

Silicone Breast Implants in Relation to Connective Tissue Diseases and Immunologic Dysfunction

**A Report by a National Science Panel
to the Honorable Sam C. Pointer Jr.,
Coordinating Judge for the Federal Breast Implant
Multi-District Litigation**

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Silicone Breast Implants in Relation to Connective Tissue Diseases and Immunologic Dysfunction

Executive Summary

Four scientific experts in the fields of immunology, epidemiology, toxicology, and rheumatology were appointed by the Honorable Sam C. Pointer, Jr., Coordinating Judge for the Federal Breast Implant Multi-District Litigation, to serve on a National Science Panel. Members of the panel include:

Betty A. Diamond, MD, Professor, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York;

Barbara S. Hulka, MS, MD, MPH, Kenan Professor, Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina;

Nancy I. Kerkvliet, MS, PhD, Professor of Toxicology and Extension Toxicology Specialist, Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon; and

Peter Tugwell, MBBS, MD, MSc, FRCP [Canada and United Kingdom], Professor and Chairman, Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada.

The panel was instructed to review and critique the scientific literature pertaining to the possibility of a causal association between silicone breast implants and connective tissue diseases, related signs and symptoms, and immune system dysfunction. The panel met, received instructions from the judge, and heard testimony from experts selected by the counsels for the plaintiffs and for the defendants in October 1996. Additional hearings were held in July 1997, when experts identified by the parties provided testimony, and in November 1997 when the panel's invited experts presented their research material.

In spring 1997, over 2000 documents were submitted to the panelists from the legal counsels for both parties. Subsequently, the counsels pared these numbers down to the approximately 40 most important documents from each side for each panel member. The source of references, whether counsel for the plaintiffs or counsel for the defendants, was not identified to the panelists. The panel members also used their own literature search strategies, and were neither limited to nor obligated to use those submitted by the respective legal counsels.

Organization of Report

The report is divided into four chapters, each based on the expertise of one of the four panelists. They follow in sequence: toxicology, immunology, epidemiology, and rheumatology. A summary and bibliography are provided at the end of each chapter and some chapters contain appendices. This executive summary precedes the chapters to state the judge's charge to the panelists, indicate the process undertaken by the panel, and provide a brief overview of the panel's main findings and conclusions.

Charge from Judge Pointer

The court-appointed experts were asked to respond to the following questions:

“(a). Issues. To what extent, if any and with what limitations and caveats do existing studies, research, and reported observations provide a reliable and reasonable scientific basis for one to conclude that silicone-gel breast implants cause or exacerbate any of the conditions described in (b) below? If, in the process of making these findings, you believe that there are related or subordinate issues that should be separately addressed, please do so.

(b). Scope. You are asked at this time to consider the relationship, if any, between implants and the following:

‘classic’ connective tissue diseases, such as systemic lupus erythematosus, Sjögren’s syndrome, etc.

‘atypical’ presentations of connective tissue diseases or symptoms immune system dysfunctions

Listed in the appendix to this order are various diseases, symptoms, conditions, or complaints that have sometimes been asserted as possibly associated with silicone-gel implants. To the extent you believe appropriate and without being asked to address separately each of these diseases, symptoms, conditions, and complaints you are encouraged to comment on the scientific basis, if any, for any such claimed linkage. You are not being asked to consider purely local complications, such as breast disfigurement, tenderness, or capsular contracture.

(c). **Contrary Opinions.** To what extent, if any, should any of your opinions referenced in (a) above be considered as subject to sufficient genuine dispute as would permit other persons, generally qualified in your field of expertise, to express opinions that, though contrary to yours, would likely be viewed by others in the field as representing legitimate and responsible disagreement within your profession?"

Background to Charge

While silicone breast implants have been in use since the early 1960s, it was not until 1976 that legislation was passed giving the Food and Drug Administration (FDA) responsibility to oversee the safety of medical devices. Because implants had been used for over a decade, their safety was presumed and their continued use was permitted. Furthermore, while it was known that local complications could occur with silicone breast implants and that rupture of the implant occurred in a portion of recipients, safety studies in animals had suggested no systemic toxicity of silicone gel. In 1982, the FDA proposed that the manufacturers of implants should provide additional evidence on the safety of breast implants. In 1988, the FDA mandated that manufacturers provide such evidence. This ruling was not enforced until 1991, when public attention became focused on the question of the risks of implants and their possible association with connective tissue diseases. The FDA convened two advisory committees in 1991. After the first, David Kessler, then head of the FDA, asked for a voluntary moratorium on the use of silicone gel-filled implants; after the second in 1992, he banned their use except in clinical trials of breast reconstruction after cancer surgery. He stated that the ban was implemented not because gel-filled implants had been shown to be unsafe, but rather, that the manufacturers had not provided adequate data proving their safety.

The first suggestion that there might be adverse systemic reactions to augmentation mammoplasty were reports of autoimmune disease in Japanese women who received liquid paraffin or silicone injections for breast augmentation. Subsequently, concerns were raised regarding an association of silicone breast implants with classic connective tissue diseases and less well-defined atypical syndromes. These initial concerns were expressed in case reports in the medical literature and raised the call for examination of the effects of silicone on the immune system. In December 1990, Connie Chung reported in a nationally televised program that breast implants might be unsafe. Although litigation against the manufacturers of breast implants started in 1982, the number of suits brought by women claiming that they had developed systemic connective tissue disease following silicone breast implantation increased markedly in the 1990s. It has been in this adversarial atmosphere, with high stakes for plaintiffs and defendants, that immunologic and epidemiologic studies of silicone and silicone breast implants have been performed.

Major Findings and Conclusions

Toxicology

Testing of chemicals, pharmaceuticals, and other products in animal models serves to prevent potentially hazardous compounds from reaching the human population. Animal toxicology studies provide information regarding the potential toxicity of a substance, the doses required to elicit toxicity, and the spectrum of possible toxic effects. Because potentially confounding variables (e.g., age, sex, environmental factors) can be controlled experimentally, animal studies provide information that often cannot be obtained directly in humans.

Toxicologic testing with silicone goes back almost 50 years. In the early years, silicone had an enviable record of safety, having been shown consistently to be inert with respect to systemic effects. Only, localized reactions analogous to those induced by other foreign bodies were observed. However, in the late 1980s, case reports of a possible link between silicone breast implants and autoimmune diseases in women reinvigorated toxicologic testing of silicone gels and related compounds. The majority of these more recent studies reaffirmed the low systemic toxicity of silicone.

Animal studies have addressed the possibility that silicone may promote systemic disease in women by acting as an adjuvant or an antigen to induce immune responses, by altering normal

regulation of the immune system, or by inducing systemic inflammation. These potential effects have been tested in specialized animal models of autoimmune diseases. The preponderance of data from these studies indicate that silicone implants do not alter incidence or severity of autoimmune disease. Although silicone gel has been shown to possess weak adjuvant activity when it is injected as an emulsified preparation with a foreign antigen, there is no evidence that silicone breast implants precipitate novel immune responses or induce systemic inflammation. The only reasonably consistent effect of silicone on the immune system in animals is a depression in natural killer cell activity. However, no physiologic consequence of this depression has been demonstrated.

Considering the broad range of testing systems that have been used in the study of silicone effects, the toxicologic and immunologic responses are few in number and questionable in significance. Yet, the results of animal testing may not fully predict the human effects.

Immunology

The evaluation of immunologic responses to silicone breast implants in humans faces significant challenges. There are large numbers of diverse immunologic responses that may be evoked in humans, whether the subjects are healthy or ill, for which the biological meaning and clinical interpretation is uncertain. Furthermore, many of the studies available for analysis are methodologically inadequate with ill-defined or inappropriate comparison subjects, unorthodox data analyses, and the potential for systematic biases in laboratory methods, exemplified by the analysis of cases and controls separately, at different time periods, by different technicians using different batches of reagents. Not surprisingly, inconsistent results in studies purporting to evaluate the same immunologic parameter are common.

While there are data showing that silicone may cause local activation of inflammatory responses, there are no consistent data to suggest systemic inflammation or systemic induction of anti-silicone or autoreactive responses in women with silicone breast implants. Immunologic responses studied include: cytokines as indicators of inflammation, natural killer cell activity, superantigen stimulation of T cells, antigen-specific T cell activation, and autoantibodies of various types (anti-nuclear antibodies, anti-collagen antibodies, and anti-microsomal antibodies), and anti-silicone antibodies. In these studies, employing different immunologic response markers, when appropriate comparisons were made, (ill women with implants compared to

healthy women with implants, or healthy women with implants compared to healthy women without implants), neither immune system activation nor autoreactivity could be reproducibly demonstrated in women with silicone breast implants. Furthermore, no unique human lymphocyte antigen haplotypes in ill women with implants have been identified. The frequency of different human lymphocyte antigen haplotypes is the same in ill women with or without implants. The main conclusion that can be drawn from existing studies is that women with silicone breast implants do not display a silicone-induced systemic abnormality in the types or functions of cells of the immune system.

In a mouse strain predisposed to the development of plasmacytomas, tumor formation was enhanced after the intraperitoneal injection of silicone gel. How this information translates to humans is currently unknown. Existing data in humans do not suggest an effect of silicone breast implants on either gammopathy or myeloma, but the number and size of studies is inadequate to produce definitive results.

Epidemiology

The evaluation of epidemiologic studies of silicone breast implants and connective tissue diseases focused on several definite connective tissue diseases (rheumatoid arthritis, systemic lupus erythematosus, scleroderma, Sjögren's syndrome, and dermatomyositis/polymyositis) and a grouping of less well-defined entities, which we labeled "other autoimmune/rheumatic conditions." The latter included a mixture of signs, symptoms, and diagnoses provided by the authors of the relevant studies. Several meta-analyses, which pool data from multiple studies, were conducted to identify a possible association between breast implants and connective tissue diseases.

No association was evident between breast implants and any of the individual connective tissue diseases, all definite connective diseases combined, or the other autoimmune/rheumatic conditions. Sjögren's syndrome was a possible exception to this statement. This entity requires salivary gland biopsy to meet the published diagnostic criteria. Whether biopsy was actually performed for cases in the studies cited is unknown. The remaining criteria based on dryness of the eyes and mouth with possible immunologic alterations are nonspecific and relatively common in any population group. Thus, the accuracy of diagnosis of Sjögren's syndrome in the studies incorporated in this meta-analysis is questionable.

One meta-analysis included only those studies that distinguished silicone gel-filled breast implants from any other type. The results from this meta-analysis were consistent with those from the other meta-analyses where breast implants were more broadly defined. There was no association between silicone gel-filled implants and any of the definite connective tissue diseases (including Sjögren's syndrome) or the other autoimmune/rheumatic conditions.

Rheumatology

The term *atypical connective tissue disease* has been used to describe constellations of signs, symptoms, and abnormal laboratory tests, insufficient by themselves to meet the specified criteria of a classic connective tissue disease. Among these descriptive groupings, mixed connective tissue disease and undifferentiated connective tissue disease are distinctive in that they have established case definitions, which include substantive and sustained symptoms. In most studies of breast implants, however, neither of these diagnostic entities has been evaluated as a separate disease category. Rather, they have been included in a combined grouping of ill-defined connective tissue diseases. The one study that specifically addressed undifferentiated connective tissue disease found no association with silicone breast implants. Another reported disease entity is "systemic silicone related disease," for which the case definition includes the presence of a silicone breast implant. This inclusion criterion makes scientific evaluation difficult, since there is no possibility of comparing the incidence of the syndrome in women with and without implants.

Breast implant patients have reported a diversity of symptoms and signs that are also associated with rheumatic or autoimmune diseases. For each sign or symptom showing an association with breast implants in a given study, other studies found no association. Symptoms associated with breast implants in at least one study included: arthralgias, swollen or tender lymph glands, myalgias, dryness of mouth or eyes, skin changes, and stiffness. Problems in analyzing these studies were numerous: the same complaint appeared in more than one disease category; self-report was not verified; timing of the complaint in relation to the implant was not known; indication for the implant was ignored; and in individual studies, the number of affected women was small. Furthermore, many of the rheumatologic complaints reported are common in the general population and as presenting complaints in physicians' offices. No distinctive features relating to silicone breast implants could be identified.

Little is known about the effect of silicone breast implants on clinical course and immunologic parameters in women with pre-existing classic connective tissue disease or in women who develop such a disease following an implant.

Contrary Opinions

The panel members are in agreement on the findings and interpretations of the data on silicone breast implants and connective tissue diseases, and their immunologic correlates, as presented in this report. The material presented represents an analysis of the most rigorous and relevant scientific information currently available. It is our informed opinion that the large majority of scientists in our respective disciplines would find merit in our reviews and analyses.

Nevertheless, as in every field of endeavor, a few individuals may find disagreements with our statements. As individual scientists and as a group, we have taken no predetermined position on the issues, nor have we designed the report to refute or enhance any point of view. On the contrary, we have allowed the existing research data to lead us to the conclusions presented. We cannot anticipate what research findings may appear in the future.

Chapter I: Review of Animal Studies Relevant to Silicone Toxicity

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Chapter I

Review of Animal Studies Relevant to Silicone Toxicity

I. What Is Silicone?

Silicone is the name given to a family of synthetic polymers composed of a repeating Si-O backbone and carbon-linked side-groups. Si-C bonds do not exist in

nature but can be formed under appropriate manufacturing conditions. The most common example of a silicone is poly(dimethylsiloxane)(PDMS), shown in Figure 1. The dimethylsiloxane units are the basic building blocks of silicones (Lane et al., 1996). Depending on the number of dimethylsiloxane units linked together as a linear polymer and degree of cross-linking between polymer chains, products of various textures and strengths are produced, including forms that mimic human body tissues. In general, straight chain polymers are liquids that increase in viscosity as the chain lengthens (liquid → gel). Increased cross-linking of the chains leads to increasingly rigid silicone materials (gel → elastomer). Substitution of methyl (-CH₃) groups with other side chains produces silicone derivatives with varied physical characteristics and chemical reactivities.

Based on a 1950 review article, initial laboratory studies of silicone oils had shown that silicones were “remarkably stable in comparison with other fluids of similar viscosity . . . and more resistant to oxidation and more water repellent than other fluids” (Barondes et al., 1950). They were also shown to have “good resistance to chlorine, nitric acid and hydrochloric acid, sodium chloride, sulfur dioxide and sulfuric acid up to 30% concentration.” This early review also cautioned that silicone polymers “are not to be confused with the silicon (Si) compounds as sodium silicate, silica gel, and siliceous earth. Silica gel, for example, is a colloidal silica that absorbs water.”

