CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Mar. 1996, p. 156–161 Vol. 3, No. 2
1071-412X/96/$04.00+0

Phenotype of Lymphocytes Associated with the Inflammatory Reaction to Silicone Gel Breast Implants

WILLIAM E. KATZIN,[1,2]* LU-JEAN FENG,[2,3] MARY ABBUHL,[1] AND MARY ANN KLEIN[1]

Department of Pathology[1] and Division of Plastic Surgery,[3] Mt. Sinai Medical Center, and Case Western Reserve University School of Medicine,[2] Cleveland, Ohio

Received 18 July 1995/Returned for modification 13 September 1995/Accepted 27 November 1995

The tissue response to silicone gel breast implants typically includes an inflammatory infiltrate that consists of macrophages, foreign body-type giant cells, and a variable number of lymphocytes and plasma cells. The phenotype of the lymphocytic component was investigated with three-color flow cytometry. Lymphocytes were obtained by collecting fluid from the space between the implant and the fibrous capsule or by washing cells from the fibrous capsule at the time of implant removal with total capsulectomy. Eighty-nine percent of the implant-associated lymphocytes were T cells. Twenty-five percent of the $CD3^+$ T cells coexpressed HLA-DR compared with only 7.9% of matched peripheral blood lymphocytes. Sixty-eight percent of the implant-associated T cells coexpressed CD4 and CD29, while only 3% of the T cells coexpressed CD4 and CD45RO. The expression of HLA-DR and the predominance of $CD29^+$ $CD4^+$ T cells indicate that there is immune activation with the potential for stimulating antigen-specific antibody production. The role of silicone gel breast implants in immune activation and its clinical significance require further investigation.

Silicone gel-containing breast implants, introduced over 30 years ago (7), have been widely used for augmentation and reconstructive mammoplasty. Recently there has been increasing concern regarding the safety of breast implants. Most notably, a variety of rheumatic diseases, including chronic arthropathy (2, 9, 39, 46), scleroderma or systemic sclerosis (6, 20, 21, 33, 36, 37, 40, 42), rheumatoid arthritis (9, 20, 40), systemic lupus erythematosus (36, 40), and Sjogren's syndrome (20, 40) have been reported in patients with silicone gel breast implants. However, the relationship between implants and these connective tissue diseases is still unclear (41). Three separate epidemiologic studies (11, 34, 47) have not found an increased incidence of specific rheumatic diseases in large populations of women with silicone gel implants. The possible relationship between silicone breast implants and systemic disease has been recently reviewed by Sanchez-Guerrero et al. (35).

The tissue response to silicone gel-containing breast implants includes formation of a fibrous capsule. On the basis of studies with an animal model, it is likely that the capsule forms in a relatively short time—probably within 2 months (27). The histologic features of the fibrous capsule in women with implants have been described previously (3, 12, 14, 15, 30, 32, 38). In addition to a band of dense fibrous tissue, the capsule includes a variable number of inflammatory cells. Macrophages with abundant vacuolated cytoplasm are a relatively constant feature. There are often cyst-like spaces that contain refractile, nonbirefringent clear material that almost certainly represents some form of silicone (1, 14, 32, 38). The inflammatory infiltrate also includes a variable number of lymphocytes and plasma cells (12, 16, 38).

Aside from the morphologic features of the fibrous capsule that surrounds silicone gel breast implants, very little is known about the nature of the inflammatory response. In an attempt to provide more specific information in this regard, we have defined the phenotype of the lymphocytic component of the capsular infiltrate by three-color flow cytometry.

* Corresponding author. Mailing address: Department of Pathology, Mt. Sinai Medical Center, One Mt. Sinai Dr., Cleveland, OH 44106. Phone: (216) 421-4403. Fax: (216) 421-3964.

MATERIALS AND METHODS

During the study period (1 May 1992 to 1 April 1993), a total of 209 patients underwent implant removal. The vast majority of these patients presented with local breast pain and systemic symptoms that they suspected were associated with their implants. Surgery in all cases was performed by one of the authors (L.-J.F.), who submitted all of the samples. All of the patients were examined by a rheumatologist, internist, or neurologist prior to implant removal. Eight of the 209 patients had documented autoimmune diseases which had developed after implantation. Three had rheumatoid arthritis, two had multiple sclerosis, one had systemic lupus erythematosus, one had systemic lupus erythematosus and polymyositis, and one had scleroderma. Only one of the 209 patients had evidence of frank infection.

In preliminary studies, we found that a sufficient number of lymphocytes for flow cytometric analysis could be obtained only from patients with either polyurethane-coated silicone gel breast implants or textured-surface silicone gel breast implants. In those patients, exudative fluid was present in the space between the implant and the surrounding fibrous capsule. Little or no exudative fluid or very few mechanically dislodged cells could be obtained from patients with smooth-shell silicone gel implants. Implant-associated lymphocytes were obtained at the time of surgical implant removal in all cases in which exudative fluid was available. In some cases, fluid was collected from the space between the implant and the surrounding fibrous capsule and sent directly for phenotyping. In the other cases, the fibrous capsule was vigorously washed in RPMI 1640 medium and the mechanically dislodged cells were immediately sent for phenotyping. In a few cases, peri-implant fluid was sent from one breast, and a capsular wash was sent from the opposite breast of the same patient. Implant-associated cells were washed twice in RPMI 1640 medium, and the cell pellet was resuspended in 1 ml of RPMI 1640 medium. The cell yield and viability were determined at this time. The resuspended cells were then mixed with RPMI 1640 medium containing 10% fetal calf serum (Hyclone, Logan, Utah) and rocked in a 37°C incubator for 30 min. Cell suspensions were filtered through a 40-μm-pore-size nylon mesh (Tetco, Briar Cliffe Manor, N.Y.) and centrifuged for 5 min at $400 \times g$. Cells were then washed once in RPMI 1640 medium and resuspended in RPMI 1640 medium to obtain a cell concentration of 2×10^6 cells per ml. The cells in samples with a low yield were resuspended in 1 ml of RPMI 1640 medium.

In addition to the implant-associated cells, heparin-anticoagulated peripheral blood was submitted for phenotyping. Peripheral blood mononuclear cells were isolated from heparinized blood by density gradient centrifugation (Histopaque-1077; Sigma, St. Louis, Mo.). The peripheral blood mononuclear cells were recovered and washed twice with Hanks balanced salt solution without Ca or Mg (Gibco, Grand Island, N.Y.) at room temperature. The cell pellet was then resuspended in 1 ml of Hanks balanced salt solution, and the cell count was adjusted to 2×10^6 cells per ml for staining. In some cases, the lymphocytes were separated from whole blood by Ficoll-Hypaque density gradient centrifugation and stored in freezing medium (25% calf serum, 8% dimethyl sulfoxide, 67%

TABLE 1. Antibody panel used in this study

CD (clone)	Major specificity group	Fluorescent label	Dilution	Source[a]
CD45 (Hle-1)	Pan-leukocyte	FITC	1:1	BD
CD14 (Leu-M3)	Monocytes	PE	1:1	BD
CD3 (Leu-4)	T cells	FITC or PERCP	1:1	BD
CD4 (Leu-3)	T-helper and/or inducer cells, monocytes	FITC or PERCP	1:1	BD
CD8 (Leu-2)	T-cytotoxic and/or suppressor cells	FITC	1:1	BD
CD16/56 (Leu-11c + Leu-19)[b]	Natural killer cells	PE	1:1	BD
CD19 (Leu-12)	B cells	PERCP	1:1	BD
CD20 (Leu-16)	B cells	PERCP	1:1	BD
CD29 (4B4)	T-cell subset	RD1	1:5	CI
CD45RO (2H4)	T-cell subset	RD1	1:64	CI
HLA-DR		PERCP	1:1	BD

[a] BD, Becton Dickinson Immunocytometry Systems, San Jose, Calif.; CI, Coulter Immunodiagnostics, Hialeah, Fla.
[b] In a few cases, CD56 (Leu-19) was used alone.

Dulbecco's modified Eagle's medium) in liquid nitrogen prior to flow cytometry. Previously frozen peripheral blood mononuclear cells were snap thawed in a 37°C water bath and washed twice with Dulbecco's phosphate-buffered saline (D-PBS) without Ca or Mg (Gibco). The cell pellet was resuspended in 1 ml of D-PBS. Viability was checked with trypan blue (Sigma).

Three-color staining was performed with 20 μl of mouse monoclonal antibodies (Table 1), at an optimum titer, directly conjugated with fluorescein isothiocynate (FITC), phycoerythrin (PE [or RD1]), or peridinin chlorophyll protein (PERCP). One hundred microliters of the cell suspension was added to the appropriate tubes and mixed. The samples were then incubated at room temperature out of direct light for 15 min with gentle vortexing at 5-min intervals. Samples with gross erythrocyte contamination or whole-blood samples were lysed by incubation with FACSLyse (Becton Dickinson, San Jose, Calif.). Cells were then washed twice with Hanks balanced salt solution with 1% bovine serum albumin (BSA) (Sigma) and 0.1% sodium azide (Fisher, Pittsburgh, Pa.). The cell pellet was then resuspended in 0.5 ml of D-PBS with 1% BSA and 1% ultrapure electron microscopy-grade formaldehyde (Methanol Free; Polysciences, Washington, Pa.) for fixation. Negative controls, consisting of isotypically matched nonimmune mouse immunoglobulin, were used to position the cursors that defined positive and negative cells. Positive controls consisted of peripheral blood lymphocytes from healthy donors. Cells were analyzed on a FACScan flow cytometer with an argon ion laser emitting at 498 nm (Becton Dickinson). Gates for acquisition of data were set by light scatter characteristics and were verified by back-gating with cells stained for CD45 and CD14. A total of 10,000 gated events were acquired with LYSYS analysis software (Becton Dickinson), yielding a percentage of total cells positive for each antigen. In the few samples that contained too few cells to acquire 10,000 events, as many gated cells as possible were acquired. Analysis of data was performed with PAINT-A-GATE software (Becton Dickinson).

Statistical comparisons between the phenotypes of the implant-associated lymphocytes and those of the peripheral blood were analyzed with the paired *t* test. Statistical comparisons between the phenotypes of the peripheral blood lymphocytes of the patients and those of the healthy controls were analyzed with the unpaired *t* test.

RESULTS

All cases accessioned between 1 May 1992 and 1 April 1993 for which cells were submitted for phenotypic analysis by flow cytometry were included in this study, with the following exceptions. In three cases, there was an insufficient number of cells to provide complete and reliable phenotypic data. In four additional cases, the percentage of events in the lymphocyte gate that were $CD45^+$ and $CD14^-$ was less than 80%. In all cases included in the study, that percentage was greater than 85%. In three cases, separate samples were submitted from the right and left breasts. In those cases, there were no significant phenotypic differences between the two samples, and only the data from the sample submitted first were considered for statistical calculations regarding the paired peripheral blood samples. There were no phenotypic differences between exudative fluid samples and capsular wash samples. In one case, the peripheral blood lymphocytes that had been frozen were not viable when the cells were thawed.

Clinical data regarding the 17 patients included in this study are summarized in Table 2. The women ranged in age from 31 to 55 years, with a mean age of 40 years. The implants had been in place for an average of 3.7 years (range, 1.1 to 9 years). All patients presented with local complaints of either pain in the breast or chest wall or capsular contracture. In addition, all of the patients subjectively reported one or more constitutional complaints such as arthritis, myalgias, or chronic fatigue. None of the 17 patients in this study had well-documented specific rheumatologic diseases, and none had evidence of infection. Eleven patients had polyurethane-coated silicone gel breast implants, and the remaining six had textured-surface silicone gel-containing breast implants (Table 2). Sixteen of the 17 patients had bilateral breast implants.

The samples analyzed by flow cytometry contained an average of 4.5×10^6 cells (range, 0.5×10^6 to 15×10^6 cells). The volume of exudative fluid averaged around 1 to 3 ml. Stained cytospin preparations from both the exudative fluids and capsular washes revealed a predominance of lymphocytes (mean, 62%) and macrophages (mean, 37%), with only a few segmented neutrophils (mean, 2%).

Flow cytometry. The immunophenotypic data regarding both the implant-associated and peripheral blood lymphocytes are summarized in Tables 3 and 4, and representative contour plots are shown in Fig. 1 and 2. The vast majority of the implant-associated lymphocytes were T cells (mean, 89%; range, 81 to 97%). The mean percentage of $CD19^+$ B cells was only 1.4% (range, 0.0 to 4.6%). A small percentage (mean, 4.1%; range, 0.0 to 16%) of the implant-associated lymphocytes were $CD16/56^+$ $CD3^-$ natural killer cells. Compared with the paired peripheral blood lymphocytes, the implant-associated lymphocyte population had a significantly greater proportion of T cells ($P < 0.001$) and significantly fewer B cells ($P < 0.001$) and natural killer cells ($P < 0.001$). In order to compensate for this increased proportion of T cells, data regarding T-cell subsets were normalized on the basis of the total percentage of $CD3^+$ cells in order to provide a meaningful

TABLE 2. Clinical data for the patients used in this study

Age (yr)	Implant type[a]	No. of yr with implant	Reason for implant[b]
36	Meme (PU)	9	Rec
41	Replicon (PU)	6	Rec
35	Meme and Replicon (PU)	1.5	Rec, Aug
35	Meme (PU)	2.4	Aug
36	Meme (PU)	6	Aug
55	Optimam (PU)	8	Rec
41	Replicon (PU)	3	Aug
46	Replicon (PU)	2.3	Aug
34	Meme (PU)	7	Aug
31	MSI (TS)	1.3	Rec
45	Biocell (TS)	2.5	Aug
40	Biocell (TS)	2	Rec
47	Biocell (TS)	1.1	Aug
51	Optimam and Meme (PU)	2.3	Rec
37	Unknown (PU)	3.3	Aug
39	Misty (TS)	1.4	Rec
32	Mentor Siltex (TS)	2.5	Aug

[a] PU, polyurethane-coated silicone gel implant; TS, textured-surface Silastic implant.
[b] Rec, reconstruction; Aug, augmentation.

TABLE 3. Phenotype of implant-associated lymphocytes compared with that of paired peripheral blood lymphocytes

Phenotype	% of lymphocytes positive[a]	
	Implant	Peripheral blood
$CD3^+$ (T cells)	89 ± 4.9	71 ± 9.1
CD19 or $CD20^+$ (B cells)[b]	0.9 ± 1.3	12 ± 8.3
$CD16/56^+$ $CD3^-$ (natural killer cells)	3.6 ± 3.8	15 ± 7.2

[a] Values are means ± standard deviations ($n = 16$). For two-tailed probability, $P < 0.001$ (paired t test).
[b] The B-lymphocytes in five of the peripheral blood samples were quantitated with antibodies to CD20 instead of CD19.

TABLE 4. Implant-associated T-cell subsets compared with paired peripheral blood T-cell subsets

Phenotype	% of T cells positive[a]		2-Tailed probability[b]
	Implant	Peripheral blood	
$HLA\text{-}DR^+$	25 ± 8.1	7.9 ± 3.8	<0.001
$CD4^+$ $CD29^+$	68 ± 12	59 ± 13	0.043
$CD4^+$ $CD29^-$	0.6 ± 0.1	3.6 ± 3.5	0.007
$CD4^+$ $CD45RO^+$	2.6 ± 5.2	19 ± 7.2	<0.001
$CD4^+$ $CD45RO^-$	63 ± 13	41 ± 8.5	<0.001
$CD8^+$	37 ± 10	42 ± 9.6	0.115

[a] Values are means ± standard deviations ($n = 16$).
[b] Paired t test.

comparison of these cell types between the two compartments. Among the implant-associated T cells, there was increased expression of HLA-DR ($P < 0.001$). Twenty-five percent of the $CD3^+$ cells coexpressed HLA-DR. There was a predominance of $CD4^+$ T cells; for the implant-associated cells, the mean CD4/CD8 ratio was 1.8. Essentially all (greater than 99%) of the CD4 cells coexpressed CD29. Furthermore, almost all of the $CD4^+$ cells were negative for CD45RO. Compared with the paired peripheral blood lymphocytes, the increase in $CD4^+$ $CD29^+$ cells was marginally significant ($P = 0.043$) and the decrease in $CD4^+$ $CD45RO^+$ cells was statistically significant ($P < 0.001$).

The peripheral blood lymphocyte subsets in the patients with implants were, in most respects, similar to those in healthy controls (Table 5). These data are only preliminary, since the patient and control populations were not matched with regard to sex or age. Nevertheless, the data do suggest that there is a decrease in the number of $CD4^+$ $CD45RO^+$ cells in patients with implants ($P = 0.022$). Other differences in lymphocyte subsets did not appear to be statistically significant.

DISCUSSION

Despite concern regarding possible immunologic abnormalities associated with silicone gel breast implants, the phenotype of implant-associated lymphocytes has not previously been clearly defined. In preliminary studies, others have noted a predominance of T cells (29, 31). In this study, three-color flow cytometry was used to define the phenotypes of the lympho-

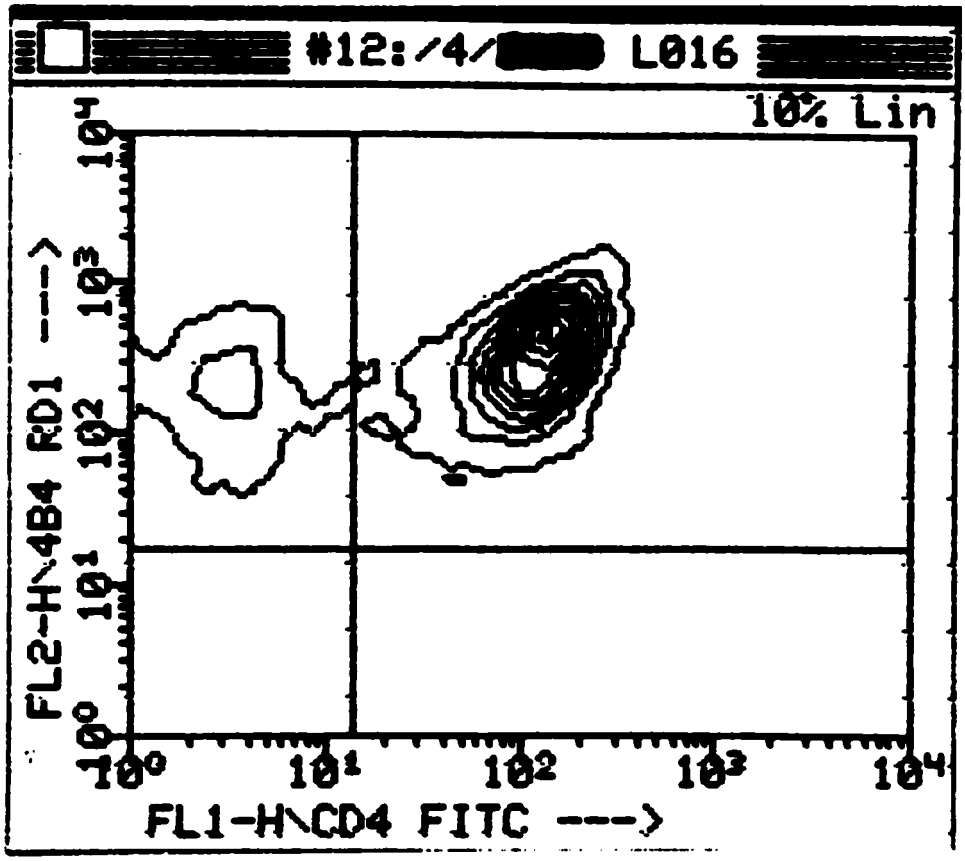

FIG. 1. Representative contour plot of implant-associated lymphocytes stained with antibodies to CD4 and CD29 (4B4). The vast majority of the cells coexpress CD4 and CD29 (upper right quadrant).

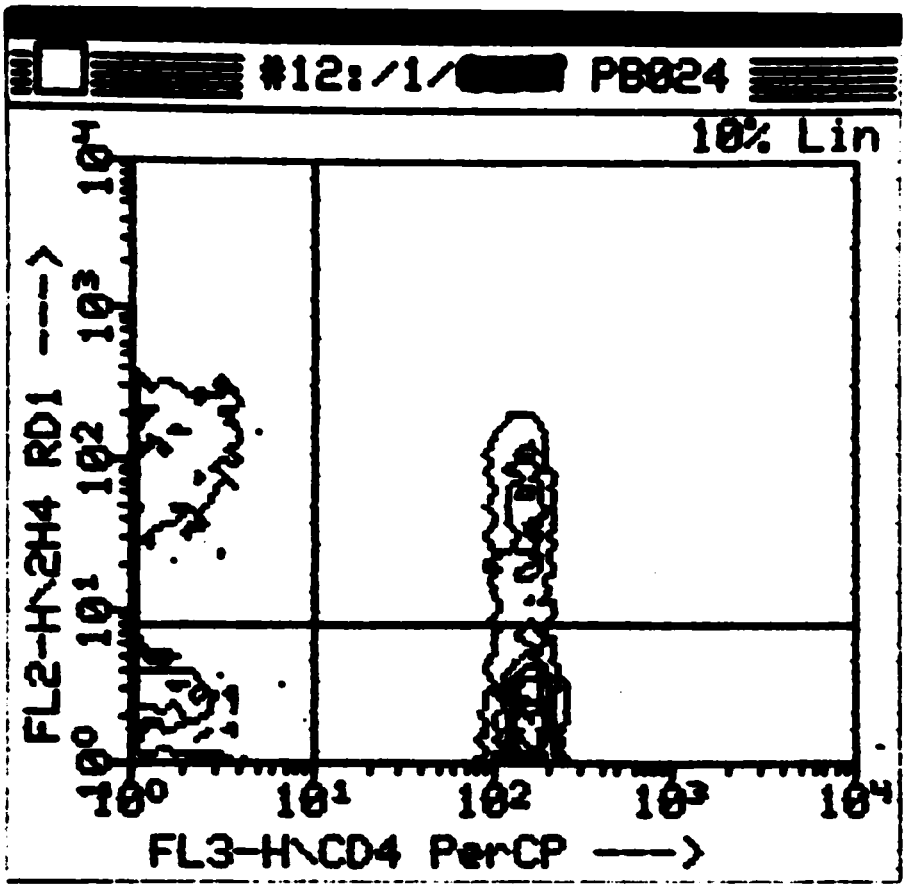

FIG. 2. Representative contour plot of peripheral blood lymphocytes stained with antibodies to CD4 and CD45RO (2H4). A significant fraction of the $CD4^+$ cells coexpress CD45RO (upper right quadrant). PerCP, PERCP.

cytes that are associated with polyurethane-coated and textured-surface silicone gel-containing breast implants. These types of implants were specifically chosen for this study because of the large number of lymphocytes that could be harvested from the capsule or from the peri-implant fluid. Whether or not the inclusion of only textured-surface and polyurethane-coated implants in our study introduced some bias cannot be determined from our available data. In all cases, there was a striking predominance of T cells. Most of the T cells had the phenotype $CD3^+$ $CD4^+$ $CD29^+$ $CD45RO^-$. Furthermore, among the T cells there was significant expression of HLA-DR. Comparison of the implant-associated lymphocytes with patient-matched peripheral blood lymphocytes, obtained at the time of surgery, showed that the increase in $CD3^+$ cells, the increased expression of HLA-DR by the T cells, and the decrease in expression of CD45RO by the $CD4^+$ cells were all statistically significant ($P < 0.001$ for each comparison). The increase in the percentage of $CD4^+$ $CD29^+$ T cells among the implant-associated lymphocytes compared with the level of the matched peripheral blood lymphocytes was of borderline statistical significance ($P = 0.043$).

TABLE 5. Patient peripheral blood lymphocytes compared with control peripheral lymphocytes

Phenotype	% of lymphocytes positive[a]		2-Tailed probability[b]
	Implant patients ($n = 16$)	Controls ($n = 12$)	
CD3	71 ± 9.1	75 ± 3.5	0.072
$CD3^+$ HLA-DR^+	5.5 ± 2.5	3.9 ± 2.3	0.097
$CD4^+$ $CD29^+$	41 ± 10	45 ± 9.2	0.301
$CD4^+$ $CD45RO^+$	13 ± 5.5	21 ± 9.8	0.022
$CD8^+$	30 ± 7.6	25 ± 7.1	0.081
$CD19^+$ (or $CD20^+$)[c]	12 ± 8.3	15 ± 4.1	0.186
$CD16/56^+$ $CD3^-$	15 ± 7.2	11 ± 5.0	0.069

[a] Values are means ± standard deviations.
[b] Unpaired *t* test.
[c] B lymphocytes in five of the patient peripheral blood samples were quantitated with antibodies to CD20 instead of CD19.

The role of textured-surface implants in eliciting an exudative host response is uncertain. It is known that textured-surface Silastic implants, as well as polyurethane-covered implants, are frequently associated with synovial metaplasia of the lining of the fibrous capsule (30). Review of histologic sections from the capsules from each of the patients in this study confirmed the presence of synovial metaplasia in all cases. In addition, in most capsules there was a marked lymphocytic infiltrate together with foamy macrophages.

The significance of the predominance of $CD29^+$ $CD4^+$ T cells among the implant-associated lymphocytes is uncertain. $CD4^+$ T cells can generally be divided into two major functional categories. $CD4^+$ $CD29^+$ T cells proliferate maximally to soluble antigen and increase antigen-specific antibody production (22). In contrast, $CD4^+$ $CD45RO^+$ T cells induce CD8

cells to exert suppressor function (23). It is of interest that $CD4^+$ $CD29^+$ T cells are also the predominant lymphocyte subset in synovial fluid of patients with rheumatoid arthritis (24). Furthermore, as we found for implant-associated lymphocytes, synovial tissue lymphocytes in patients with rheumatoid arthritis have increased expression of HLA-DR antigens (8). The apparent depletion of $CD4^+$ $CD45RO^+$ T cells from the peripheral blood of patients with silicone gel-containing breast implants will require confirmation by more extensive controlled studies.

To date, there is very little information that sheds light on the manner whereby silicone gel-containing breast implants could potentially cause connective tissue diseases. Some authors have suggested that silicone or silicone-protein complexes may themselves be antigenic (13, 18, 19, 44). Others have drawn attention to the fact that a significant percentage of women with silicone breast implants have antinuclear antibodies (5, 28). The results from animal studies have been conflicting (4, 25). Clinical studies of patients with silicone gel breast implants suffer from the fact that patients are often referred because of symptoms of rheumatic disease. Large population-based epidemiologic studies are limited by the fact that "diseases" potentially caused by silicone gel-containing implants have not been clearly defined. Most of the patients in this study reported a symptom complex similar to fibromyalgia. A preliminary study of 144 patients with breast implants also reported clinical manifestations characteristic of fibromyalgia (45). Certainly there is no reproducibly specific marker for patients with silicone gel-associated rheumatic disease. In one study, which has yet to be substantiated, Vasey and colleagues reported clinical observations suggesting that rheumatic disease symptoms in patients with silicone gel implants may be reversible after implant removal (43). This finding should at least provide the impetus for continued studies of the immunologic effects of silicone.

There are several inherent limitations in our study regarding its ability to address the questionable relationship between silicone breast implants and immunologic diseases. First, our patient population includes only those patients who are currently having problems with their implants. Because it is difficult to form a control group of patients with no local or systemic problems and who wish to undergo implant removal and capsulectomy, this study does not resolve the question of whether T-cell activation around polyurethane or textured-surface breast implants is in fact a pathologic phenomenon. Although polyurethane-covered implants have been known to elicit greater inflammatory responses in the capsule than smooth-shell implants, it is not clear from previous studies what type of inflammation they elicit (16). Nor is it clear from previous studies how long the inflammatory responses persist. Our study has shown that T-cell activation around these implants occurs as early as 1 year after implantation and can persist for as long as 9 years.

The other unresolved question which this study could not address is the significance of local T-cell activation in eliciting local pain and systemic illness. Although all patients studied had significant pain around their implants and all of the patients had some concomitant constitutional symptoms, without a large symptom-free control group, the effect of local T-cell activation on the systemic immune system remains elusive.

In summary, this study is the first to define the phenotype of the lymphocytes isolated from the fluid or tissue surrounding silicone gel-containing breast implants. The lymphocytes are almost all $CD3^+$ T cells, most of which express CD4 and CD29. Compared with peripheral blood lymphocytes, the T cells have significantly increased expression of HLA-DR and significantly reduced expression of CD45RO. Histologic examination of capsular tissue, as well as examination of the exudative fluid, has shown that most of the implant-associated lymphocytes are present in association with foamy macrophages that are known to ingest droplets of silicone gel. We have found that these macrophages strongly and uniformly express HLA-DR (17). Our results are consistent with the hypothesis that the silicone-containing macrophages act as antigen-presenting cells to CD4 cells which become activated and subsequently function to up-regulate an immune response, as indicated by expression of CD29. Admittedly, this simplistic hypothesis requires extensive further testing and refinement. In particular, it will be important to determine whether the implant-associated $CD4^+$ T cells belong to the Th1 or Th2 subset. A recent study by Ojo-Amaize et al. (26) provides additional evidence in support of the central role of T cells in the immunologic reaction to silicone breast implants. In any case, if science is to prevail in the silicone controversy, as Fisher rightly insists (10), then further analysis of the inflammatory reaction to silicone breast implants will be necessary.

ACKNOWLEDGMENTS

This research was supported in part by a grant from the Haas Fund, The Mount Sinai Medical Center, Cleveland, Ohio.

We express our gratitude to David Hom of the Department of Epidemiology and Biostatistics at Case Western Reserve University School of Medicine, Cleveland, Ohio, for assistance with the statistical analyses.

REFERENCES

1. **Baker, J. L., R. R. LeVier, and D. E. Spielvogel.** 1982. Positive identification of silicone in human mammary capsular tissue. Plast. Reconstr. Surg. **69:**56–60.
2. **Baldwin, C. M., and E. N. Kaplan.** 1983. Silicone-induced human adjuvant disease? Ann. Plast. Surg. **10:**270–273.
3. **Brandt, B., V. Breiting, L. Christensen, M. Nielsen, and J. L. Thomsen.** 1984. Five years experience of breast augmentation using silicone gel prostheses with emphasis on capsule shrinkage. Scand. J. Plast. Reconstr. Surg. **18:**311–316.
4. **Brantley, S. K., S. F. Davidson, P. A. St. Arnold, M. B. Johnson, P. J. Talbot, J. B. Grogan, M. A. Cuchens, H. S. Hsu, and S. K. Das.** 1990. Assessment of the lymphocyte response to silicone. Plast. Reconstr. Surg. **86:**1131–1137.
5. **Bridges, A. J., C. Conley, G. Wang, D. E. Burns, and F. B. Vasey.** 1993. A clinical and immunologic evaluation of women with silicone breast implants and symptoms of rheumatic disease. Ann. Intern. Med. **118:**929–936.
6. **Brozena, S. J., N. A. Fenske, C. W. Cruse, C. G. Espinoza, F. B. Vasey, B. F. Germain, and L. R. Espinoza.** 1988. Human adjuvant disease following augmentation mammoplasty. Arch. Dermatol. **124:**1383–1386.
7. **Cronin, T. D., and F. J. Gerow.** 1964. Augmentation mammoplasty: a new "natural feel" prosthesis, p. 41. *In* Transactions of the Third International Congress of Plastic Surgery. Excerpta Medica, Amsterdam.
8. **Cush, J. J., and P. E. Lipsky.** 1988. Phenotypic analysis of synovial tissue and peripheral blood lymphocytes isolated from patients with rheumatoid arthritis. Arthritis Rheum. **31:**1230–1238.
9. **Endo, L. P., N. L. Edwards, S. Longley, L. C. Corman, and R. S. Panush.** 1987. Silicone and rheumatic diseases. Semin. Arthritis Rheum. **17:**112–118.
10. **Fisher, J. C.** 1992. The silicone controversy: when will science prevail? N. Engl. J. Med. **326:**1696–1698.
11. **Gabriel, S. E., W. M. O'Fallon, L. T. Kurland, C. M. Beard, J. E. Woods, and L. J. Melton.** 1994. Risk of connective-tissue diseases and other disorders after breast implantation. N. Engl. J. Med. **330:**1697–1702.
12. **Gayou, R. M.** 1979. A histological comparison of contracted and non-contracted capsules around silicone breast implants. Plast. Reconstr. Surg. **63:**700–707.
13. **Goldblum, R. M., R. P. Pelley, A. A. O'Donell, D. Pyron, and J. P. Heggers.** 1992. Antibodies to silicone elastomers and reactions to ventriculoperitoneal shunts. Lancet **340:**510–513.
14. **Hardt, N. S., L. T. Yu, G. La Torre, and B. Steinbach.** 1994. Fourier transform infared microspectroscopy used to identify foreign materials related to breast implants. Mod. Pathol. **7:**669–676.
15. **Hester, T. R., F. Nahai, J. Bostwick, and J. Cukic.** 1988. A 5-year experience with polyurethane-covered mammary prostheses for treatment of capsular contracture, primary augmentation, mammoplasty, and breast reconstruction. Clin. Plast. Surg. **15:**569–585.
16. **Jabaley, M. E., and S. K. Das.** 1986. Late breast pain following reconstruc-

tion with polyurethane-covered implants. Plast. Reconstr. Surg. **78:**390–395.
17. **Katzin, W. E., and L. J. Feng.** 1994. Phenotype of lymphocytes associated with the inflammatory reaction to silicone-gel breast implants. Mod. Pathol. **7:**17A. (Abstract.)
18. **Kossovsky, N., and C. J. Freiman.** 1994. Silicone breast implant pathology: clinical data and immunologic consequences. Arch. Pathol. Lab. Med. **118:** 686–693.
19. **Kossovsky, N., J. P. Heggers, and M. C. Robson.** 1987. Experimental demonstration of the immunogenicity of silicone-protein complexes. J. Biomed. Mater. Res. **21:**1125–1133.
20. **Kumagai, Y., A. Chiyuki, and Y. Shiokawa.** 1979. Scleroderma after cosmetic surgery: four cases of human adjuvant disease. Arthritis Rheum. **22:**532–537.
21. **Marik, P. E., A. L. Kark, and A. Zambakides.** 1990. Scleroderma after silicone augmentation mammoplasty: a report of 2 cases. S. Afr. Med. J. **77:**212–213.
22. **Morimoto, C., N. L. