter and colleagues (1995) have described the induction of plasmacytomas in BALB/c mice following intraperitoneal injection of paraffin oils. In their studies, injection of silicone gel, but not oil, caused plasmacytomas in BALB/c mice. Tumors developed in 20–55% of mice given 0.2–0.4 ml gel with a latency of 200–300 days. It appears that tumors develop at the site of granulomas. These tumors display chromosomal translocations similar to those seen in paraffin oil-induced tumors (Potter et al., 1995). Chromosomal translocations are present in 10 to 20% of malignant plasma cells in humans also, but are rarely the same as those seen in plasmacytomas in BALB/c mice (Chesi et al., 1998; Lai et al., 1998; Yamamoto et al., 1998). The data from Potter's laboratory on the induction of plasmacytomas in BALB/c mice by silicone gel raised the question of an increase in myeloma or monoclonal gammopathy in women with SBI.

Monoclonal gammopathy is a nonmalignant proliferation of a clonal B cell population. It is said to occur in 0.5% of the population. Thirty-year follow up of patients with monoclonal gammopathy shows that 16% will develop multiple myeloma, with an annual actuarial risk of

0.8%. The percentage of monoclonal gammopathies that disappear over time is not known, although it is thought to be small (Herrinton, 1996; Kyle, 1996).

The National Cancer Institute has established a registry for myeloma in women with SBI. In a 1996 report, they had recorded 18 cases (Rabkin et al., 1996). The time from implant to diagnosis was two to 25 years. Of note, seven women were less than 45 years old. The age of onset for myeloma, in general, is somewhat older, but epidemiological studies to determine if there is an earlier age of onset of the disease in women with SBI have not been published.

Published data address three questions: (1) Is there increased immunoglobulin production in women with SBI?; (2) Is there an increase in monoclonal gammopathy?; and (3) Is there an increase in myeloma? Silverman et al. (1996b) studied 630 symptomatic women with SBI. IgG was increased in 10.8%, IgA was increased in 3.2%, and IgM was increased in 6.3%. There was no control group of symptomatic women without SBI. Sera from 284 women (18% with increased immunoglobulin levels) were examined for the presence of monoclonal gammopathy. Five had a monoclonal gammopathy with one progressing to myeloma. Three women underwent explantation of SBI and received saline implants; in two of these women the monoclonal gammopathy disappeared. This study is uncontrolled and it is not possible from these data to know if SBI cause increased immunoglobulin levels, monoclonal gammopathy, or myeloma.

Another study examined sera from 82 women with SBI (Garland et al., 1996). Thirty percent had immunoglobulin levels greater than the normal range; none displayed a monoclonal gammopathy. The health status of these women was not recorded. Brunner et al. (1996) examined 239 women with breast implants. IgG levels were increased in 12.6% of the women (normal range 643–1610 mg/dl) and IgM levels were elevated in 7.5% of the women (normal range 36–300 ug/dl). There was no difference between women with silicone implants and women with saline implants.

Kyle performed a retrospective analysis of 749 women with SBI seen at the Mayo Clinic. None had myeloma or a documented monoclonal gammopathy. A control group matched for sex and age included four cases of monoclonal gammopathy and one case of myeloma (Kyle, 1996). Deapen et al. (1997) found no cases of myeloma in 3182 women with SBI followed up for over 14 years. As the normal incidence of myeloma is three to four per 100,000 individuals per year, these studies are too small to be definitive.

The nature of the genetic susceptibility of BALB/c mice to plasmacytoma induction is

unknown. It is, therefore, not possible to look for a similar genetic susceptibility in a subset of women to ask whether this subset has an increased risk of monoclonal gammopathy or myeloma following SBI. The data on immunoglobulin levels in women with SBI might be of interest as an indicator of inflammation, but studies comparing symptomatic women with SBI to symptomatic women without SBI or healthy women without SBI to healthy women with SBI have not been performed. The comparison of symptomatic women with SBI to symptomatic women without SBI is especially important since many connective tissue diseases are associated with hypergammaglobulinemia. The studies on monoclonal gammopathy are uncontrolled and the data on myeloma with few exceptions represent case reports. There are, at present, no data that associate SBI with monoclonal gammopathy or myeloma.

VII. Summary

Overall, when analyzing studies of the innate immune response and inflammatory mediators, and of antigen-specific cell-mediated and humoral immunity in women with SBI, it must be concluded that there are no consistent data to suggest systemic inflammation or systemic induction of anti-silicone or autoreactive responses. There is no HLA antigen found with unexpectedly high frequency in symptomatic women with SBI to suggest an autoimmune disease. There has been no site distal to the breast that has been demonstrated to be a target organ for immune attack; no tissue except the breast has been demonstrated to have an inflammatory infiltrate. A reproducible autoantibody specificity has not been found; autoreactive T cells have also not been identified. As discussed in the previous section, studies to determine if disease can be transferred by transfer of lymphoid cells are not possible even in animals, as disease manifestations are primarily subjective. Furthermore, there is no consistent credible evidence for exposure to SBI causing an immune response to components of the implant, and no evidence exists for a role for implant rupture and increased exposure to antigen in the induction of any immunologic abnormality.