II. Utility and Significance of Animal Studies for Human Toxicity Assessment

Experimental animal studies are used for safety assessment purposes prior to the introduction of a chemical or device for use in humans. These studies primarily use laboratory rats and mice, dogs, and rabbits, with additional animal species tested to address specific toxicology questions.

The data obtained from animal studies provide three main types of information. The first

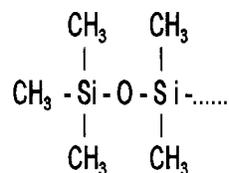


Figure 1. Poly (dimethylsiloxane)

tests conducted in animals are generally referred to as “hazard identification.” These studies are carried out to determine the *possible* biological/toxicological effects the chemical is *capable* of causing, and often incorporate very high exposure levels or unnatural routes of exposure. These studies are not intended to address the likelihood of effects in humans, but allow scientists to understand the basic ways in which the particular chemical interacts with the cells and tissues in a living mammalian organism.

The second major purpose of animal studies is to establish the relationship between exposure and effects and to characterize the dose-response for those effects. Animal studies are carried out using nearly identical groups of animals that differ only in their exposure to the test substance of interest. By controlling for as many other variables as possible (for example, age, sex, genetic background, environment, diet, etc.), any differences in responses between the controls and treated groups can be causally linked to exposure to the test substance.

Furthermore, by testing different levels of exposure (doses), it is possible to see the relationship between severity of effect and dose; that is, how much chemical is necessary to cause specific effects. The results of this phase of testing are useful in predicting human effects to the extent that appropriate animal models are used and good scientific methods are employed. Applicability of results to humans is also enhanced when similar effects are reported by different laboratories and when consistent effects of exposure are seen in more than one animal species. The results of such studies often determine the fate of products prior to marketing. Once a product is marketed, if problems appear to arise from human exposure, the animal data are valuable to support or refute limited or conflicting evidence in humans.

The third main value of animal toxicity studies is to determine the mechanisms by which a chemical interacts with living cells to produce its toxicity, an important factor in understanding how the chemical might induce or aggravate disease. Such mechanistic studies are particularly important if the benefits of the chemical (e.g., drug) outweigh the toxicity (e.g., side-effects) and the product will be marketed in spite of its recognized toxicity. By understanding the mechanisms for the toxicity, measures can be instituted to prevent or reduce the risk of toxicity. In this phase of testing, the approaches used are not dictated by government regulations and are limited only by the ingenuity of investigators and the amount of funding available for such studies. The relevance of such mechanistic studies in animals will depend on how well-defined the toxicity is in humans and the ability to reproduce the same toxic effects in animals.

III. Rationale for Analysis of Specific Animal Studies Relative to Silicone Toxicity

When considering the whole data base of animal studies relating to silicone toxicity, several decision points were used during this analysis to determine the relevance of specific papers to SBI toxicity. The rationale for these decisions was as follows:

1. The term *silicone* has been used to represent many different types of materials that may have very different chemical characteristics when compared to PDMS. Therefore, toxicity studies of silicones that differ significantly from PDMS, are not present in SBIs, and are known to have different chemical reactivities than PDMS, were not included in this analysis. However, in many papers given to the Panel to review, the specific silicone materials tested were not described other than by code. In this case, it was assumed that a relevant form of PDMS was tested, and the data were reviewed and incorporated into this analysis.
2. Studies that examined the toxicity of silicone species that are found only as minor contaminants of SBIs were evaluated, but more critically in terms of their dose-response relationships. In general, minimal effects by minor species that were seen in animals only at high levels of exposure were considered not applicable to the SBI issue.
3. Studies in which relatively large doses of silicone were directly injected into tissues that would not be accessible by the silicone from SBIs in any significant concentration (eg., silicone injected into the brain) have been judged not relevant to the SBI issue.
4. Studies that are based on the oral route of exposure have been judged not applicable. Most silicones are poorly absorbed and do not result in appreciable systemic exposure from this route (Chenoweth et al., 1956). Thus, a lack of toxicity following dietary exposure cannot be used to infer lack of toxicity from SBIs. On the other hand, the possible hydrolysis of some small silicone molecules (e.g., D4) in the acid environment of the stomach also introduces a variable that would not be applicable to the SBI issue .
5. Based on the lack of any definitive evidence that silicone can be degraded to silicon or silica in the body, the toxicology of silicon or silica has not been reviewed for this report. Although very recent studies have provided evidence that D4 can be metabolized via

oxidative demethylation (Varaprath et al., 1997), probably through the action of hepatic mixed function oxidase activity (McKim et al., 1988), there is no evidence that PDMS fluid, gel, or elastomer induce hepatic enzymes or are metabolized.

6. This review does not specifically address the issues of silicone leakage or metabolism since there is sufficient animal toxicity data available in which silicone fluids and gels were injected directly into tissue, modeling a worst-case scenario in which all of the silicone in the SBI, including minor species, had leaked through the elastomer and was free in the tissue. Furthermore, the results observed in long-term exposure studies of free fluid and gel would have reflected any migration or metabolism that might have occurred.
7. All literature forms provided to the Panel were reviewed, including peer-reviewed journal articles and non-peer-reviewed book chapters, abstracts, theses and reports. When only the abstract of a report was available, it was not used to provide the sole basis for any conclusions drawn. In judging the quality of individual studies, scientific credibility was strengthened by clearly written reports based on experiments that were hypothesis-driven, had adequate control groups (positive and negative), used well-documented and validated assays, evaluated the dose-response relationship, and were analyzed by accepted statistical tests. Credibility was also increased when conclusions drawn were biologically plausible.

IV. Animal Models for Atypical Connective Tissue Diseases

When considering the question of silicones and “atypical connective tissue diseases” (ACTD), the relevance of animal models to human disease becomes an issue. Because most of the symptoms of ACTD are subjective, the disease constellation cannot be modeled in animals unless a surrogate marker for the disease can be identified. However, since the biological basis for the subjective symptoms is not known, only hypothetical causes of ACTD can be examined in animal studies. These hypothetical causes have been articulated by the plaintiffs to the Science Panel and are also found in various publications provided to the Panel. The evidence from animal studies to support or refute these hypotheses has been critically evaluated.

V. Historical Perspectives on Silicone Toxicity

The first review of silicone toxicology was published in 1950 wherein the results of standard testing of various PDMS fluids (DC200 series) in rats, rabbits, and mice were summarized (Barondes et al., 1950). Routes of exposure to silicone included oral intubation or intraperitoneal (ip) injection in rats; intradermal (id) or subcutaneous (sc) injection in mice and rabbits; intravenous (iv) injection in mice; and eye instillation or skin application in rabbits. The overall conclusions drawn from these studies was that the silicone fluids tested “are practically inert physiologically and nontoxic to the body tissues. When fed to laboratory animals in doses as high as 2%, no discernable ill effects were noted. There is little if any reaction when administered intradermally, subcutaneously or intramuscularly.”

Based on the low toxicity of silicone fluids, and the development of a medical grade silicone rubber, the medical uses of silicone greatly expanded during the 1950s and early 1960s (Agnew et al., 1962; Andrews, 1966 ; Ballantyne et al., 1965; Braley, 1972;). When certain adverse reactions to injected silicone fluid were reported, they tended to be attributed to the use of nonmedical grade or otherwise adulterated products. This position appears to have evolved from the fact that most clinical experience with silicone was very good, and most animal studies showed little reaction to pure silicone fluid (e.g., Dow Corning IND 2702, Informational Materials, 1968).

In the United States, the first SBI made of a silicone elastomer (rubber) envelope containing silicone gel was developed in 1960 and marketed in 1963 (Braley, 1972). The silicone gel matrix was composed of high molecular weight linear PDMS polymers cross-linked via the presence of intermittent methyl vinyl groups within the linear chain (Lane and Burns, 1996). Based on the belief that the envelope protected the patient from exposure to the gel, clinical trials on SBIs were not conducted, and their use in humans was apparently allowed based on already- established successful clinical use of silicone rubber and other silicone prostheses. The major concern regarding silicone toxicity at this time appeared to be possible tumor development at the implant site, predicated on a mechanical theory of tumor induction. However, animal studies in the early 1960s described the tissue response to silicone rubber in rats as a fibrotic capsule formation that was accompanied by a mild chronic inflammatory response in some animals. Histiocytes and giant cells were observed (Agnew et al., 1962). These findings were not considered serious detriments to the clinical use of silicone because of the focus on carcinogenesis and the fact that few tumors were observed (Agnew et al., 1962).

In 1966, the cellular response to silicone fluid was described by Andrews in a preliminary report. In this study, silicone fluid was injected directly into the subcutaneous tissue of mice. Tissue responses were compared to mice injected with saline. Tissue sections were reported to show macrophages that had phagocytosed silicone. Similarly, Rees et al.(1966) and Ben-Hur et al. (1967) reported that silicone fluid injected ip or sc appeared to be phagocytosed and distributed systemically, likely via the lymphatics. Other studies by Ballantyne et al. (1965) showed that massive injections of silicone fluid in guinea pigs, while accompanied by phagocytosis, were well-tolerated by the animals. Sparchu and Clashman (1970) also reported evidence of systemic distribution of ip or sc injected silicone fluid in rats, but noted that much of the silicone appeared to be in extracellular vacuoles and not associated with inflammatory cells.

As reviewed by Braley (1973), the use of silicone devices continued to expand in the 1960s, and by 1973, thousands of patients had received various forms of silicones in medical applications, including their growing use as mammary prostheses. Although complications from the clinical use of silicone fluid were recognized, few papers addressed complications from silicone gel implants and those were primarily related to local contracture. It is presumed that there was little concern over the safety of SBIs during this time. This viewpoint was supported by a report by Lilla and Vistnes (1975) who found little reaction to the long-term implantation of various types of SBIs in rabbits. Similarly, two-year dog and rat studies showed little reaction and no toxicity to multiple im, sc or id injections of silicone fluid (DC-360) (West and Jolly, 1976). Similar innocuous effects were seen in dogs that received various implant materials (presumably silicones) over a six-year period (Mastalski et al., 1977). In 1982, a conference at the National Institutes of Health on the safety of clinical applications of biomaterials noted the success of soft tissue augmentation of the breast in its Consensus Statement.

Additional toxicology studies continued to be carried out in the 1980s and into the 90s. The results of two independent two-year chronic toxicity studies of different silicone gels in rats indicated that tissue changes were observed locally at the site of the implant but no systemic toxicity was seen, based on the absence of changes in body weight and food consumption data, or clinical, gross or microscopic pathology results, including data from interim sacrifices (Goodman et al., 1988; Lemen et al., 1992). Tumors were observed at the site of implantation but tumor development was related to the process known as solid-state tumorigenesis. This effect appears to be a process unique to rats injected with free gel since rats injected with liquid silicone (Agnew

et al., 1966) or implanted with elastomer (King et al., 1989) did not develop tumors. Tumors were also not found in rabbits implanted with elastomer-covered gel for up to 18 months (Lilla and Vistnes, 1976) or in mice implanted with silicone fluid, gel or elastomer for 180 days (Bradley et al., 1994). Long-term implantation of various synthetic (presumably silicone-based) materials in dogs for as long as six years did not result in tumor development (Mastalski et al., 1977).

VI. Silicone and “Adjuvant Disease”

In the late 1980's, a number of articles began to appear suggesting a possible link between SBIs and autoimmune disorders in women. The concern seems to have been initiated by reports of delayed adverse reactions in some Japanese women that had been injected with silicone fluid admixed with other substances such as paraffin in the breast tissue many years previously (see citations in Picha and Goldstein, 1996). The connective tissue disease that was observed in these women was termed “human adjuvant disease” based on the theory that silicone could act like the experimental adjuvant known as “Complete Freund’s Adjuvant” (CFA). CFA is an emulsified preparation of heat-killed mycobacteria in mineral oil. When an antigen is incorporated into CFA, the immune response to that antigen is increased and prolonged, a desirable situation for vaccine delivery. However, CFA itself is not used clinically because of severe local inflammatory reactions as well as possible sensitization to the mycobacterium. The mineral oil component of CFA functions to provide a depot effect for the water-in-oil emulsified antigen; the mycobacterial component induces an inflammatory response that facilitates the immune response to the antigen (Broderick, 1989).

“Adjuvant arthritis” is an experimental inflammatory joint disease in rats that is induced by a single injection of CFA (Glenn and Gray, 1965; Pearson, 1956). The clinical manifestations of the disease resemble some inflammatory rheumatic diseases in humans such as rheumatoid arthritis, ankylosing spondylitis, and Reiter’s disease (Muir and Dumonde, 1982). However, “adjuvant arthritis” appears to be a disease unique to rats. Among experimental animals tested, including mice, guinea pigs, rabbits, sheep and monkeys, CFA induces the disease only in the rat, and only in certain strains of rat, indicating that a specific genetic predisposition is required. In some rats (e.g., Dark Agouti [DA]), arthritis can even be induced by the injection of mineral oil alone in the absence of mycobacteria (i.e., Incomplete Freund’s Adjuvant [IFA]).

The mineral oil component of CFA functions nonspecifically, and many different types of

oils were shown to be effective in inducing “adjuvant disease” in rats when emulsified with killed mycobacteria (Whitehouse et. al., 1974). In these studies, a commercial silicone oil was also shown to be a “potent arthritogen.” However, the silicone oil was described as “a lubricating oil of unknown composition” sold as a lock lubricant, which caused severe weight loss in the rats. Thus, this oil is not representative of the medical grade of silicone found in SBIs.

As summarized in Table 1, more recent studies have shown that neither silicone gel nor silicone oil (PDMS) was capable of eliciting arthritis in Lewis rats when injected alone or emulsified with mycobacteria (Chang, 1993; Picha and Goldstein, 1997). Similarly, in the DA rat, a mixture of silicone gel and oil was not effective in inducing arthritis (Naim et al., 1995) unless injected directly into the joint (Yoshino, 1994). Thus, there are no experimental data to support the labeling of silicone-associated disorders as “human adjuvant disease.”

VII. Adjuvant Activity of Silicone

The term *adjuvant* is a label more widely applied in recent years to describe “any substance that enhances the immune response to an antigen with which it is mixed” (Janeway and Travers, 1994). Effective vaccines for human diseases often depend on the incorporation of an adjuvant in order to generate and enhance the development of protective immunity. Under this broad definition, many diverse substances, acting by diverse mechanisms, have been shown to function as adjuvants. Oily substances that prolong antigen half-life in tissues and may enhance cellular uptake are especially effective adjuvants.

As summarized in Table 2, several studies have examined the ability of silicones to function as adjuvants to increase antibody production or cell-mediated immune responses when injected as an emulsified preparation with the antigen. In general, it appears that adjuvant activity is seen more often with silicone gel than with silicone oil. The low molecular weight cyclosiloxane D4 has also been shown to possess adjuvant activity in terms of enhancing antibody production to some antigens.

Adjuvants have also been used experimentally to induce autoimmune disease in animals following immunization with autoantigens or cross-reacting foreign antigens. For example, an arthritic disease can be induced in animals following immunization with either homologous or heterologous type II collagen (Ellis et al., 1992). In this collagen-induced arthritis model, collagen protein is emulsified in CFA or IFA and injected into rats or mice. After one or more

immunizations, the onset of the disease is identified by severe swelling and erythema in the paws, which is associated with a massive inflammatory infiltrate into the synovium (Ellis et al., 1992). The histological changes in the joints of these animals resemble those observed in rheumatoid arthritis patients.

The ability of silicone to substitute for mineral oil in the induction of collagen-induced arthritis has been examined in both rats and mice (see Table 2). Following the injection of bovine collagen emulsified in a silicone oil:gel mixture, Naim et al. (1995) reported that arthritis was induced in 4/10 rats compared to 8/9 rats injected with collagen in IFA. When silicone oil was tested independently from silicone gel, the incidence of arthritis was higher with the gel(7/10) than the oil (3/10). In contrast, D4 was not an effective adjuvant for induction of arthritis (Naim et al., 1995). Using the DBA/1 mouse model, Schaefer (1997) reported that silicone oil was not an effective adjuvant for the induction of collagen-induced arthritis, even if the inoculum included killed mycobacteria, whereas 80% of the mice injected with collagen in CFA developed arthritis.

It is important to recognize that the successful induction of arthritis in rats with collagen emulsified in silicone does not reflect silicone-induced “adjuvant arthritis”, which develops in the absence of active immunization. Rather, these results point to the successful immunization of the rat to the foreign collagen protein when silicone gel or oil was used as the adjuvant. On the other hand, in an animal model of experimental autoimmune thyroiditis, the injection of rat thyroglobulin (Tg) emulsified in a silicone oil:gel mixture was unable to induce thyroiditis, while 100% of rats injected with Tg in CFA developed thyroid disease (Naim et al., 1993).

VIII. Effects of Silicone in Animal Models of Autoimmune Disease

The question of whether or not silicone is capable of causing or aggravating autoimmune disease can be addressed most directly by laboratory animal studies using different experimental models of autoimmune disease. These established models of autoimmune disease have been developed to study the biological processes responsible for the symptoms associated with the disease, the predisposing genetic and environmental factors that influence the disease process, and the effectiveness of potential therapies. While no single animal model perfectly matches human disease, there are usually many parallels, and data obtained from different animal models can provide insight into the disease process in humans.

Animal models in which autoimmune disease develops spontaneously are most relevant for

the evaluation of the ability of silicone to exacerbate (promote) autoimmune disease. Promotion could be associated with the early appearance, increased severity, and/or increased incidence of autoimmune disease in animals that were destined to develop autoimmune disease due to genetic predisposition. Animal models in which autoimmune disease is induced by specific antigen injection are also useful to evaluate promotion when the severity of the induced disease in controls can be minimized. In contrast, it is much more difficult to evaluate whether or not silicone *causes* an autoimmune disease because of the multifactorial nature of disease induction. Causation could perhaps be deduced if a novel disease developed in an autoimmune-prone strain or if autoimmune disease was induced in a non-susceptible strain. As summarized in Table 3, the effects of silicone have been assessed in several experimental paradigms of autoimmunity.

Arthritis-prone DBA/1 Mice

Using arthritis-prone DBA/1 mice, Schaefer et al (1997) examined the ability of silicones to induce arthritis. The results of these studies showed that mice implanted with silicone oil, gel or elastomer for as long as 12 months did not develop arthritis. However, it should be noted that DBA/1 mice injected with CFA also failed to develop arthritis in this study; thus the positive control group failed to document the sensitivity of the model.

In a different animal model, genetically susceptible BALB/cAnPt mice injected in the peritoneal cavity with various silicone gels did not develop the arthritis that is frequently found in this strain when they are treated with pristane oil (Potter et al., 1994). Similarly, single or multiple sc injections of silicone gel failed to induce arthritis in BALB/cAnPt mice even if the implant site was co-injected with *Staphylococcus* bacteria (MacDonald et al., 1998).

Taken together with the previously described ineffectiveness of silicone in rat adjuvant arthritis models, these findings indicate that silicones do not directly induce arthritis in arthritis-prone mice or rats.

MRL^{lpr/lpr} Model of Lupus

MRL/lpr mice carry a spontaneous lymphoproliferative mutation (*lpr/lpr*) that results in the development of an autoimmune syndrome at approximately eight weeks of age. The disease progresses over 16–24 weeks and is characterized by high levels of circulating autoantibodies leading to an immune-complex mediated glomerulonephritis, diffuse vasculitis and arthritis (Hang

et al., 1982). Approximately 50% of the mice die by 24 weeks of age due to renal failure. The clinical symptoms in MRL ^{*lpr/lpr*} mice closely resemble systemic lupus erythematosus (SLE) in humans. The arthritis that develops in MRL ^{*lpr/lpr*} mice is similar to RA in humans. The mice also develop a Sjögren's- like inflammation of the conjunctiva. MRL ^{*+/+*} mice, which lack the *lpr* gene mutation, develop a milder autoimmune disease later in life as compared to MRL ^{*lpr/lpr*} mice. Schaefer (1997) investigated the ability of silicones to modify disease in the MRL strain. At five weeks of age, prior to the onset of autoimmune symptoms, MRL ^{*lpr/lpr*} and MRL ^{*+/+*} mice received sc implants of silicone gel, silicone oil or a sham implant. During the next 12 weeks, clinical parameters of disease were measured by palpation of lymph nodes, urinary protein, and serum titers of collagen and DNA antibodies. Serum levels of several cytokines were also monitored. At sacrifice, kidneys were fixed and stained for immune complex deposition.

All MRL ^{*lpr/lpr*} mice had severe glomerulonephritis by the time they were sacrificed, and silicone exposure did not influence the severity of the disease. MRL ^{*+/+*} mice showed minimal renal changes, and this too was unaffected by the silicone implants. Lymph node enlargement was also not influenced by silicone. Anti-DNA antibody titers were significantly higher in MRL ^{*lpr/lpr*} mice that received silicone gel and in MRL ^{*+/+*} mice that received gel or oil implants as compared to sham controls. Some differences in the levels of certain cytokines were noted at various times during the experimental time period, but no pattern of change was revealed that would suggest that silicone altered disease by modifying cytokine production.

In these and other experiments, Schaefer (1997) presents data that purport to demonstrate the presence of autoantibodies to silicone-bound proteins. However, the unorthodox procedures that were used to quantify the proteins, the lack of positive controls, and the manner in which the data were presented, do not allow such conclusions to be made. The data are not convincing of anything more than nonspecific binding of protein to the implant.

In similar studies using a different strain of mouse with the *lpr* mutation, Osborn et al. (1995) reported that silicone oil containing 5% D4 did not alter the incidence of mortality at 48 weeks of age when compared to saline-injected mice. The frequency and latency of other disease symptoms also did not differ between the groups.

New Zealand Black (NZB) x New Zealand White (NZW) F1 Murine Model of SLE

NZB/W mice spontaneously develop severe systemic autoimmune disease characterized by

elevated titers of anti-nuclear antibodies (ANA), increased levels of serum IgG, polyclonal activation of B cells and the subsequent development of a fatal immune-complex mediated glomerulonephritis (Rose and Bhatia, 1995). The disease symptoms resemble human SLE.

White et al. (1998) evaluated the ability of silicone gel implanted in the mammary region of female NZB/W mice to alter the course of the disease over a 78-day period. The effects of silicone were compared to two known inducers of autoimmune disease, mercuric chloride and D-penicillamine. These positive control groups are helpful in demonstrating the sensitivity of the model to exogenous autoimmune-inducing substances. The results of these studies showed that silicone gel-implanted mice did not differ from their sham controls in terms of total IgG levels or antibody titers to dsDNA, laminin, DNP-HSA or SRBC. Spleen weight was also not affected by silicone exposure. In contrast, all of these parameters were significantly elevated in the positive control groups when compared to their own controls. Although actual disease was not measured in this study, silicone gel exposure did not appear to be promoting the clinical signs that have been associated with development of the disease in this model.

Tight Skin Mouse Model of Scleroderma

Mice bearing the TSK mutation (TSK/+) spontaneously develop skin fibrosis and characteristic autoantibodies which resemble human scleroderma. Frondoza et al. (1995) evaluated the influence of silicone on the pathogenesis of the disease in this mouse model. TSK/+ mice as well as their phenotypically normal TSK/- litter mates were injected with low molecular weight silicone fluid, high molecular weight silicone gel, IFA as a positive control, or saline as a negative control. One month later, skin was examined histologically for development of hyperplasia and thickening. Other tissues (kidney, liver, spleen) were examined for pathological changes. Circulating autoantibodies to RNA polymerase I, topoisomerase and bovine serum albumin (BSA) were also measured.

The results indicated that the normal progression of the disease seen in saline-treated control TSK/+ mice as revealed by histological examination was not altered by silicone exposure. It was also not altered by IFA. No evidence of hyperplasia or pathology was seen in the TSK/- mice with any of the treatments. None of the mice showed pathological changes in the other organs. Circulating antibodies to RNA polymerase I, topoisomerase or BSA were not altered in TSK/+ mice treated with silicone or IFA as compared to saline-treated controls. The lack of effect in the

IFA-treated positive control group limits the ability to interpret the lack of effects with silicone.

Type II Collagen-induced Arthritis

Because collagen provides the basic framework of cartilage, the experimental induction of an immune response to collagen can produce the symptoms of arthritis. As previously described in Section VII, animal models of collagen-induced arthritis have been characterized in which the intradermal injection of bovine type II collagen emulsified with CFA or IFA induces an arthritis in genetically susceptible rats or mice (Holmdahl et al., 1989). The lesions found in the affected joints are quite similar to those found in humans with rheumatoid arthritis. Anti-collagen antibodies develop in immunized rats and mice and are also found in RA patients, but the specific role they play in the pathogenesis of the disease is controversial. Circulating levels of inflammatory cytokines such as IL-1 β , TNF- α , and IL-6 are elevated during the disease process, and experimental manipulation of cytokine activity (production or receptor blockade) modifies the disease. Thus, proinflammatory substances might be expected to promote arthritic disease.

Schaefer et al. (1997) used the mouse model of collagen-induced arthritis to examine the ability of various forms of silicone implants to promote the disease process. Mice were injected with silicone oil, gel or elastomer for three days or nine months prior to immunization with collagen in CFA. The results showed that silicone in all forms had no influence on the incidence or severity of arthritis as compared to sham- treated mice. However, because the incidence and severity of disease was high in the controls, significant promotional effects would have been difficult to demonstrate. Time-to-onset of disease was not reported.

In a second study, Schaefer (1997) used collagen immunization with IFA instead of CFA to induce a lower incidence of disease in the controls. In this study, 9/10 mice implanted with silicone elastomer nine months prior to immunization exhibited disease compared to 3/10 control mice. The severity of the disease was also increased in the silicone elastomer-treated mice. In mice treated with silicone gel or oil, 6/9 mice in each group developed disease and their arthritic scores also tended to be higher than the controls, although these changes were not statistically different from controls. Taken together, the results suggest that exposure to different forms of silicone may promote the development of arthritis in this model of autoimmune disease. However, these results must be considered preliminary and interpreted cautiously in light of the small number of animals tested and the fact that only 3 control mice developed disease. Furthermore, the findings

are less than compelling based on the fact that anti-collagen antibody titers were not altered by silicone, and cytokine levels were not consistently altered in a manner that might explain the increased incidence of disease.

NZB Mouse Model of Autoimmune Hemolytic Anemia

NZB mice develop a form of autoimmune hemolytic anemia that closely resembles the human disease. The disease begins at about three months of age and by nine months almost all mice show reduced hematocrits as evidence of the disease process (Howie and Helyer, 1968).

MacDonald et al.(1998) used the NZB model to study the influence of silicone gel implants on this autoimmune disease process. Groups of mice were injected with saline as a negative control, pristane as a positive control, or silicone gel. Injections given one time were compared to injections given three times to examine the effect of multiple exposures. Some mice were given an injection of silicone followed three months later by a capsulotomy to evaluate the effect of “traumatic rupture.” Other mice were given an injection of silicone followed three months later by a low dose of *Staphylococcus epidermidis*, intended to mimic infection with “a common contaminant found on the surface of breast implants and hypothesized to be involved in capsular contracture.” Appropriate controls were included for all of the procedures except the capsulotomy. Mice were examined daily for ten months after which time all surviving mice were sacrificed. Blood was used to measure hematocrits and serum was analyzed for autoantibody production. Urinary protein levels were monitored bi weekly during the course of the study for the possible development of glomerulonephritis.¹

By ten months of age, some mortality had occurred in all groups, including the controls (20-30%). Only multiple treatments with pristane, the positive control, significantly increased the mortality of the mice to 80%. Increased mortality (60%, $P < 0.085$) was also noted after multiple treatments of silicone. Hematocrits were much lower in all NZB mice compared to normal BALB/c mice. Hematocrits were further reduced in mice given pristane three times, and in all mice injected with silicone, although it was not clear what pairwise comparisons were made to determine statistical significance. In contrast, hemagglutination titers were similar in all groups compared to controls. ANA titers were elevated in silicone-implanted mice that had undergone a

¹It is not clear why this endpoint was included in this study. This NZB model should not be confused with the NZB/NZW F1 mice that spontaneously develop an SLE-like disease, as described in part 2 of this section.

capsulotomy at three months. Anti-collagen IgM titers were elevated in mice that were injected with silicone and infected with *S. epidermidis* whereas anti-collagen IgG titers were similar in all groups. Multiple injections of pristane or silicone increased urinary protein levels.

Based primarily on the mortality and hematocrits, the results of this study provide limited evidence for a promotion of autoimmune hemolytic anemia by silicone in NZB mice. However, the results would have to be repeated before such a conclusion could be drawn. The data would also be more compelling if additional endpoints relevant to the disease process had been measured, as it is not clear what caused the death of the animals. In addition, the clinical data might have been more insightful if it had been collected prior to the onset of mortality. On the other hand, the relevance of this disease model to women with SBIs is unclear.

Normal BALB/c mice were also tested in parallel with the NZB mice and given identical pristane and silicone treatments to determine if a non-genetically disposed mouse could be induced to develop disease by exposure to silicone. However there was no significant mortality in any treatment group, and all had normal hematocrits. These data indicate silicone was not able to induce autoimmune disease in genetically resistant mice.

IX. Immunotoxicity of Silicone in Animals

Although the majority of results from the animals models of autoimmune disease do not support an enhancement of the disease process by silicone, which was tested in several different forms and for various periods of time, arguments can be put forth as to why the animal models are different from the human situation and therefore not reflective of the human response to silicone. Thus, it is appropriate to review the animal studies that have examined the ability of silicone to alter processes that are believed to contribute to the development of autoimmunity. Based on a general conceptual understanding of autoimmune disease pathogenesis, several hypothetical mechanisms have been proposed by which silicone could induce or exacerbate the process. These include:

1. Silicone causes immune system “dysregulation” resulting in abnormal T cell and/or B cell activity leading to the generation of the autoimmune response. For example, polyclonal B cell activation or loss of suppressor T cell functions have been associated with some autoimmune diseases.
2. Silicone induces specific T cell activation by modification of self-proteins resulting in a novel autoimmune disease.

3. Silicone causes inflammation and the resulting inflammatory cytokine production initiates or exacerbates autoimmune disease development.

The evidence available to support or refute these hypotheses will be summarized below.

Evidence that Silicone Alters Immune Responsiveness of Animals

Several studies have been conducted in laboratory animals in an effort to determine the influence of silicone exposure on immune function. Comprehensive immunotoxicology studies were carried out in the early 1990s by the Medical College of Virginia under contract to the National Toxicology Program. A standard immunotoxicology screen was utilized in mouse studies to examine the immunomodulatory effects of various doses² of silicone oil, silicone gel, or silicone elastomer disks implanted subcutaneously in the breast area of female B6C3F1 mice.

Polyurethane disks were also tested. Controls were injected with saline. Immunological testing was carried out ten days or 180 days after initiation of silicone exposure (Bradley et al., 1994a,b). The ten-day period was selected to represent the peak inflammatory response. The 180-day exposure was intended to reflect the chronic condition of SBIs wherein a well-developed fibrous capsule had formed. Endpoints assessed included body weight, histopathology, hematology, serum complement levels, bone marrow colony formation, spleen cell subpopulations (B cells, T cells and T cell subsets); primary antibody response to SRBC (IgM and IgG PFCs); proliferative responses to B and T cell mitogens and to allogeneic lymphocytes, cytotoxic T lymphocyte response, NK cell cytotoxicity, reticuloendothelial system clearance of antigenic particles, peritoneal macrophage phagocytosis and IFN- γ production. The ability of the animals to resist infection by *Streptococcus pneumoniae* or *Listeria monocytogenes* and to control B16 melanoma metastases were assessed as holistic measurements of overall immune status.

The results of these immunotoxicology studies showed that silicone in any form tested did not induce systemic toxic effects or alter immune function. The only noteworthy silicone-related treatment effect was a decrease in NK cell activity in the spleen of mice injected with silicone gel

²According to Wilson and Munson, (1996): Assuming a specific density of 1 g/ml for the gel, a dose of 1-3 ml of gel in the mouse constitutes approximately 5-15% of the body weight. In the rat, a gel implant ranging from 1 to 20 ml represents approximately 0.6 to 13% of body weight. In women, the implantation of two 300 ml mammary implants is not unusual. In a 50-kg woman, this volume of implanted material would correspond to 1.2% of her body weight.

or implanted with the elastomer disk for 180 days. However, this effect on NK activity did not translate into an increase in B16 tumor metastasis, which is the host resistance model considered sensitive to changes in NK cell activity.

Follow-up studies were carried out to validate the effect of silicone on NK activity in a dose-response study. The results of the study confirmed the suppression of NK cell activity but only at the highest dose level. Comparative studies using F344 rats also showed a suppressive effect on NK cell activity from silicone treatment. In rats, NK activity could be boosted in silicone-implanted rats by polyI:C but not to levels observed in controls (Wilson and Munson, 1996).

Taken together, these results indicate a modest and somewhat consistent effect of silicone exposure on NK cell activity. The importance of the finding may derive from the fact that any systemic alteration in an immunological endpoint could be induced by the presence of a silicone implant. However, the importance of the finding in terms of autoimmune disease is not known since a role for NK cells in autoimmune disease development is not widely recognized. The mechanism by which silicone alters NK cell activity was not elucidated.

Although the NTP-sponsored immunotoxicity assessment of silicone was thorough and of high quality, there were some shortcomings in the experimental design. For example, these studies utilized a standard screening procedure to identify immunotoxic substances. The assays were previously validated to detect immunosuppressive substances and were not specifically oriented to address autoimmune-relevant endpoints. Another potential shortcoming is that the animals used in the studies were not predisposed to develop autoimmune disease and may therefore be more resistant to the effects of silicone than an autoimmune-prone strain.

Evidence for Antigenicity of Silicone

The question of silicone antigenicity has been addressed in a limited number of animal studies that have attempted to demonstrate silicone-specific antibodies or silicone-specific T cell responses, including the possible development of specific immunologic memory. The data from these studies will be reviewed here.

Delayed-type hypersensitivity (DTH) responses are useful measures of T-cell mediated immunity in animals. A true DTH response follows delayed kinetics that reflect the response of pre-sensitized antigen-specific memory T cells. These T cells proliferate in response to their specific antigen and secrete cytokines that activate macrophages, causing tissue swelling that peaks

48–72 hours after antigen injection. The kinetics of the DTH response is crucial for differentiating T cell immunity from nonspecific inflammatory responses that occur within a few hours of injection and are not antigen-specific. The DTH response is also distinguished by its kinetics from a rapid contact hypersensitivity response that is mediated by antigen-specific IgE antibodies.

Brantley et al. (1990) used a creative approach to ask if there is a cell-mediated immune response to silicone. They “immunized” rats to silicone by injecting silicone in CFA. Four weeks later, the animals were given silicone implants. Reactions to the implants were measured by capsule formation and histology of the capsule. Based on the similar histology of the capsules of immunized (CFA + silicone) and nonimmunized (CFA only) rats, there was no evidence of an immune response to silicone. One would have expected a characteristic tissue reaction if the rats had been immune to the silicone. However, these negative results must also be interpreted cautiously given that a positive control was not included to demonstrate the sensitivity of the technique.

In related studies, Brantley et al. (1990) immunized rats with silicone gel sonicated in CFA. Four weeks later, lymphocytes from the spleen were obtained and tested for their ability to respond to silicone *in vitro*. There was no difference in the proliferative response from silicone-immunized mice compared to mice injected only with CFA. Thus, there was no measurable memory response to silicone, the most unambiguous measure of antigen-specific immunity.

Smith et al. (1990) immunized rats with fine particles of solid silicone from a bone prostheses emulsified with CFA. After six sensitizing injections, they reported evidence for an immune response to the silicone in a positive skin reaction to challenge and IgG deposition around the silicone implant. However, the study lacked appropriate controls.

LeBeau (1967) reported that silicone gel strips did not induce a hypersensitivity response in the skin of guinea pigs. Naim et al (1993) found no evidence for a DTH response to silicone in rabbits.

Silicone-specific antibody production by B lymphocytes has also been examined to determine if there is a specific immune response to silicone. In order to demonstrate antigen specificity, one must be able to show specific binding of the induced antibody to the antigen *in vitro*. In regard to silicone antibodies, these *in vitro* assays have been fraught with problems associated with nonspecific protein binding to the silicone (Butler et al., 1996; Rosenau et al., 1996). The studies are also complicated by the hydrophobic nature of silicone materials and the difficulties of working

with them in antibody assays . Unfortunately, because of the unconventional methods that have been used in past studies in an attempt to circumvent these problems, along with a failure to provide adequate assay validation, the data currently available are not convincing of silicone-specific antibody production.

Evidence that Silicone Induces Inflammation

Several studies have reported that subcutaneous or intramuscular injection of various types of silicone in experimental animals induces an inflammatory response similar to a foreign body reaction leading to the formation of a fibrous capsule around the implant (Lilla and Vistnes, 1975; Goodman et al., 1988; Lemen et al., 1992). The extent of the inflammatory response in any particular study depends on the type of silicone material that is implanted, the size and shape of the implant, its location, as well as other unidentified factors (Picha and Goldstein, 1991). In addition, differences in surgical techniques cannot be minimized since surgical trauma alone can account for a part of the early inflammation noted, and inadvertent bacterial contamination would likely increase the severity of the inflammatory response. However, in most studies, the inflammatory response declines over time, and the implants are usually found surrounded by a mature connective tissue capsule of varying thickness containing minimal numbers of macrophages, neutrophils and lymphocytes (Grasso et al., 1965; Malczewski, 1984; Mudgett et al., 1990; Picha and Goldstein, 1991; Rasmussen, 1988). Recently, Fabre et al. (1998) used a novel technique to demonstrate this process. They measured the cellular response to a cylindrical elastomer implant using flow cytometry. Two days after implantation, a mixture of monocytes and neutrophils predominated in the exudate that formed inside the tube. At day nine, cell subpopulations could still be identified, whereas by day 23, the cellular components had declined to undetectable levels. Fibrinogen levels rose progressively during this time. These results indicate a resolution of the active inflammatory response to the silicone elastomer within the 23-day time frame.

Injection of silicone fluid directly into the joint of DA rats induced arthritis, suggesting that a local inflammatory reaction was induced by silicone (Yoshino, 1994). This response is not particularly surprising since the DA rat is highly susceptible to arthritis induction. However, there is no evidence that silicone from SBIs is transported to joints at any significant concentration to directly induce arthritis. This is supported by the animal studies discussed previously that show silicone injections outside of the joint do not induce arthritis, even in the DA rat (see Table 1).

While it is generally accepted that silicones elicit varying degrees of local inflammation at the site of the implant, there is little evidence from controlled animal studies that suggest silicone causes systemic inflammatory responses. The most extensive examination of this possibility was carried out by Schaefer (1997), who measured levels of circulating cytokines at various times after silicone implantation in relationship to the development of autoimmune disease. The results of these studies, documented in Table 3, failed to provide evidence that silicones induced a systemic alteration in inflammatory cytokine production.

Evidence that Silicone Activates Macrophages

As previously discussed, studies carried out in the late 1960s had shown that silicone fluid injected ip or sc appeared to be phagocytosed and distributed systemically via the lymphatics (Ben Hur et al., 1967; Rees et al., 1966; Sparchu and Clashman, 1970). In more recent studies, macrophages containing silicone were also found in rats injected with silicone fluid (Hill et al., 1996; Malczewski et al., 1994), but not in rats implanted with silicone gel or elastomer (Malczewski et al., 1994). Likewise, several other studies on silicone gel have found no evidence for the phagocytosis or systemic distribution of silicone gel in rats that had been implanted for as long as two years (Goodman et al., 1988; Raposo do Amaral et al., 1993; Tiziani et al., 1995). Taken together, these results suggest that phagocytosis of silicone by macrophages and systemic distribution of silicone is a phenomenon primarily associated with the injection of free silicone fluid rather than gel.

However, because macrophages have been shown to be capable of engulfing silicone in any form, many of the hypotheses related to silicone-induced disease invoke a role for silicone-induced activation of macrophages. These activated macrophages are then hypothesized to secrete cytokines that lead to the disease symptomology. Unfortunately, there are no definitive data available that have characterized the influence of silicone on such macrophage activation or cytokine production.

Das et al. (1990) examined the ability of silicone sonicated with CFA to cause long-term activation of macrophages as measured by their secretion of IL-1. At eight months after silicone injection, there was no difference in IL-1 production compared to mice injected with CFA or saline. However, the negative results must be tempered by the fact that no relevant positive control was included. The addition of LPS *in vitro* to activate macrophages was not an appropriate positive control

MacDonald et al. (1996) reported that silicone gel injected into the peritoneal cavity of certain strains of mice induced a population of predominately macrophages that was able to induce a small degree of proliferation in CD4⁺ T cells in a nonantigen-specific manner. The authors suggested that these “silicone-laden macrophages” induce a proliferative response that is “unique to silicone” because macrophages from pristane- or thioglycollate-injected mice did not induce proliferation. However, the authors failed to speculate on what unique factor silicone-laden cells produce that other inflammatory macrophages do not, nor did they demonstrate by any other criteria that the macrophages were indeed activated. Furthermore, they did not demonstrate that the activity was mediated by the macrophage component of the peritoneal exudate cells, nor that the macrophages indeed contained silicone. Thus, the data do not support the conclusions of the authors and provide no insight into the effect of silicone on macrophage activity.

The studies of Bradley et al. (1994a,b) addressed the potential for silicone to alter systemic macrophage activity in mice that received short-term(ten day) or long-term (180 day) implants of silicone oil, gel, or elastomer. Reticuloendothelial clearance of particles by tissue macrophages present in the liver, spleen, lymph nodes and lungs was evaluated by measuring the vascular clearance and tissue uptake of radioactively labeled foreign particles (SRBC or Covaspheres). The functional activity of adherent peritoneal cells (primarily macrophages) was evaluated by their ability to phagocytose radiolabeled particles *in vitro*, and by their ability to be activated by IFN γ and LPS to kill tumor cells *in vitro*.

The results of these studies revealed no change in any of these parameters ten days after the implant surgery except for a decrease in macrophage tumoricidal function under various *in vitro* conditions. However, this decrease in tumoricidal function did not translate into a change in the resistance of the mice to tumor growth. On the other hand, in one experiment, all of the mice in the silicone groups were more resistant to infection by *Listeria* bacteria, which could possibly reflect enhanced phagocytic activity.

In mice that had been implanted with silicone materials 180 days previously, there was increased uptake of SRBC by the liver of mice exposed to silicone gel, but this effect was not confirmed in a subsequent dose-response study. Peritoneal cells from silicone fluid- implanted mice had significantly increased phagocytic activity for Covaspheres, but this too was not confirmed in a dose-response study. Finally, the growth of iv-injected tumor cells in the lung was decreased in all silicone-treated mice compared to vehicle controls, which could reflect increased

macrophage tumoricidal activity. However, since macrophage tumoricidal activity was not evaluated, additional studies would be required to document this effect.

Recently, Rhie et al. (1998) published a study in which macrophages were cultured on silicone gel that was centrifuged onto the bottom of tissue culture plates, allowing for direct contact between the cells and the gel. Subsequent analysis of the function of these macrophages demonstrated a significantly enhanced responsiveness compared to macrophages cultured directly on the plastic plate. The authors conclude that silicone gel activated the macrophages to augment immune function. However, the authors do not consider an equally plausible explanation for the data; that is, that by preventing the adherence of the macrophages to the plastic plate with the gel coating, the suppressive effect of adherence-induced activation was prevented. This possibility arises from the well-known and widely used technique of coating tissue culture plates and tubes with silicone to prevent macrophage adherence, and the equally well-known fact that an excess of activated macrophages usually suppresses immune function *in vitro*. Unfortunately, Rhie et al. failed to provide an important control group that would have characterized a normal immune response without any added macrophages. They also failed to provide any direct evidence for the state of activation of the macrophages cultured on gel vs plastic (e.g., adhesion molecule expression or cytokine production). Thus, these studies do not provide convincing evidence that silicone gel induces the activation of macrophages.

X. Potential Contribution of Other Materials in SBIs to Toxicity

Low Molecular Weight Cyclosiloxanes

D4 and D5 are low molecular weight, cyclic silicones that have been detected in SBIs. D4 has been analyzed at levels of approximately 500 ppm in the gel and in the elastomer at a level of 100-300 ppm³ (Van Dyke et al., 1993). D5 levels are similar. There has been some speculation that these molecules play a part in the health effects of SBIs.

³If two 300-ml implants contain gel with D4 at a concentration of 500 ppm, this equals a total of 0.3 g D4. If a 50 kg woman were exposed to all of the D4, it would represent a total dose of 6 mg/kg. Any exposure to D4 from a leaking implant would be acquired over a long exposure period, such that on a daily basis the dose is much lower.

The toxicities of D4 and D5 have been tested independently of silicone because they were formulated and are marketed for use in a variety of products. Inhalation of relatively large doses of D4 and D5 have been shown to produce few toxic effects other than liver enlargement and induction of hepatic drug metabolizing enzymes (McKim et al., 1988; Mehendale, 1989; Siddiqui, 1989). Such hepatic effects are not seen following the implantation of silicone gel (Bradley et al., 1994b; Selwyn and Danner, 1988). High doses of D4 have also been reported to enhance NK cell activity (Wilson and Munson, 1997), whereas exposure to silicone gel suppresses NK function (Bradley et al., 1994a,b). Thus, based on current studies, there is no data to implicate these low molecular weight silicones in any health effect associated with SBIs.

Silanols

The toxicity of silanols is considered not relevant to the SBI issue. Silanols are highly toxic chemicals with a profile of toxicity in rats that includes liver, kidney, and neural damage, and bleeding (Dow Report No. 2964). However, none of these toxicities are seen in rats injected with large amounts of silicone fluid, gel or elastomer.

Platinum

Platinum (Pt) is used as a catalyst in the preparation of silicone gels and elastomers. According to Lykissa et al. (1997), Pt was detectable in silicone gel at a level of approximately 700 µg/kg (parts per billion) using ICP-MS. Furthermore, they indicate that when the gel was incubated in lipid-rich media, Pt diffused into the media at a rate of approximately 20–25 µg/day/250g implant. Since the whole implant used in these studies would only contain 175 µg of Pt, it suggests that all of the Pt would diffuse from the gel into the media within seven days. This is not logical. There are no data available that address the level of Pt in blood or tissues of animals or humans who have SBIs. However, if a worst-case exposure scenario was calculated based on the value published by Lykissa, wherein all of the Pt in two 300-ml implants was released into the body of a 50-kg woman, the Pt dose would be 8.4 µg/kg or 8.4 ppb. This concentration of Pt is approximately equivalent to the five ppb level found naturally in the environment. Pt toxicity is dependent on its chemical form. Pt salts primarily cause liver and kidney toxicity, which have not been associated with SBI materials. Thus, there is no evidence that Pt plays any role in the health effects

associated with SBIs.

XI. Conclusions

This chapter has reviewed the experimental animal data to evaluate the evidence that silicone breast implants have the potential to cause systemic disease in humans. The results of this review indicate that the silicones used in SBIs are of very low toxicity to animals. Although there is documented evidence of local inflammatory reactions to silicone breast implant materials in animals, there is no convincing evidence for a significant systemic inflammatory response. The local reaction to silicone is similar to other “foreign body reactions” described with other implanted materials.

There is some evidence that macrophages can phagocytose small droplets of silicone which may then be transported via the lymphatics to other tissues in the body. This process appears to occur primarily with low molecular weight silicone fluid rather than the high molecular weight gel. However, even with phagocytosis, there is currently no definitive evidence for systemic effects on the immune system or for processing of silicone as an antigen for T cell activation. There is also no convincing evidence from animal studies that T cells can be activated by silicone.

The ability of silicone to act as an adjuvant has received a lot of attention. Even though some silicone gels and fluids have been shown to possess adjuvant activity when antigen is emulsified with the silicone prior to immunization, this capability has little bearing on the issue of silicone- induced autoimmune disease. It most likely reflects a depot effect of the non-degradable silicone. There are no convincing data that show silicone acts like an adjuvant when it is present at a site distant from the antigen injection, and there is no biologically plausible mechanism for antigen emulsification to take place in the body.

Immunotoxicity testing of silicones revealed only one fairly reproducible effect which was suppression of NK activity in both rats and mice. The degree of suppression was variable between experiments and not of sufficient magnitude to affect a disease model responsive to NK cell activity. Although these results are of interest, the specific role that NK cells may play in autoimmune disease development is not well understood.

The greatest weight of evidence in this review has been given to studies that evaluated the ability of silicone to induce or promote autoimmune disease in whole animal models. Such animal models provide the most holistic approach to identifying biologically relevant effects induced by silicone exposure that might lead to autoimmune disease induction or promotion in humans. The use of animals that are genetically predisposed to develop autoimmune disease provide the advantage of a high and predictable incidence of spontaneous disease. If an alteration in disease induction occurs with silicone treatment and it correlates with changes in relevant clinical endpoints, the evidence for a cause-effect relationship becomes more credible. If clinical correlates of a promotional effect by silicone can be identified in animals, it would provide a focus for human clinical investigations.

Several adequately designed animal studies have been published that address the question of silicone's ability to induce or exacerbate autoimmune disease. The human autoimmune diseases that are simulated in these animal models include rheumatoid arthritis, systemic lupus erythematosus, scleroderma, and autoimmune hemolytic anemia. In the 17 experimental regimens outlined in Tables 1 and 3, 15 indicate that silicone did not induce or promote the development of autoimmune disease and/or alter diagnostic clinical endpoints. The other two experiments must be viewed as providing weak but suggestive preliminary evidence of a promotional effect by silicone exposure. However, these preliminary findings must be confirmed in independent studies. Curiously, one of models that shows some evidence of promotion by silicone is the model of autoimmune hemolytic anemia, which is not of obvious relevance to the SBI issue. The other is a bovine collagen-induced model of arthritis using incomplete Freund's adjuvant to lower the degree of disease in the controls. Limitations of these studies relate to the small number of animals in the treatment groups and the lack of clinical endpoints that verify the exacerbation of disease.

On the other hand, there are also limitations with some of the studies that show no effect of silicone on autoimmune disease. The biggest problem with several of the studies is that disease incidence is so high in the controls, it would be difficult to demonstrate an increase in disease in the silicone-treated mice. Although a promotional effect in such cases might be evidenced by an early appearance of the disease, latency was not an endpoint that

was documented in most of the studies. The second limitation of several of these studies is the lack of positive controls that demonstrate the sensitivity of the model to exogenous modulation. Without a positive control, it is difficult to put the failure of silicone to alter disease in a relevant context. Nevertheless, the data from these studies cannot be ignored for their null effects on the disease process.

In conclusion, the preponderance of evidence from animal studies indicates little probability that silicone exposure induces or exacerbates systemic disease in humans.

References

- Agnew WF, Todd EM, Richmond H, et al. Biological evaluation of silicone rubber for surgical prosthesis. *J Surg Res* 2:357–63, 1962.
- Andrews JM. Cellular behavior to injected silicone fluid: a preliminary report. *Plast Reconstr Surg* 38:581–83, 1966.
- Ballantyne DL, Jr., Rees TD, Seidman I. Silicone fluid: response to massive subcutaneous injections of dimethylpolysiloxane fluid in animals. *Plast Reconstr Surg* 36:330–38, 1965.
- Barondes R. Silicones in medicine: New organic derivatives and some of their unique properties. *Military Surgeon* 106:379–87, 1950.
- Ben-Hur N. Local and systemic effects of dimethylpolysiloxane fluid in mice. *Plast Reconstr Surg* 39:423–26, 1967.
- Bradley SG, Munson AE, McCay JA, et al. Subchronic 10 day immunotoxicity of polydimethylsiloxane (silicone) fluid, gel and elastomer and polyurethane disks in female B6C3F1 mice. *Drug Chem Tox* 17:175–220, 1994a.
- Bradley SG, White KL, McCay JA, et al. Immunotoxicity of 180 day exposure to polydimethylsiloxane (silicone) fluid, gel and elastomer and polyurethane disks in female B6C3F1 mice. *Drug Chem Tox* 17:221–69, 1994b.
- Braley SA. The use of silicones in plastic surgery. *Plast Reconstr Surg* 51:280–88, 1973.
- Brantley SK, Davidson SF., St. Arnold PA, et al. The effects of prior exposure to silicone on capsule formation, histology, and pressure. *Ann Plastic Surg* 25:44–47, 1990.

- Brantley SK, Davidson SF, St. Arnold PA, et al. Assessment of the lymphocyte response to silicone. *Plast Reconstr Surg* 86:1131–37, 1990.
- Broderson JR. A retrospective review of lesions associated with the use of Freund's Adjuvant. *Lab Animal Sci* 39:400–5, 1998.
- Butler JE, Lu EP, Navarro P, et al. The adsorption of proteins on a polydimethylsiloxane elastomer (PEP) and their antigenic behavior. In: Potter M, Rose NR, eds., *Immunology of Silicones*. Springer, pp 75–84, 1996.
- Chang Y. Adjuvanticity and arthritogenicity of silicone. *Plast Reconstr Surg* 92:469–73, 1993.
- Chenoweth MB, Holmes B, Stark F. *Dow Corning Report No. 1377. The Physiological Assimilation of Dow Corning 200 Fluid*. Midland, MI, 1956.
- Das SK, Johnson M, Ellsaesser C, et al. Macrophage interleukin 1 response to injected silicone in a rat model. *Ann Plast Surg* 28:535–37, 1992.
- Fabre T, Bertrand-Barat J, Freyburger G, et al. Quantification of the inflammatory response in exudates to three polymers implanted in vivo. *J Biomed Mater Res* 155:637–41, 1998.
- Fronzoza C, Jones L, Rose NR, et al. Development of scleroderma-like syndrome in Tsk/+ mice is not enhanced by silicone administration. In: Potter M, Rose NR, eds., *Immunology of Silicones*, Springer, pp 299–306, 1996.
- Gish J, Gaspari AA, Klykken P, et al. Adjuvancy of octamethylcyclotetrasiloxane (D4) in the skin immune system of normal mice. *J Invest Dermatol* 106:924 (Abstract).
- Glenn EM, Gray J. Adjuvant-induced polyarthritis in rats: biologic and histologic background. *Am J Vet Res* 26:1180–94, 1998.
- Goodman DG, Gail RG, Kitchen DN. Two-year gel implant study (B7811) in Sprague-Dawley rats. Jamesville, MD: Pathco, Inc., 1988.
- Grasso P, Fairweather FA, Golberg L. A short-term study of epithelial and connective tissue reactions to subcutaneous injection of silicone fluid. *Food Cosmet Toxicol* 3:263–69, 1965.
- Hang L, Theofilopoulos AN, Dixon FJ. A spontaneous rheumatoid arthritis-like disease in

- MRL/1 mice. *J Exp Med* 155:1690–1701, 1982.
- Hill SL, Landavere MG, Rose NR. The adjuvant effect of silicone gel and silicone elastomer particles in rats. In: Potter M, Rose NR, eds., *Immunology of Silicones*, Springer, pp123–37, 1996.
- Holmdahl R, Andersson ME, Goldschmidt TJ, et al. Collagen induced arthritis as an experimental model for rheumatoid arthritis. *APMIS* 97:575–84, 1989.
- Howie JB, Helyer BJ. The immunology and pathology of NZB mice. *Adv Immunol* 9: 215–66, 1968.
- Janeway, CA, Travers P. *Immunobiology. The Immune System in Health and Disease*. New York: Garland Publishing, 1994.
- King DW, Zimmer MA, Rasmussen CR. Two-year tissue implant study with Q7-4750, X7-4780 and Q7-4840 in Fischer 344 rats. Dow Corning Report reference 226, 1989.
- Klykken P, White KL. The adjuvancy of silicones: dependency on compartmentalization. In: Potter M, Rose NR, eds., *Immunology of Silicones*, Springer, pp.113–21,1996.
- Lane TH, Burns SA. Silica, silicon and silicones. Unraveling the mystery. In: Potter M, Rose NR, eds., *Immunology of Silicones*, Springer, pp 3–12, 1996.
- LeBeau JE. Evaluation of Dow Corning Gel Strip as to its ability to produce a sensitization phenomena upon guinea pig skin. Report I0065-1306-4, 1967.
- Lemen J, Wolfe GW. *Combined Chronic Toxicity and Oncogenicity Study in Rats*. Hazleton Washington, Inc. Report, 1992.
- Lilla JA, Vistnes LM. Long-term study of reactions to various silicone breast implants in rabbits. *Plast Reconstr Surg* 57:637–49, 1976.
- Lykissa ED, Kala SV, Hurley JB, et al. Release of low molecular weight silicones and platinum from silicone breast implants. *Anal Chem* 69:4912–16, 1997.
- Malcrewski R. Ninety-day implant study of Dow Corning Q7-2146/50 gel. Report 2332-6, 1985.
- Mastalski K, Jenkins DH, Kinoshita FK, et al. Subcutaneous implant study with 114-189171-22571, 114-189171A-113070, 114-189171B-113070, Prosthesis, 114-P847F-22371 and 114-1102B1/2-22371 in female beagle dogs. Report IBT No.

8580-09424, 1977.

- McDonald AH, Weir K, Schneider M, et al. Silicone gel enhances the development of autoimmune disease in New Zealand black mice but fails to induce it in BALB/cAnPt mice. *Clin Immunol Immunopath* 87:248–55, 1998.
- McKim JM, Wilga PC, Kolesar GB, et al. Evaluation of octamethylcyclotetrasiloxane (D4) as an inducer of rat hepatic microsomal cytochrome P450, UDP-glucuronosyltransferase, and epoxide hydrolase: a 28-day inhalation study. *Tox Sci* 41:29–41, 1998.
- Mehendale HM. Evaluation of the liver microsomal enzyme induction potential of D-5. Report 3724-10, 1989.
- Mudgett SL, Zimmer MA, Ruhr LP. A 90-day implant study of Dow Corning Q7-2412 elastomer. Report 1990-I0000-35219, 1990.
- Muir VY, Dumonde DC. Different strains of rats develop different clinical forms of adjuvant disease. *Ann Rheum Dis* 41:538–43, 1982.
- Naim JO, Ippolito KML, Lanzafame RJ, et al. Induction of type II collagen arthritis in the DA rat using silicone gel as adjuvant. In: Potter M, Rose NR, eds, *Immunology of Silicones*, Springer, pp 103–11, 1996.
- Naim JO, Ippolito KML, Lanzafame RJ, van Oss CJ. The effect of molecular weight and gel preparation on humoral adjuvancy of silicone oils and silicone gels. *Immunol Invest* 24: 537–47, 1995.
- Naim JO, Ippolito KML, van Oss CJ. Adjuvant effect of different types of silicone gel. *J Biomed Mater Res* 37:534–38, 1997.
- Naim JO, Lanzafame RJ, van Oss CJ. The adjuvant effect of silicone-gel on antibody formation in rats. *Immunol Invest* 22:151–61, 1993.
- Naim JO, van Oss CJ, Lanzafame RJ. The induction of autoantibodies to thyroglobulin in rats with silicone gel as adjuvant. *Surg Forum* 44:676–78, 1993.
- Nicholson JJI, Wong GE, Frondoza CG, et al. Silicone gel and octamethylcyclotetrasiloxane potentiate antibody production to bovine serum albumin in mice. In: Potter M, Rose NR, eds., *Immunology of Silicones*, Springer, pp. 140–44, 1996.

- Osborn TG, Neshar G, Moore TL. Effect of silicone injection of skin thickness and antinuclear antibody in the tight-skin mouse. *Arth Rheum* 37(9 suppl.):S271, 1995 (Abstract)
- Osborn T, Moore T, McMurtry P. Effect of polydimethylsiloxane on autoimmune parameters in C57Bl/6 *lpr/lpr* (B6-*lpr*) mice. *Am Coll Rheum* 38:Abstract 1033,1995.
- Pearson CM. Development of arthritis, peri-arthritis and periostitis in rats given adjuvants. *Proc Soc Exp Biol Med* 91:95–101, 1956.
- Picha GJ, Goldstein JA. Investigation of silicone oil and fumed silica in an adjuvant animal model. *Plast Reconstr Surg* 100:643–52, 1997.
- . Analysis of the soft-tissue response to components used in the manufacture of breast implants: rat animal model. *Plast Reconstr Surg* 87: 490–500, 1991.
- Potter M, Morrison S, Wiener F, et al. Induction of plasmacytomas with silicone gel in genetically susceptible strains of mice. *J Natl Cancer Inst* 86:1058–65, 1994.
- Potter M, Wax JS. Genetics of susceptibility to pristane-induced plasmacytomas in BALB/cAn: reduced susceptibility in BALB/cJ with a brief description of pristane-induced arthritis. *J Immunol* 127:1591–95, 1981.
- Raposo do Amaral CM, Tiziani V, Cintra ML, et al. Local reaction and migration of injected silicone gel: experimental study. *Aesth Plast Surg* 17:335–38, 1993.
- Rasmussen CR. A 90-day implant test of Dow Corning Q7-2423 in rabbits. Report 5194-7, 1988.
- Rees TD, Ballantyne DL, Jr., Seidman I, et al. Visceral response to subcutaneous and intraperitoneal injections of silicone in mice. *Plast Reconstr Surg* 39: 402–10, 1967.
- Rhie JW, Han SB, Byeon JH, et al. Efficient in vitro model for immunotoxic assessment of mammary silicone implants. *Plast Reconstr Surg* 102:73–77, 1998.
- Rose NR, Bhatia S. Autoimmunity: Animal models of human autoimmune disease. In: *Methods in Immunotoxicology*. Wiley-Liss, Inc., 427–45, 1995.
- Rosenau B, Schneebaun AB, Schur PH. The development of an ELISA method for the detection of "antibodies" to silicone. In: Potter M, Rose NR, eds., *Immunology of Silicones*, Springer, pp. 69-74, 1996.

- Schaefer CJ. The influence of silicone implantation on experimental models of autoimmunity. PhD dissertation, Wayne State University, 1997.
- Schaefer CJ, Whalen JD, Knapp T, et al. The influence of silicone implantation on type II collagen-induced arthritis in mice. *Arthr Rheum* 41:1064–72, 1997.
- Selwyn MR, Danner VM. Statistical analyses for two-year gel-implant study of Q7-2159A and MDF-0193 in Sprague-Dawley rats. Report M8518-0:M8518-0, 1988.
- Siddiqui WH. Evaluation of liver microsomal enzyme induction potential of decamethyl-cyclopentasiloxane in the rat. Report I-0005-2586, 1989.
- Smith DJ, Jr., Sazy JA, Crissman JD, et al. Immunogenic potential of carpal implants. *J Surgical Res* 48:13–20, 1990.
- Sparschu GL, Clashman A. Pathology Report on the Effects of Dow Corning 360 Fluid-350 Centistokes after Administration to Rats Intraperitoneally or Subcutaneously. Midland, MI: Dow Chemical Co., 1970.
- Van Eden W, Holoshitz J, Nevo Z, et al. Arthritis induced by a T-lymphocyte clone that responds to *Mycobacterium tuberculosis* and to cartilage proteoglycans. *Proc Natl Acad Sci USA* 82:5117–20, 1985.
- Varaparth S, Salyers KL, Plotzke KP. Non-regulated study: identification of major metabolites of octamethylcyclotetrasiloxane (D4) in rat urine. Report 1997-I0000-43454, 1997.
- West B, Jolly ER. Two year safety evaluation study in dogs with DOW Corning 360 liquid. 1976.
- Whitehouse MW, Orr KJ, Beck FWJ, et al. Freund's adjuvants: relationship of arthritogenicity and adjuvanticity in rats to vehicle composition. *Immunology* 27:311, 1974.
- Wilson SD, Munson AE. Modulation of NK1.1 splenocytes after exposure to octamethylcyclotetrasiloxane (D4). *The Toxicologist* (Abstract), 1996
- Yoshino S. Silicone-induced arthritis in rats and possible role for T cells. *Immunobiol* 192:40–47, 1994.

Table 1. Influence of Silicone in Adjuvant Arthritis Disease Models

Model	Description	Treatment	Design	Significant Results	Interpretation/conclusions
1. Lewis rat model of adjuvant arthritis Chang (1993)	Genetically susceptible strain of rat; develops arthritis in response to CFA injection.	<i>M. tuberculosis</i> was emulsified in mineral oil or silicone gel that was liquified by homogenization Rats were injected subplantar with: a) saline b) silicone gel c) <i>M. tuberculosis</i> in silicone gel d) <i>M. tuberculosis</i> in mineral oil (CFA)	There were 7 rats/group. Hind paws were measured on the day of injection and on the 16 th and 23 rd day after injection.	Rats injected with CFA developed arthritis by day 16. Rats injected with silicone gel with or without mycobacteria did not develop arthritis.	This study directly tested the ability of silicone gel to induce "adjuvant arthritis". Silicone gel was not effective.
2. Lewis rat model of adjuvant arthritis. Picha and Goldstein (1997)	Genetically susceptible strain of rat, develops arthritis in response to CFA injection.	Rats were injected id into the plantar aspect of the right foot with: a) CFA b) silicone oil + <i>M. tuberculosis</i> c) silica in IFA d) silicone oil + <i>M. tuberculosis</i> + silica + IFA	There were 15 rats/treatment. Rats were examined over a 4 month period for development of arthritis.	Rats responded to CFA injection with prolonged arthritis Rats injected with silicone oil + <i>M. tuberculosis</i> developed a response at day 8 that declined by day 15, followed by a second response at 120 days. Silicone oil injected with silica appeared to decrease the inflammatory reaction to silica.	This study directly tested the ability of silicone oil to induce "adjuvant arthritis". Silicone oil was ineffective. The significance of the modified response seen in silicone-treated rats is difficult to evaluate due to the subjective description of the data.
3. Dark Agouti (DA) rat adjuvant arthritis model Naim et al. (1995)	Genetically susceptible strain of rat, develops arthritis in response to injection of IFA	Rats were injected id at base of tail with: a) silicone oil:gel (1:1) b) IFA	There were 10 rats/treatment The DTH response to collagen was measured on day 18 Rats were killed 89 days after injection Sera obtained for anti-collagen titers	IFA induced arthritis in 8/10 rats Silicone-treated rats did not develop arthritis No DTH reaction or antibody response to collagen in either group	This experiment directly tested the ability of a mixture of silicone oil and gel to induce "adjuvant arthritis" in a highly susceptible strain of rat. Silicone oil/gel was ineffective.

Table 2. Adjuvant Activity of Silicones

Model	Description	Immunization	Design	Significant Results	Interpretations/Conclusions
1. Antibody response to bovine serum albumin (BSA) in rats Naim et al. (1993)	Immune response to a foreign protein (e.g., BSA) is enhanced when the protein is injected as an emulsified preparation in an adjuvant	Sprague-Dawley rats were injected im with 50 ug BSA mixed or emulsified with: a) saline b) silicone oil (20 cs) c) silicone oil:gel (1:1) d) CFA e) IFA	There were 10 rats/group. Rats were bled on day 12, 22, 40 and 56 days after immunization. Sera were tested for anti-BSA antibodies by ELISA.	High titers of anti-BSA antibodies were found when CFA, silicone gel, and IFA were used as adjuvant. Antibody titers were low when silicone oil was used as adjuvant.	The adjuvanticity of silicone gel was as great as CFA. Silicone oil possessed only weak adjuvant activity.
2. Antibody response to BSA in rats Naim et al. (1995)	Immune response to a foreign protein (e.g., BSA) is enhanced when the protein is injected as an emulsified preparation in an adjuvant	Sprague-Dawley rats were immunized im with 50 ug BSA mixed with a) saline b) D4 c) silicone oil, 100cs d) silicone oil, 350 cs e) silicone oil, 1000 cs f) silicone oil, 12,500 cs g) silicone gel in 20 cs oil h) CFA A booster injection was given on day 71 in same adjuvant except CFA group was injected with IFA	There were 8 rats/group. Rats were bled at 14, 29, 49, 71, 79 and 98 days after immunization. Sera were tested for anti-BSA antibodies by ELISA.	Antibody titers to BSA were detectable in all rats following immunization. All silicones were much poorer adjuvants than CFA.	Silicone oils and gel demonstrated weak adjuvant activity for antibody responses to BSA. Silicone gel was a better adjuvant than the oils. There was a trend toward increased antibody titers with higher molecular weight oils.
3. Antibody response to BSA in rats Naim et al. (1995)	Immune response to a foreign protein (e.g., BSA) is enhanced when the protein is injected as an emulsified preparation in an adjuvant	BSA was admixed with homogenized silicone gel in 3 separate preparations that were subjected to varying applied shear force.	Rats were bled periodically. Sera were tested for anti-BSA antibodies by ELISA	Low titers of anti-BSA antibodies were found in all rats immunized with BSA in silicone gel. There was no difference in titers between the treatment groups.	The homogenization process did not influence the adjuvancy of silicone gel.

Table 2 continued. Adjuvant Activity of Silicones

Model	Description	Immunization	Design	Significant Results	Interpretations/Conclusions
<p>4. Antibody response to BSA in rats and mice.</p> <p>Klykken and White (1996)</p>	<p>Immune response to a foreign protein (e.g., BSA) is enhanced when the protein is injected as an emulsified preparation in an adjuvant</p>	<p>Sprague Dawley rats or B6C3F1 mice were injected im with BSA emulsified in</p> <p>a) saline b) CFA c) silicone gel bleed d) silicone gel e) D4</p>	<p>The number of animals in each group was not indicated</p> <p>Serum samples were obtained from rats at 2,4,6 and 8 weeks post-immunization. Serum samples were obtained from mice at 4, 7 and 12 weeks after immunization.</p>	<p>Administration of BSA in CFA, gel or D4 resulted in an enhanced antibody response in both rats and mice. Gel bleed tested in rats did not boost the antibody response above the saline control.</p>	<p>Silicone gel and D4 but not gel bleed were effective adjuvants to increase the antibody response to BSA</p>
<p>5. Anti-ovalbumin (OVA) antibody production in rats.</p> <p>Naim et al. (1997)</p>	<p>Immune response to a foreign protein (e.g. OVA) is enhanced when the protein is injected as an emulsified preparation in an adjuvant</p>	<p>OVA was emulsified in</p> <p>a) CFA b) Dow Corning gel lot # HH019581 c) McGhan gel lot # DP9339 d) McGhan gel lot # S0400488</p> <p>Each rat was injected id at base of tail. A booster injection was given after 48 days in same adjuvant except CFA group was injected with IFA.</p> <p>On day 14, rats were challenged in the right ear with OVA and in left ear with saline.</p>	<p>There were 4-6 rats/group.</p> <p>Serum samples were taken at 21, 48, 62 and 84 days after immunization for measurement of anti-OVA titers.</p> <p>Delayed-type hypersensitivity (DTH) response to OVA was measured on day 14 by ear swelling test.</p>	<p>Antibody and DTH responses to OVA were observed in all rats except those in group c.</p> <p>The antibody and DTH responses with silicone as adjuvant were lower than the response with CFA.</p>	<p>Different types of silicone gel were compared for their adjuvant activity. Two of three silicone gels had adjuvant activity in terms of boosting the antibody response to OVA.</p> <p>The DTH response did not follow classic kinetics.</p>

Table 2 continued. Adjuvant Activity of Silicones

Model	Description	Immunization	Design	Significant Results	Interpretations/Conclusions
<p>6. Immune response to keyhole limpet hemocyanin (KLH) in mice</p> <p>(Abstract only available)</p> <p>Gish et al. (1997)</p>	<p>Immune response to a foreign protein (eg. KLH) is enhanced when the protein is injected as an emulsified preparation in an adjuvant</p>	<p>KLH was emulsified in</p> <p>a) saline</p> <p>b) CFA</p> <p>c) D4</p> <p>and injected id in the footpad of mice</p>	<p>There were 3 mice/group.</p> <p>Footpad swelling was measured at 96 hr</p> <p>Mice were killed after 1 week and lymph node cells were cultured with KLH to evaluate proliferation response and cytokine production</p> <p>Antibody production was measured after 21 days</p>	<p>Footpad swelling occurred in mice injected with CFA or D4, independent of KLH. Swelling with D4 was greater than with CFA.</p> <p>D4 was as effective as CFA in inducing immunity to KLH as measured by lymph node proliferation, IL-2, IL-4 and antibody production</p>	<p>The study evaluated the ability of D4, a low molecular weight cyclosiloxane detected in silicone gel, to act as an adjuvant.</p> <p>D4 was as effective as CFA in inducing immunity to KLH</p>
<p>7. Antibody response to BSA in rats.</p> <p>Hill et al. (1996)</p>	<p>Immune response to a foreign protein (eg. BSA) is enhanced when the protein is injected as an emulsified preparation in an adjuvant</p>	<p>Sprague-Dawley rats were immunized with BSA emulsified in:</p> <p>a) IFA</p> <p>b) silicone oil</p> <p>c) IFA/silicone oil</p> <p>d) silicone oil:gel (1:1)</p> <p>e) silicone oil + 1000 μ elastomer particles</p> <p>f) silicone oil + 500 μ elastomer particles</p>	<p>There were 10 rats/group</p> <p>Blood samples were obtained on days 0, 14, 28, 42, and 55 for measuring anti-BSA IgG levels</p>	<p>Antibody titers to BSA were enhanced groups a, c, and d indicating that silicone gel mixed with silicone oil is an effective adjuvant but silicone oil alone or mixed with elastomer particles is not.</p>	<p>This study directly tested the ability of different forms of silicone to act as an adjuvant in the antibody response to BSA.</p> <p>Silicone gel but not silicone oil or silicone elastomer particles was an effective adjuvant.</p>
<p>8. Immune response to EL₄ tumor cells in rats</p> <p>Chang (1993)</p>	<p>The antibody and cell-mediated immune response of Lewis rats to mouse EL₄ tumor cells is greatly enhanced when the cells are mixed with CFA</p>	<p>Rats were immunized ip with EL₄ tumor cells in:</p> <p>a) saline</p> <p>b) mineral oil</p> <p>c) CFA</p> <p>d) silicone + <i>M. tuberculosis</i></p>	<p>There were 7 rats/treatment group.</p> <p>Rats were killed 16 days after immunization and spleen cells were tested for cytotoxic activity to EL₄ cells.</p> <p>Rat serum was tested for antibodies to EL₄ cells. (The paper failed to state when sera were collected.)</p>	<p>Rats that were injected with EL₄ cells in saline developed a low level of cell-mediated cytotoxicity which was boosted to a high level by FCA. The response was not boosted by mineral oil alone or by silicone + <i>M. tuberculosis</i></p> <p>The antibody response was boosted by CFA but by silicone + <i>M. tuberculosis</i> (mineral oil alone was not tested)</p>	<p>Silicone gel failed to act as an adjuvant in a novel model of immunity</p>

Table 2 continued. Adjuvant Activity of Silicones

Model	Description	Immunization	Design	Significant Results	Interpretations/Conclusions
<p>9. Antibody response to BSA in mice.</p> <p>Nicholson et al. (1996)</p>	<p>Immune response to a foreign protein (eg., BSA) is enhanced when the protein is injected as an emulsified preparation in an adjuvant</p>	<p>A/J mice were immunized with BSA emulsified in</p> <p>a) saline b) IFA c) silicone oil d) IFA/silicone oil e) 1:1 silicone gel:oil f) D4 g) IFA/D4</p>	<p>There were 10 mice/group</p> <p>The mice were bled on days 0, 15, 30, 45, 60, 75 and 90 following immunization.</p> <p>Serum was tested for IgG antibody to BSA</p>	<p>Enhanced antibody production compared to the saline group was observed with all treatments except silicone oil alone.</p>	<p>D4 and silicone gel but not silicone oil were effective adjuvants for the antibody response to BSA in mice.</p>
<p>10. Collagen-induced arthritis in DBA/1 mice</p> <p>Schaefer (1997)</p>	<p>A genetically susceptible strain of mouse that develops progressive, inflammatory arthritis in response to immunization with bovine collagen.</p>	<p>Mice were immunized with:</p> <p>a) collagen emulsified in CFA b) collagen emulsified in silicone oil+ <i>M. tuberculosis</i> c) silicone oil + <i>M. tuberculosis</i></p>	<p>There were 18 mice/group for the silicone treatments and 10 mice for the CFA treatment.</p> <p>Mice were observed for signs of arthritis for 10 weeks after immunization; joints measured 3x/week.</p> <p>Sera obtained for measurement of anti-collagen antibodies</p>	<p>The incidence of arthritis was 80% in mice immunized with collagen in CFA. Arthritis did not develop in any mice injected with silicone oil as adjuvant.</p> <p>High titers of collagen antibodies developed in mice immunized with collagen in CFA. Mice immunized with silicone oil as adjuvant did not develop antibodies to bovine collagen</p>	<p>This experiment was designed to test the adjuvanticity of silicone using disease as an endpoint as well as antibody titers. It does not address the potential of silicone to induce or exacerbate autoimmune disease.</p> <p>Silicone oil containing mycobacteria was unable to act as an adjuvant for collagen in the induction of arthritis.</p>

Table 2 continued. Adjuvant Activity of Silicones

Model	Description	Immunization	Design	Significant Results	Interpretations/Conclusions
11. Collagen-induced arthritis in Dark Agouti (DA) rat Naim et al. (1995)	DA rats are a genetically susceptible strain that develops arthritis in response to immunization with bovine collagen.	Rats were injected id at the base of the tail with <u>six µg</u> bovine collagen emulsified in a) saline b) silicone oil:gel (1:1) c) IFA Groups a and b received a booster injection of the emulsified collagen on day 45	There were 10 rats/group Rats were observed periodically for signs of arthritis and scored for severity of symptoms. All rats were killed on day 89. Serum samples were obtained on days 21, 59 and 89 after immunization for anti-collagen titers	The incidence of arthritis was: a) saline 0/10 b) silicone oil:gel 4/10 c) IFA 8/9 Disease severity was lower when silicone was used as adjuvant compared to IFA Very low antibody titers were found in both silicone- and IFA-injected mice. They could not be directly compared because of the booster injection given to group b but not c.	This experiment was designed to test the ability of silicone to act as an adjuvant when emulsified with a foreign protein (bovine collagen) using disease and antibody titers as endpoints in a highly susceptible strain of rat. However, it does not directly address the potential of silicone to induce or exacerbate spontaneous autoimmune disease. Silicone gel was an effective adjuvant for collagen-induced arthritis
12. Collagen-induced arthritis in DA rat using high dose of collagen Naim et al. (1995)	DA rats are a genetically susceptible strain that develops arthritis in response to immunization with bovine collagen.	Rats were injected id at the base of the tail with <u>125 µg</u> bovine collagen emulsified in: a) saline b) silicone gel c) IFA d) silicone oil e) D4 f) 1% D4 in silicone oil	There were 10 rats/group Rats were observed periodically for signs of arthritis and scored for severity of symptoms. Serum samples were obtained on days 21, 59 and 89 after immunization for anti-collagen titers All rats were killed on day 69	The incidence of arthritis was: a) saline 0/10 b) silicone gel 7/10 c) IFA 10/10 d) silicone oil 3/10 e) D4 0/10 f) D4 in silicone oil 1/10 The severity of arthritis was significantly greater with IFA compared to any other group. Only IFA was an effective adjuvant for inducing high titer anti-collagen antibodies. Antibody production was lower and delayed with silicone gel as adjuvant. No antibody was produced with D4 as adjuvant.	This experiment was designed to test the ability of silicone to act as an adjuvant when emulsified with a foreign protein (bovine collagen) using disease and antibody titers as endpoints in a highly susceptible strain of rat. However, it does not directly address the potential of silicone to induce or exacerbate spontaneous autoimmune disease. Silicone gel and to a lesser extent silicone oil but not D4 was an effective adjuvant for collagen-induced arthritis and production of anti-collagen antibodies.

Table 2 continued. Adjuvant Activity of Silicones

<p>13. Experimental autoimmune thyroiditis (EAT) in Wistar rats</p> <p>Naim et al. (1993)</p>	<p>Thyroiditis is induced in several species of animals by the injection of thyroglobulin (Tg) emulsified in CFA. The induction of disease in the rat is rapid and predictable.</p>	<p>Rats were injected once id at base of tail with 2 mgTg emulsified in</p> <p>a) saline b) CFA c) silicone oil:gel (1:1)</p>	<p>There were 6-7 rats/group.</p> <p>Rats were bled on day 15 and 28 after immunization for measurement of anti-Tg antibodies</p> <p>Rats were killed on day 28 and thyroids were processed for histological exam.</p>	<p>The incidence of thyroiditis was:</p> <p>a) saline 0/6 b) CFA 7/7 c) silicone oil:gel 0/7</p> <p>All rats immunized with Tg in CFA developed high antibody titers to Tg. Three of 7 rats injected with Tg in silicone produced low titers of anti-Tg antibodies</p>	<p>This experiment was designed to test the adjuvanticity of silicone using disease and antibody production as endpoints. It does not address the potential of silicone to induce or exacerbate spontaneous autoimmune disease.</p> <p>Silicone gel was a weak adjuvant for induction of anti-Tg antibodies but not for induction of disease.</p>
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Table 3. Influence of silicone injections/implants on spontaneous or induced autoimmune diseases

Model	Description	Treatment	Design	Significant Results	Interpretation/Conclusions
<p>1. DBA/1 mouse model of arthritis</p> <p>Schaefer (1997) Schaefer et al. (1997)</p>	<p>An arthritis-prone strain; spontaneously develops arthritis at low incidence. Increased incidence of arthritis with mineral oil (pristane) injection.</p>	<p>Mice were injected ip with: silicone elastomer (0.1 mg segment) silicone gel (0.1 ml) sham injection</p> <p>Some of the mice were injected with CFA at the base of tail 3 days after implant surgery because these mice served as controls for collagen-immunized mice reported in entry 12, this table)</p>	<p>There were 10 mice/treatment</p> <p>Mice were observed for signs of arthritis for 12 weeks; joints were measured.</p> <p>Sera were obtained: a) after 28 days, b) at onset of arthritis (when applicable) c) at termination, for measurement of anti-collagen antibodies, cytokines, and "silicone-binding antibodies"</p>	<p>No arthritis observed in any treatment group</p> <p>No anti-collagen antibodies observed in any group</p> <p>No differences in cytokine levels in any group</p>	<p>This experiment directly tested the ability of silicone to induce arthritic disease.</p> <p>The absence of a positive control group (e.g., ip pristane) limits interpretation of the negative data. CFA injected did not induce arthritis in this mouse model (in contrast to the DA rat model) The data on "silicone binding antibodies" and "silicone-bound proteins" were not convincing of anything more than nonspecific protein binding.</p>
<p>2. DBA/1 mouse model of arthritis</p> <p>Schaefer (1997)</p>	<p>An arthritis-prone strain; spontaneously develops arthritis at low incidence. Increased incidence of arthritis with mineral oil (pristane) injection.</p>	<p>Mice were injected ip with: silicone oil silicone gel silicone elastomer sham injection</p> <p>(Some of the mice in each treatment group were injected with CFA at base of tail 9 months after implant surgery because these mice served as controls for collagen-immunized mice reported in entry 13, this table)</p>	<p>There were 18-20 mice/ treatment</p> <p>Implants were in place for 12 months prior to termination.</p> <p>(Many of the specific details of this study were not included in the chapter of the thesis from which these data were obtained. It was assumed that the same experimental methods as described in entry 1, this table, were used.)</p>	<p>No arthritis observed in any treatment group (data not shown)</p> <p>No anti-collagen antibodies were observed in any group.</p> <p>Several changes in cytokine levels were noted in different groups of silicone-treated mice but effects were not consistent between mice that were injected with CFA and those that were not.</p>	<p>This experiment directly tested the ability of silicone to induce arthritic disease. The absence of a positive control group (e.g., ip pristane) limits interpretation of the negative data.</p> <p>The significance of altered cytokines is not known.</p> <p>Silicone implantation for as long as 12 months did not induce arthritis in this susceptible strain of mouse.</p>

Table 3 continued. Influence of silicone injections/implants on spontaneous or induced autoimmune diseases

Model	Description	Treatment	Design	Significant results	Interpretation/conclusions
<p>3. Balb/cAnPt mouse model of arthritis</p> <p>MacDonald et al. (1998)</p>	<p>This strain of mouse develops plasmacytomas and arthritis from ip pristane; otherwise not considered autoimmune-prone</p>	<p>Mice were injected sc with:</p> <p>0.2 ml saline</p> <p>0.2 ml silicone gel</p> <p>0.5 ml pristane</p> <p>0.2 ml silicone gel + capsulotomy</p> <p><i>Staph. epidermidis</i></p> <p>0.2 ml gel + <i>Staph</i></p> <p>0.2 ml saline 3X</p> <p>0.2 ml silicone gel 3X</p> <p>0.5 ml pristane 3X</p>	<p>There were 10 mice/group.</p> <p>Survival was recorded daily for 10 mos.</p> <p>Urinary protein was measured biweekly.</p> <p>At termination: hematocrit, anti-RBC titers, anti-nuclear antibody titers; anti-Type I collagen titers</p>	<p>None of the treatment groups developed signs of arthritis. Survival was 100% in all groups except for group injected 3x with silicone in which survival was 80%. The cause of death was not stated.</p> <p>There were no effects of any treatment on hematocrit values or urinary protein levels, and no antibodies to RBC were found.</p> <p>Pristane 3x induced ANA in 5/5 mice at a titer of 96. No other group had more than 1 out of 5 animals with a low ANA titer.</p> <p>Anti- collagen titers were increased in mice that were injected 3x with pristane or 3x with silicone.</p>	<p>This experiment directly addressed the ability of silicone to induce autoimmune disease in a mouse model that is not considered autoimmune prone.</p> <p>No evidence of autoimmune disease was found with any treatment.</p> <p>The presence of a bacterial infection or multiple injections of silicone did not induce disease.</p> <p>The lack of response in the positive control group (pristane) limits interpretation of the negative data.</p>
<p>4. BALB/cAnPt-A mouse model of arthritis</p> <p>Potter et al. (1994)</p>	<p>BALB/cAnPt mice injected ip with pristane frequently develop arthritis (Potter and Wax, 1981)</p>	<p>Mice received multiple ip injections of different silicone gels, silicone oil, corn oil or pristane for the primary purpose of assessing plasmacytoma induction.</p>	<p>The mice were examined for plasmacytoma development over a period of 125-400 days.</p>	<p>The authors noted that " silicone-treated mice did not develop arthritis frequently found in pristane-treated mice". The frequency of arthritis in the pristane-treated mice was not reported.</p>	<p>These studies indirectly provided evidence regarding the inability of various silicone gels to induce arthritis in this genetically susceptible mouse strain.</p>

Table 3 continued. Influence of silicone injections/implants on spontaneous or induced autoimmune diseases

Model	Description	Treatment	Design	Significant results	Interpretation/conclusions
5. MRL ^{lpr/lpr} mouse model of SLE Schaefer (1997)	By 8 weeks of age, MRL ^{lpr/lpr} mice spontaneously develop lymphadenopathy, arthritis, proteinuria and glomerulonephritis. Death is at 16-24 weeks due to renal failure.	At 5 weeks of age, mice received sc implants of a) sham b) silicone gel c) silicone oil	There were 6 mice/treatment. Mice were killed 12 weeks after implant surgery. Disease was assessed by palpation of cervical lymph nodes, urinary protein levels, and immune complex deposition in kidney. At termination, serum was tested for antibodies to collagen and DNA, rheumatoid factor, and total Ig. IL-1, IL-2, IL-4, TNF- α levels in serum were measured at 3 time points.	After 15 weeks of age, disease severity was similar in all three groups. Anti-collagen antibodies were similar in all groups. Anti-DNA antibodies were higher in silicone gel implanted mice. IL-2 levels in serum were elevated in mice with silicone oil implants.	This model directly tested the ability of silicone gel and oil to modify genetically-determined autoimmune disease. However, because of the severity of the disease in control mice, it may be difficult to detect exacerbation in this model. The authors did not report time-to-onset data. The significance of anti-DNA antibodies and IL-2 to disease in this model are not known.
6. MRL ^{+/+} mouse model of SLE Schaefer (1997)	MRL ^{+/+} mice spontaneously develop mild autoimmune glomerulonephritis late in life.	At 5 weeks of age, mice received sc implants of a) sham b) silicone gel c) silicone oil	There were 6 mice/treatment. Mice were killed 12 weeks after implantation. Disease was assessed by palpation of cervical lymph nodes, urinary protein levels, immune complex deposition in kidney. At termination, serum was tested for antibodies to collagen and DNA, rheumatoid factor, and total Ig. IL-1, IL-2, IL-4, TNF- α levels in serum were measured at 3 time points.	Lymphadenopathy was not detected in any group. Immune complex deposition in glomeruli was minimal in all groups. Antibodies to DNA were slightly higher in silicone gel and oil implanted mice.	Because of the minimal disease that develops in control MRL ^{+/+} mice, this model directly tested the ability of silicone to exacerbate genetically-determined autoimmune disease. Silicone failed to induce disease. The significance of the anti-DNA antibodies to disease in this model are not known.

Table 3 continued. Influence of silicone injections/implants on spontaneous or induced autoimmune diseases

Model	Description	Treatment	Design	Significant results	Interpretation/conclusions
7. C57Bl/6 ^{lpr/lpr} model of SLE Osborn et al. (1995) (abstract)	C57Bl/6 ^{lpr/lpr} mice spontaneously develop autoimmune disease characterized by lymphadenopathy, antinuclear antibodies and early mortality	At 6 weeks of age, mice were injected sc with: a) silicone oil containing 5% D4 b) saline	There were 20 mice/group. Animals were monitored at 0, 1, 3, 6, and 12 mos. for antinuclear antibodies, rheumatoid factor, lymph node enlargement and death.	At 48 weeks, mortality was: silicone 10/20 saline 11/20 The frequency and latency of other disease symptoms did not differ between the groups.	This model directly tested the ability of silicone to exacerbate genetically-determined autoimmune disease. Silicone failed to alter disease.
8. TSK/+ mouse model of scleroderma Fronzoza et al. (1996)	Tight skin (TSK/+) mice spontaneously develop skin fibrosis and characteristic auto-antibodies which resemble human scleroderma	1 month old TSK/+ mice or their normal litter-mates were injected sc with: a) silicone oil b) silicone gel c) IFA d) saline	There were 5-6 mice/group Mice were bled on day 0 and day 30 after implant surgery for measurement of antibodies to BSA, RNA polymerase protein, and topoisomerase. Samples of skin, kidney and liver were examined histologically.	There were no significant differences in skin histology or antibody titers in the silicone injected mice compared to those that received saline or IFA.	This study directly assessed the ability of silicone to modify the development of a spontaneous autoimmune disease. Silicone oil or gel injected sc did not alter the progression of disease in the TSK mouse model of scleroderma. The positive control group (IFA-treated mice) also failed to alter disease progression.
9. NZB/W F1 model of SLE White et al. (1997)	The NZB/W ♀ F1 mouse develops a gradual systemic autoimmune disease with characteristics including elevated titers of antinuclear antibodies and serum IgG, polyclonal B cell activation, and ultimately a fatal immune-complex mediated glomerulo-nephritis.	Mice, 7-8 weeks of age, were implanted sc with 1, 2 or 3 ml of silicone gel for 78 days. Saline (3 ml) was injected in control mice. Two positive control groups of mice were also evaluated in separate studies: 1 mg/kg HgCl ₂ injected sc 3x/week for 2 wks or 450 mg/kg d-penicillamine orally for 28 days	All mice were bled on day 79 following implantation of silicone gel. It was not stated when the positive control mice were sampled.	Exposure to silicone gel did not alter serum levels of IgG, or levels of antibodies to dsDNA, laminin, DNP-HSA, or SRBC. Spleen weight was not increased. All of these endpoints were increased in mice treated with HgCl ₂ or d-penicillamine. Proteinuria was noted in HgCl ₂ and d-penicillamine treated mice but not in silicone-treated mice (data was not shown)	This study did not measure autoimmune disease; only clinical endpoints that correlate with disease progression. The data from the positive controls demonstrate that clinical measurements of disease can be influenced by exposure to chemicals that are known to induce autoimmune disease. However, these data were obtained in independent studies and are thus not directly comparable to the silicone-treated mice. The responses of the sham controls differed between the studies.

Table 3 continued. Influence of silicone injections/implants on spontaneous or induced autoimmune diseases

<p>10. TSK/+ mouse model of scleroderma Osborn et al. (1995)</p>	<p>Tight skin (TSK/+) mice spontaneously develop skin fibrosis and characteristic auto-antibodies which resemble human scleroderma</p>	<p>3 week-old TSK mice were injected sc with: a) saline b) silicone + 5% D4</p>	<p>There were 12 mice/group Mice were sacrificed at 1, 6, and 12 months. Skin thickness of 6 mm biopsies and serum levels of antinuclear antibodies were measured.</p>	<p>There were no significant differences in skin thickness measurements or antinuclear antibody titers between saline-treated and silicone-injected mice.</p>	<p>This study directly assessed the ability of silicone to modify the development of a spontaneous autoimmune disease. Silicone gel containing 5% D4 when injected sc did not alter the progression of disease in the TSK mouse model of scleroderma. There was no positive control group.</p>																				
<p>11. NZB mouse model of autoimmune hemolytic anemia MacDonald et al. (1998)</p>	<p>NZB mice spontaneously develop autoantibodies and autoimmune hemolytic anemia. Death is due to anemia.</p>	<p>Mice were given the following sc treatments: sham 0.2 ml saline 0.2 ml silicone gel 0.5 ml pristane 0.2 ml silicone gel + capsulotomy at 3 mos <i>Staph. epidermidis</i> 0.2 ml silicone gel + <i>Staph.</i> 0.2 ml saline 3X 0.2 ml silicone gel 3X 0.5 ml pristane 3X</p>	<p>There were 10 mice/group Survival was recorded daily for 10 months and proteinuria biweekly. At termination, hematocrits and hemagglutination titers were measured. Other endpoints included: anti-nuclear antibody titers and anti-Type I collagen titers</p>	<p>At 12 months of age, the percentage mortality was:</p> <table border="0" data-bbox="1243 690 1566 961"> <tr><td>untreated</td><td>20</td></tr> <tr><td>saline 1x</td><td>30</td></tr> <tr><td>pristane 1x</td><td>20</td></tr> <tr><td>silicone gel 1x</td><td>50</td></tr> <tr><td><i>Staph. epidermidis</i></td><td>20</td></tr> <tr><td>gel + <i>Staph.</i></td><td>40</td></tr> <tr><td>gel + capsulotomy</td><td>20</td></tr> <tr><td>saline 3X</td><td>30</td></tr> <tr><td>pristane 3X</td><td>80</td></tr> <tr><td>silicone gel 3X</td><td>60</td></tr> </table> <p>Mortality was significantly increased compared to controls only in mice given 3 injections of pristane. Hematocrits in survivors at 12 mos of age were lower compared to controls in all silicone-treated mice and in mice injected 1x with pristane. Multiple injections of silicone or pristane increased urinary protein levels</p>	untreated	20	saline 1x	30	pristane 1x	20	silicone gel 1x	50	<i>Staph. epidermidis</i>	20	gel + <i>Staph.</i>	40	gel + capsulotomy	20	saline 3X	30	pristane 3X	80	silicone gel 3X	60	<p>This model directly tested the ability of silicone to exacerbate an autoimmune disease other than arthritis. The data on clinical endpoints reflect only survivors which likely changes the significance of effects. It is not clear what statistical comparators were used to determine the significance of the clinical data (eg., 3x saline as comparator for 3x pristane and 3x silicone) The relevance of certain endpoints measured (eg., urinary protein) in this model are not known.</p>
untreated	20																								
saline 1x	30																								
pristane 1x	20																								
silicone gel 1x	50																								
<i>Staph. epidermidis</i>	20																								
gel + <i>Staph.</i>	40																								
gel + capsulotomy	20																								
saline 3X	30																								
pristane 3X	80																								
silicone gel 3X	60																								

Table 3 continued. Influence of silicone injections/implants on spontaneous or induced autoimmune diseases

Model	Description	Treatment	Design	Significant results	Interpretation/conclusions
<p>12) Collagen-induced arthritis in DBA/1 mice (short-term implants)</p> <p>Schaefer (1997) Schaefer et al. (1997)</p>	<p>A genetically susceptible strain that develops progressive inflammatory arthritis in response to immunization with bovine collagen.</p>	<p>Mice were implanted ip with: Sham elastomer (0.1 mg segment) silicone gel (0.1 ml)</p> <p>3 days after implant surgery, all mice were injected at base of tail with 100 µg bovine collagen type II emulsified in CFA.</p>	<p>There were 10 mice/group.</p> <p>Mice were observed for signs of arthritis for 10 weeks after immunization; joints measured 3x/week.</p> <p>Sera obtained: a) after 28 days b) at onset of arthritis (when applicable) c) at termination for measurement of anti-collagen antibody, cytokines (IL-1, TNF, IFNγ, IL-2), and silicone-binding antibodies</p>	<p>The incidence of arthritis was high in all groups.</p> <p>The severity of disease was not different between groups.</p> <p>Titers of anti-collagen antibodies were similar between groups.</p> <p>TNF levels were lower in silicone gel-implanted mice. IL-2 levels were elevated in elastomer-implanted mice.</p>	<p>This model directly tested the ability of silicone to modify collagen-induced arthritis. However, because of the high incidence of disease in control mice, the model was not sensitive to detect increased frequency of disease. The authors did not report time-to-onset data.</p> <p>The cytokine data are contrary to a hypothesized proinflammatory effect of silicone</p> <p>The data on "silicone binding antibodies" and "silicone-bound proteins" were not convincing of anything more than nonspecific protein binding.</p>
<p>13. Collagen-induced arthritis in ♀ DBA/1 mice (long-term implants)</p> <p>Schaefer (1997)</p>	<p>A genetically susceptible strain that develops progressive, inflammatory arthritis in response to immunization with bovine collagen.</p>	<p>Mice were implanted ip with: sham silicone elastomer (0.1 mg segment) silicone gel (0.1 ml) silicone oil</p> <p>9 months after implantation, all mice were injected with bovine collagen in CFA at base of tail.</p>	<p>There were 10 mice/treatment</p> <p>Mice were observed for signs of arthritis for 10 weeks after immunization; joints measured 3x/week.</p> <p>Sera obtained for measurement of anti-collagen Abs, cytokines, silicone-binding antibodies</p>	<p>The incidence of arthritis was high in all groups.</p> <p>The severity score was higher in silicone oil implanted mice compared to all other groups.</p> <p>Titers of anti-collagen antibodies were similar between groups</p> <p>IL-1 was higher in oil-implanted mice. IL-2 levels were lower in gel-implanted mice. IL-4 levels were lower in oil- and elastomer-implanted mice. IL-5 levels were higher in gel- and elastomer-implanted mice. IFN levels were lower in oil- and higher in elastomer-implanted mice.</p>	<p>This experiment directly tested the ability of silicone to exacerbate collagen-induced autoimmune disease. However, the high incidence and severity of disease in the control mice limits the sensitivity of the model to detect exacerbation.</p> <p>The significance of altered cytokine levels is not known but did not correlate with disease severity in any recognizable pattern.</p>

Table 3 continued. Influence of silicone injections/implants on spontaneous or induced autoimmune diseases

Model	Description	Treatment	Design	Significant results	Interpretation/conclusions
<p>14. Collagen-induced arthritis in DBA/1 mice (long-term implants) (collagen emulsified in IFA).</p> <p>Schaefer (1997)</p>	<p>A genetically susceptible strain of mouse that develops progressive, inflammatory arthritis in response to immunization with bovine collagen.</p>	<p>Mice were implanted ip with: sham silicone elastomer (0.1 mg segment) silicone gel (0.1 ml) silicone oil</p> <p>9 months after immunization, all mice were injected with bovine collagen emulsified in IFA at base of tail</p>	<p>There were 9-10 mice/treatment.</p> <p>Mice were observed for signs of arthritis for 3 months following immunization; joints were measured.</p> <p>Sera obtained for measurement of anti-collagen antibodies, cytokines, and silicone-binding antibodies</p>	<p>The incidence of arthritis was: sham 3/10 elastomer 8/9 silicone gel 6/9 silicone oil 6/9</p> <p>Average severity score was higher in silicone elastomer-implanted mice</p> <p>Titers of anti-collagen antibodies were similar between groups.</p> <p>IL-2 levels were lower in gel-implant mice. IL-4 and IL-5 levels were lower in oil-injected mice. IL-10 levels were higher in elastomer implanted mice.</p>	<p>This experiment directly tested the ability of silicone to exacerbate collagen-induced autoimmune disease. The sensitivity to detect exacerbation was improved by using IFA as adjuvant, which reduced the incidence and lowered the severity of arthritis in the control mice.</p> <p>The data indicate enhanced disease in silicone elastomer implanted mice.</p> <p>The significance of altered cytokines is not known. However, overall levels of cytokines were similar to levels seen in mice immunized with CFA indicating lack of correlation with disease severity.</p>