Overall, the data do not demonstrate antigenicity of silicone, immune system activation in women with SBI, or presence of autoreactivity in women with SBI. An unanswered question is whether SBI can cause sufficient local inflammation to account for the symptoms women report. With no evidence of systemic activation of the immune system, this seems unlikely. The concern has also been raised that many SBI are colonized by bacteria (Ahn et al., 1996; Dobke et al.,

1995; Jennings et al., 1991; Netscher et al., 1995) and that symptoms might represent a response to infection or colonization. The data, however, do not support an association of bacterial colonization of SBI with particular symptoms or even a sense of unwellness (Ahn et al., 1996; Dobke et al., 1995; Netscher et al., 1995).

Most studies on the immune system in women with SBI have design flaws such that they do not, in fact, address the existence of an SBI-induced immunologic abnormality. These problems include a poor choice of control group or no control group, unorthodox data analysis, and often an uncertain relationship of the antigen used in the study to the compounds present in SBI. Most studies yield negative data, and, where one can make inferences from poorly designed studies, the conclusions are that women with SBI do not display an SBI-induced abnormality in phenotype or function of cells of the immune system. It remains possible that we do not yet know how to recognize an affected subset of individuals or that we have not analyzed the relevant parameters of immune activation. Furthermore, no studies have examined whether the immunologic abnormalities present in women with defined CTD are enhanced or exaggerated in women with defined CTD who have SBI. Thus, the potential for SBI to exacerbate disease is an unresolved issue. Nonetheless, to date, there are no consistent data that support the hypothesis that SBI cause an alteration in systemic immune responses in women.

References

- Ahmed, SA, TB Aufdemorte, JR Chen, AI Montoya, D Olive, N Talal. Estrogen induces the development of autoantibodies and promotes salivary gland lymphoid infiltrates in normal mice. *J Autoimmun.* 2:543–52e, 1989.
- Ahn, CY, CY Ko, EA Wagar, RS Wong, WW Shaw. Microbial evaluation: 139 implants removed from symptomatic patients. *Plast Reconstr Surg.* 98:1225 –29, 1996.
- Aho, K, P Koskela, R Makitalo, M Heliovaara, T Palosuo. Antinuclear antibodies heralding the onset of systemic lupus erythematosus. *J Rheumatol* 19:1377–79, 1992.
- Alarcon, GS, RF Willkens, JR Ward, DO Clegg, JG Morgan, KN Ma, JZ Singer, VD Steen, HE Paulus, ME Luggen, et al. Early undifferentiated connective tissue disease. IV. Musculoskeletal manifestations in a large cohort of patients with undifferentiated connective tissue diseases compared with cohorts of patients with well- established connective tissue diseases: followup analyses in patients with unexplained polyarthritis and patients with

- rheumatoid arthritis at baseline. *Arthritis Rheum* 39:403–14, 1996.
- Bar-Meir, E, SS Teuber, HC Lin, I Alosacie, G Goddard, J Terybery, N Barka, B Shen, JB Peter, M Blank. Multiple autoantibodies in patients with silicone breast implants. *J Autoimmun* 8:267–77, 1995.
- Biasi, G, A Fioravanti, M Galeazzi, R Marcolongo. Absence of correlation between HLA antigens and fibromyalgia syndrome in Italian patients. *Ann Ital Med Int.* 9:228–30, 1994.
- Blackburn, WDJ, JC Grotting, MP Everson. Lack of evidence of systemic inflammatory rheumatic disorders in symptomatic women with breast implants. *Plast Reconstr Surg* 99:1054–60, 1997.
- Blank, M, S Mendlovic, H Fricke, E Mozes, N Talal, Y Shoenfeld. Sex hormone involvement in the induction of experimental systemic lupus erythematosus by a pathogenic anti-DNA idiotypic in naive mice. *J Rheumatol* 17:311–17, 1990.
- Blaszczyk, M, S Majewski, M Wasik, T Chorzelski, S Jablonska. Natural killer cell activity of peripheral blood mononuclear cells from patients with various forms of lupus erythematosus. *Br J Dermatol* 117:709–14, 1987.
- Bradley, SG, KLJ White, JA McCay, RD Brown, DL Musgrove, S Wilson, M Stern, MI Luster, AE Munson. Immunotoxicity of 180 day exposure to polydimethylsiloxane (silicone) fluid, gel and elastomer and polyurethane disks in female B6C3F1 mice. *Drug Chem Toxicol* 17:221–69, 1994.
- Bridges, AJ, JD Anderson, DE Burns, K Kemple, JD Kaplan, T Lorden. Autoantibodies in patients with silicone implants. *Curr Top Microbiol Immunol* 210:277–82, 1996.
- Bridges, AJ, C Conley, G Wang, DE Burns, FB Vasey. A clinical and immunologic evaluation of women with silicone breast implants and symptoms of rheumatic disease. *Ann Intern Med* 118:929–36, 1993.
- Brunner, CA, AM Feller, R Groner, E Dees, K Biefel, E Biemer. Increase of immunologically relevant parameters in correlation with Baker classification in breast implant recipients. *Ann Plast Surg* 36:512–18, 1996.
- Burda, CD, FR Cox, P Osborne. Histocompatibility antigens in the fibrositis (fibromyalgia) syndrome. *Clin Exp Rheumatol* 4:355–58. 1986.
- Campbell, A, N Brautbar, A Vojdani. Suppressed natural killer cell activity in patients with silicone breast implants: reversal upon explantation. *Toxicol Ind Health* 10:149–54, 1994.

- Chang, YH. Adjuvanticity and arthritogenicity of silicone. *Plast Reconstr Surg* 92:469–73, 1993.
- Chesi, MP, L Bergsagel, OO Shonukan, ML Martelli, LA Brents, T Chen, E Schrock, T Ried, WM Kuehl. Frequent dysregulation of the c-maf proto-oncogene at 16q23 by translocation to an Ig locus in multiple myeloma. *Blood* 12:4457–63, 1998.
- Ciapetti, G, D Granchi, S Stea, E Cenni, P Schiavon, R Giuliani, A Pizzoferrato. Assessment of viability and proliferation of in vivo silicone-primed lymphocytes after in vitro re-exposure to silicone. *J Biomed Mater Res* 29:583–90, 1995.
- Claman, HN, AD Robertson. Antinuclear antibodies and breast implants. *West J Med* 160:225–28, 1994.
- . Antinuclear antibodies in apparently healthy women with breast implants. *Curr Top Microbiol Immunol* 210:265–68, 1996.
- Clegg, DO, HJ Williams, JZ Singer, VD Steen, S Schlegel, C Ziminski, GS Alarcon, ME Luggen, RP Polisson, RF Willkens. Early undifferentiated connective tissue disease. II. The frequency of circulating antinuclear antibodies in patients with early rheumatic diseases. *J Rheumatol* 18:1340–43, 1991.
- Cuellar, ML, O Gluck, JF Molina, S Gutierrez, C Garcia, R Espinoza. Silicone breast implant--associated musculoskeletal manifestations. *Clin Rheumatol* 14:667–72, 1995a.
- Cuellar, ML, E Scopelitis, SA Tenenbaum, RF Garry, LH Silveira, G Cabrera, LR Espinoza. Serum antinuclear antibodies in women with silicone breast implants. *J Rheumatol* 22:236–40, 1995b.
- de Vlam, K, F De Keyser, G Verbruggen, M Vandenbossche, B Vanneuville, D D'Haese, EM Veys. Detection and identification of antinuclear autoantibodies in the serum of normal blood donors. *Clin Exp Rheumatol* 11:393–97, 1993.
- Deapen, DM, L Bernstein, GS Brody. Are breast implants anticarcinogenic? A 14-year follow-up of the Los Angeles Study. *Plast Reconstr Surg* 99:1346–53, 1997.
- Dinerman, H, DL Goldenberg, DT Felson. A prospective evaluation of 118 patients with the fibromyalgia syndrome: prevalence of Raynaud's phenomenon, sicca symptoms, ANA, low complement, and Ig deposition at the dermal-epidermal junction. *J Rheumatol* 13:368–73, 1986.
- Dobke, MK, JK Svahn, VL Vastine, BN Landon, PC Stein, CL Parsons. Characterization of microbial presence at the surface of silicone mammary implants. *Ann Plast Surg* 34:563–69,

1995.

- Edworthy, SM, L Martin, SG Barr, DC Birdsell, RF Brant, MJ Fritzler. A clinical study of the relationship between silicone breast implants and connective tissue disease. *J Rheumatol* 25:254–60, 1998.
- Ellis, TM, NS Hardt, L Campbell, DA Piacentini, MA Atkinson. Cellular immune reactivities in women with silicone breast implants: a preliminary investigation. *Ann Allergy Asthma Immunol* 79:151–54, 1997.
- Emlen, W, L O'Neill. Clinical significance of antinuclear antibodies: comparison of detection with immunofluorescence and enzyme-linked immunosorbent assays. *Arthritis Rheum* 40:1612–18, 1997.
- Fitzgerald, O, EV Hess, GT O'Connor, G Spencer-Green. Prospective study of the evolution of Raynaud's phenomenon. *Am J Med* 84:718–26, 1988.
- Freundlich, B, C Altman, N Snadorfi, M Greenberg, J Tomaszewski. A profile of symptomatic patients with silicone breast implants: a Sjogrens-like syndrome. *Semin Arthritis Rheum* 24:44–53, 1994.
- Fritzler, MJ, JD Pauls, TD Kinsella, TJ Bowen. Antinuclear, anticytoplasmic, and anti-Sjogren's syndrome antigen A (SS- A/Ro) antibodies in female blood donors. *Clin Immunol Immunopathol* 36:120–28, 1985.
- Gabriel, SE, WM O'Fallon, LT Kurland, CM Beard, JE Woods, LJ Melton. Risk of connective-tissue diseases and other disorders after breast implantation. *N Engl J Med* 330:1697–02, 1994.
- Garland, LL, OF Ballester, FB Vasey, K Benson, LC Moscinski, MJ Farmelo, MJ Rodriguez, DP Rapaport. Multiple myeloma in women with silicone breast implants. Serum immunoglobulin and interleukin-6 studies in women at risk. *Curr Top Microbiol Immunol* 210:361–66, 1996.
- Gerbracht, DD, VD Steen, GL Ziegler, TAJ Medsger, GP Rodnan. Evolution of primary Raynaud's phenomenon (Raynaud's disease) to connective tissue disease. *Arthritis Rheum* 28:87–92, 1985.
- Goldblum, RM, D Pyron, M Shenoy, Modulation of IgG binding to silicone by human serum albumin. *FASEB J abstract* 9:5967, 1995.
- Goldblum, RM, RP Pelley, AA O'Donell, D Pyron, JP Heggers. Antibodies to silicone elastomers

- and reactions to ventriculoperitoneal shunts [published erratum appears in *Lancet* 340:800, 1992]. *Lancet* 340:510–13, 1992.
- Granchi, D, D Cavedagna, G Ciapetti, S Stea, P Schiavon, R Giuliani, A. Pizzoferrato. Silicone breast implants: the role of immune system on capsular contracture formation. *J Biomed Mater Res* 29:197–202, 1995.
- Herrinton, LJ. The epidemiology of monoclonal gammopathy of unknown significance: a review. *Curr Top Microbiol Immunol* 210:389–95, 1996.
- Hill, SL, MG Landavere, NR Rose. The adjuvant effect of silicone gel and silicone elastomer particles in rats. *Curr Top Microbiol Immunol* 210:123–37, 1996.
- Homburger, HA. Cascade testing for autoantibodies in connective tissue diseases. *Mayo Clin Proc* 70:183–84, 1995.
- Horven, S, TC Stiles, A Holst, T Moen. HLA antigens in primary fibromyalgia syndrome. *J Rheumatol* 19:1269–70, 1992.
- Imai, M, C Yamada, S Saga, S Nagayoshi, M Hoshino. Immunological cross reaction between sera from patients with breast cancer and mouse mammary tumor virus. *Gann* 70:63–74, 1979.
- Irwin, M, A Mascovich, JC Gillin, R Willoughby, J Pike, TL Smith. Partial sleep deprivation reduces natural killer cell activity in humans. *Psychosom Med* 56:493–98, 1994.
- Irwin, M, J McClintick, C Costlow, M Fortner, J White, JC Gillin. Partial night sleep deprivation reduces natural killer and cellular immune responses in humans. *FASEB J* 10:643–53, 1996.
- Jennings, DA, MJ Morykwas, WW Burns, ME Crook, WP Hudson, LC Argenta. In vitro adhesion of endogenous skin microorganisms to breast prostheses. *Ann Plast Surg* 27:216–20, 1991.
- Juby, AG, P Davis, JE McElhaney, S Gravenstein. Prevalence of selected autoantibodies in different elderly subpopulations. *Br J Rheumatol* 33:1121–24, 1994.
- Kallenberg, CG, AA Wouda, MH Hoet, WJ van Venrooij. Development of connective tissue disease in patients presenting with Raynaud's phenomenon: a six year follow up with emphasis on the predictive value of antinuclear antibodies as detected by immunoblotting. *Ann Rheum Dis* 47:634–41, 1988.
- Katzin, WE, LJ Feng, M Abbuhl, MA Klein. Phenotype of lymphocytes associated with the inflammatory reaction to silicone gel breast implants. *Clin Diagn Lab Immunol* 3:156–61,

- 1996.
- Kavanaugh, DY, DP Carbone. Immunologic dysfunction in cancer. *Hematol Oncol Clin North Am* 10:927–51, 1996.
- Klajman, A, B Kafri, T Shohat, I Drucker, T Moalem, A Jaretzky. The prevalence of antibodies to histones induced by procainamide in old people, in cancer patients, and in rheumatoid-like disease. *Clin Immunol Immunopathol* 27:1–8, 1983.
- Klykken, PC, KLJ White. The adjuvancy of silicones: dependency on compartmentalization. *Curr Top Microbiol Immunol* 210:113–21, 1996.
- Kossovsky, N, M Zeidler, G Chun, N Papasian, A Nguyen, S Rajguru, J Stassi, A Gelman, E Sponsier. Surface dependent antigens identified by high binding avidity of serum antibodies in a subpopulation of patients with breast prostheses. *J Applied Biomaterials* 4:281–88, 1993.
- Kyle, RA. Monoclonal gammopathy of undetermined significance. *Curr Top Microbiol Immunol* 210:375–83, 1996.
- Lai, JL, L. Michaux, N Dastugue, F Vasseur, A Daudignon, T Facon, F Bauters, M Zandecki. Cytogenetics in multiple myeloma: a multicenter study of 24 patients with t(11;14)(q13;q32) or its variant. *Cancer Genet Cytogenet* 2:133–38, 1998.
- Lewy, RI, E Ezrailson. Laboratory studies in breast implant patients: ANA positivity, gammaglobulin levels, and other autoantibodies. *Curr Top Microbiol Immunol* 210:337–53, 1996.
- Luggen, M, L Belhorn, T Evans, O Fitzgerald, G Spencer-Green. The evolution of Raynaud's phenomenon: a longterm prospective study. *J Rheumatol* 22:2226–32, 1995.
- McDaniel, DO, GS Alarcon, PW Pratt, JD Reveille. Most African-American patients with rheumatoid arthritis do not have the rheumatoid antigenic determinant (epitope). *Ann Intern Med* 123:181–87, 1995.
- Mena, EA, N Kossovsky, C Chu, C Hu. Inflammatory intermediates produced by tissues encasing silicone breast prostheses. *J Investigative Surgery* 8:31–42, 1994.
- Morse, JH, M Fotino, Y Zhang, ER Flaster, CL Peebles, H Spiera. Position 26 of the first domain of the HLA-DQB1 allele in post-silicone implant scleroderma. *J Rheumatol* 22:1872–75, 1995.
- Naim, JO, KM Ippolito, CJ van Oss. Adjuvancy effect of different types of silicone gel. *J Biomed*

- Mater Res* 37:534–38, 1997.
- Naim, JO, KM Ippolito, RJ Lanzafame. The effect of molecular weight and gel preparation on humoral adjuvancy of silicone oils and silicone gels. *Immunol Invest* 24:537–47, 1995.
- Nepom, GT, BS Nepom. Prediction of susceptibility to rheumatoid arthritis by human leukocyte antigen genotyping. *Rheum Dis Clin North Am* 18:785–92, 1992.
- Netscher, DT, G Weizer, P Wigoda, LE Walker, J Thornby, D Bowen. Clinical relevance of positive breast periprosthetic cultures without overt infection. *Plast Reconstr Surg* 96:1125–29, 1995.
- O'Hanlon, TP, S Okada, LA Love, G Dick, VL Young, FW Miller. Immunohistopathology and T cell receptor gene expression in capsules surrounding silicone breast implants. *Curr Top Microbiol Immunol* 210:237–42, 1996.
- Ojo-Amaize, EA, V Conte, HC Lin, RF Brucker, MS Agopian, JB Peter. Silicone-specific blood lymphocyte response in women with silicone breast implants. *Clin Diagn Lab Immunol* 1:689–95, 1994.
- Ojo-Amaize, EA, OJ Lawless, JB Peter. Elevated concentrations of interleukin-1 beta and interleukin-1 receptor antagonist in plasma of women with silicone breast implants. *Clin Diagn Lab Immunol* 3:257–59, 1996.
- Park, AJ, RJ Black, NS Sarhadi, U Chetty, AC Watson. Silicone gel-filled breast implants and connective tissue diseases. *Plast Reconstr Surg* 101:261–68, 1998.
- Pedersen, BK, JM Beyer. Characterization of the in vitro effects of glucocorticoids on NK cell activity. *Allergy* 41:220–24, 1986.
- Pedersen, BK, H Ullum. NK cell response to physical activity: possible mechanisms of action. *Med Sci Sports Exerc* 26:140–46, 1994.
- Pedersen, BK, M Kappel, M Klokke, HB Nielsen, NH Secher. The immune system during exposure to extreme physiologic conditions. *Int J Sports Med.* 15:S116–21, 1994.
- Peters, W, D Smith, V Fornasier, S Lugowski, D Ibanez. An outcome analysis of 100 women after explantation of silicone gel breast implants. *Ann Plast Surg* 39:9–19, 1997.
- Peters, W, E Keystone, K Snow, L Rubin, D Smith. Is there a relationship between autoantibodies and silicone-gel implants? *Ann Plast Surg* 32:1–5, 1994.
- Potter, M, S Morrison, F Miller. Induction of plasmacytomas in genetically susceptible mice with silicone gels. *Curr Top Microbiol Immunol* 194:83–91, 1996.

- Press, RI, CL Peebles, Y Kumagai, RL Ochs, EM Tan. Antinuclear autoantibodies in women with silicone breast implants. *Lancet* 340:1304–07, 1992.
- Rabkin, CS, S Silverman, G Tricot, LL Garland, O Ballester, M Potter. The National Cancer Institute silicone implant/multiple myeloma registry. *Curr Top Microbiol Immunol* 210:385–89, 1996.
- Riera, G, M Vilardell, J Vaque, V Fonollosa, B Bermejo. Prevalence of Raynaud's phenomenon in a healthy Spanish population. *J Rheumatol* 20:66–69, 1993.
- Rose, NR, C Bona. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol Today* 14:426–30, 1993.
- Rose, NR, M Landavere, RC Koppers. Silicone binding immunoglobulins in human sera. *Curr Top Microbiol Immunol* 210:269–76, 1996.
- Rosenau, B, AB Schneebaum, PH Schur. The development of an ELISA method for the detection of "antibodies" to silicone. *Curr Top Microbiol Immunol* 210:69–74, 1996.
- Rowley, MJ, AD Cook, SS Teuber, ME Gershwin. Antibodies to collagen: comparative epitope mapping in women with silicone breast implants, systemic lupus erythematosus and rheumatoid arthritis. *J Autoimmun* 7:775–89, 1994.
- Ruffatti, A, L Rossi, A Calligaro, T Del Ross, M Lagni, P Marson, S Todesco. Autoantibodies of systemic rheumatic diseases in the healthy elderly. *Gerontology* 36:104–11, 1990.
- Sheiner, NM, P Small. Isolated Raynaud's phenomenon--a benign disorder. *Ann Allergy* 58:114–17, 1987.
- Shen, GQ, EA Ojo-Amaize, MS Agopian, JB Peter. Silicate antibodies in women with silicone breast implants: development of an assay for detection of humoral immunity. *Clin Diagn Lab Immunol* 3:162–66, 1996.
- Shepard, RJ, S Rhind, PN Shek. Exercise and the immune system. Natural killer cells, interleukins, and related response. *Sports Med* 18:340–69, 1994.
- Shoenfeld, Y, DA Isenberg. *The Mosaic of Autoimmunity : (The Factors Associated with Autoimmune Disease)*. Research Monographs in Immunology, vol. 12. Amsterdam: Elsevier, 1989.
- Sibbitt, WLJ, AD Bankhurst. Natural killer cells in connective tissue disorders. *Clin Rheum Dis* 11:507–21, 1985.
- Silverman, S, O Gluck, D Silver, J Tesser, D Wallace, K Neumann, A Metzger, R Morris. The

- prevalence of autoantibodies in symptomatic and asymptomatic patients with breast implants and patients with fibromyalgia. *Curr Top Microbiol Immunol* 210:317–22, 1996a.
- Silverman, S, R Vescio, D Silver, S Renner, S Weiner, J Berenson. Silicone gel implants and monoclonal gammopathies: three cases of multiple myeloma and the prevalence of multiple myeloma and monoclonal gammopathy of undetermined significance. *Curr Top Microbiol Immunol* 210:367–74, 1996b.
- Slater, CA, RB Davis, RH Shmerling. Antinuclear antibody testing. A study of clinical utility. *Arch Intern Med* 156:1421–25, 1996.
- Smalley, DL, DR Shanklin, MF Hall, MV Stevens, A Hanissian. Immunologic stimulation of T lymphocytes by silica after use of silicone mammary implants. *FASEB J* 9:424–27, 1995.
- Solomon, G. A clinical and laboratory profile of symptomatic women with silicone breast implants. *Semin Arthritis Rheum* 24:29–37, 1994.
- Struyf, NJ, HW Snoeck, CH Bridts, LS De Clerck, WJ Stevens. Natural killer cell activity in Sjogren's syndrome and systemic lupus erythematosus: stimulation with interferons and interleukin-2 and correlation with immune complexes. *Ann Rheum Dis* 49:690–93, 1990.
- Tan, EM, TE Feltkamp, JS Smolen, B Butcher, R Dawkins, MJ Fritzler, T Gordon, JA Hardin, JR Kalden, RG Lahita, et al. Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum* 40:1601–11, 1997.
- Teller, K, L Budhai, M Zhang, N Haramati, HD Keiser, A Davidson. HLA-DRB1 and DQB typing of Hispanic American patients with rheumatoid arthritis: the "shared epitope" hypothesis may not apply. *J Rheumatol* 23:1363–68, 1996.
- Tenenbaum, SA, JC Rice, LR Espinoza, ML Cuellar, DR Plymale, DM Sander, LL Williamson, AM Haislip, OS Gluck, JR Tesser. Use of antipolymer antibody assay in recipients of silicone breast implants [published erratum appears in *Lancet* 349:1558, 1997]. *Lancet* 349:449–54, 1997.
- Teuber, SS, MJ Rowley, SH Yoshida, AA Ansari, ME Gershwin. Anti-collagen autoantibodies are found in women with silicone breast implants. *J Autoimmun.* 6:367–77, 1993.
- Thomas, C, JA Robinson. The antinuclear antibody test. When is a positive result clinically relevant? *Postgrad Med* 94:55–58, 63, 66, 1993.
- Turnbull, AR, DT Turner, JD Fraser, RS Lloyd, CJ Lang, R Wright. Autoantibodies in early breast cancer: a stage-related phenomenon? *Br J Cancer* 38:461–63, 1978.

- Ueki, A, M Yamaguchi, H Ueki, Y Watanabe, G Ohsawa, K Kinugawa, Y Kawakami, F Hyodoh. Polyclonal human T-cell activation by silicate in vitro. *Immunology* 82:332–35, 1994.
- van Ierssel, GJ, MA Mieremet-Ooms, AM van der Zon, RA van Hogezaand, MJ Wagtmans, V van der Sluys, CB Lamers, HW Verspaget. Effect of cortisol and ACTH on corticosteroid-suppressed peripheral blood natural killer cells from healthy volunteers and patients with Crohn's disease. *Immunopharmacology* 34:97–104, 1996.
- Vasey, FB, DL Havice, TS Bocanegra, MJ Seleznick, PH Bridgeford, P Martinez-Osuna, LR Espinoza. Clinical findings in symptomatic women with silicone breast implants. *Semin Arthritis Rheum* 24:22–28, 1994.
- Vojdani, A, A Campbell, N Brautbar. Immune functional impairment in patients with clinical abnormalities and silicone breast implants. *Toxicol Ind Health* 8:415–29, 1992.
- von Muhlen, CA, EM Tan. Autoantibodies in the diagnosis of systemic rheumatic diseases. *Semin Arthritis Rheum* 24:323–58, 1995.
- Williams, HJ, GS Alarcon, R Neuner, VD Steen, K Bulpitt, DO Clegg, CM Ziminski, ME Luggen, RP Polisson, RF Willkens, et al. Early undifferentiated connective tissue disease. V. An inception cohort 5 years later: disease remissions and changes in diagnoses in well established and undifferentiated connective tissue diseases. *J Rheumatol* 25:261–68, 1998.
- Wilson, SD, AE Munson. Silicone-induced modulation of natural killer cell activity. *CurrTop Microbiol Immunol* 210:199–208, 1996.
- Wolf, LE, M Lappe, RD Peterson, EG Ezrailson. Human immune response to polydimethylsiloxane (silicone): screening studies in a breast implant population. *FASEB J* 7:1265–68, 1993.
- Xavier, RM, Y Yamauchi, M Nakamura, Y Tanigawa, H Ishikura, T Tsunematsu, S Kobayashi. Antinuclear antibodies in healthy aging people: a prospective study. *Mech Ageing Dev* 78:145–54, 1995.
- Yadin, O, B Sarov, L Naggan, H Slor, Y Shoenfeld. Natural autoantibodies in the serum of healthy women—a five-year follow-up. *Clin Exp Immunol* 75:402–6, 1989.
- Yamamoto, K, H Hamaguchi, K Nagata, M Taniwaki. A variant Burkitt-type translocation (8;22) (q24;q11) in multiple myeloma. Report of a new case and review of the literature. *Cancer Genet Cyogenet* 2:98–103, 1998.
- Yoshino, S. Silicone-induced arthritis in rats and possible role for T cells. *Immunobiology*

192:40–47, 1994.

Young, VL. Testing the test: an analysis of the reliability of the silicone sensitivity test (SILS) in detecting immune-mediated responses to silicone breast implants. *Plast Reconstr Surg* 97:681–83, 1996.

Young, VL, JR Nemecek, BD Schwartz, DL Phelan, MW Schorr. HLA typing in women with and without silicone gel-filled breast implants. *Curr Top Microbiol Immunol* 25:209–25, 1996.

Zazgornik, J, H Piza, W Kaiser, P Bettelheim, G Steiner, J Smolen, G Biesenbach, W Maschek. Autoimmune reactions in patients with silicone breast implants. *Wien Klin Wochenschr* 108:781–87, 1996.

Table 1 Studies of anti-nuclear antibodies in women with silicone breast implants

Author+	*Women with SBI	*Healthy controls	*SBI well	*SBI CTD	*Symptomatic no SBI	ANA dilution/intensity	Comments
Peters 1994	53/200	28/100	ni	ni	ni	100/ns	Controls age and sex matched
Peters 1997	24/100	28/100	ni	ni	ni	100/ns	100 patients requesting explant and 100 age matched controls (historical). 8 SBI had inflammatory rheumatic disease and 10 had FM
Press 1992	7/13	ni	ni	10/11	ni	40/ns	State that normals in their lab have 5-6% + rate. Women with SBI are symptomatic or have CTD
Zazgornick 1996	ni	3/36	12/36	ni	ni		Done on liver substrate. Only + are confirmed on Hep-2. Patients are mostly reconstruction post mastectomy. Controls age and sex matched
Brunner 1996	6/239	ni	ni	ni	ni	80/ns	151 gel, 41 saline, 46 unknown .
Tenenbaum 1997	17/80	4/23	ni	3/15	14/20 (CTD)	40/ns	15 patients had definite CTD type not specified. Healthy controls non-age matched.
Claman 1994	21/82	0/19	7/38	7/11	ni	256/1+	Controls are sex matched, but 6-7 years younger
Claman 1996	ni	1/17	13/37	ni	ni	80/1+	
Bridges 1993	34/156 20/127 (CTD excluded)	ni	1/12	14/29	43/174 (FM)	80/ns	29 SBI CTD included in 156. Of others, 32 with joint swelling and 95 joint and muscle pain. Controls age and sex matched
Freundlich 1993	10/50	ni	ni	ni	ni	ns/ns	All the patients in these studies are described as "sick" and some have classic CTD
Vasey 1994	13/37	ni	ni	ni	ni	20/ns	
Solomon 1994	44/176	ni	ni	ni	ni	40/ns	
Bridges 1996	150/500	2/25	7/25	ni	25/100 (FM)	80/ns	
Cuellar a 1995	123/265	ni	ni	ni	20/264 (FM, STR)	40/2+	Controls not age matched. Controls were 122 with STR, 142 with FM.
Cuellar b 1995	470/813	ni	ni	ni	20/264	40/2+	All women with SBI were symptomatic. Same controls as above.

Author	Women with SBI	Healthy controls	SBI well	SBI CTD	Symptomatic no SBI	ANA dilution/intensity	Comments
Lewy 1996	861/3890	ni	ni	ni	ni	40/ns	Numbers don't correlate with text. No medical verification. Patients were assessed by 2 physicians by standard protocol and questionnaire but the only data presented is percent complaining of each symptom. No control group.
Gabriel 1994	11/749	27/1498	ni	ni	ni	ns	Case control study. Age and sex matched controls. ANA gathered from chart.
Edworthy 1998	324/1426	162/635	ni	ni	ni	40/1+	Case control study. Sera collected prospectively. Gender but not quite age matched. No difference when age was taken into account. ENA showed no differences. RF showed no differences. Saline and other implants included (about 30% of group).
Park 1998	Aug 3/110 Rec 5/207	0/128 5/88	ni	ni	ni	40/ns	Sera collected prospectively. All silicone gel. Reconstruction and augmentation analyzed separately. Controls age and sex matched.
Silverman 1996	1121/3184	2/40	1/37	ni	56/200 (FM)	80/ns	Controls aged matched. Patients are "symptomatic" women not further described. "Concerned" but not symptomatic SBI and 200 fibromyalgia verified by ACR criteria.
Teuber 1993	16/46	ni	ni	ni	ni	80/ns	Patients self referred "concerned" women. Some had defined CTD and some had UCTD
Rowley 1994	19/70	ni	ni	ni	ni	80/ns	70 self referred includes many of the same patients as study Teuber above but definite CTD excluded.
Blackburn 1997	8/70	ni	ni	ni	ni	40/ns	Patients referred for "complaints or concerns". 27 had FM and 3 had definite CTD.

+ reports are identified by first author only

* number positive / number tested

ni - group not included or not analyzed separately

ns - not stated

ANA - anti-nuclear antibody

CTD - connective tissue disease

FM - fibromyalgia

SBI - silicone breast implant

STR - soft tissue rheumatism

Aug - augmentation

Rec - reconstruction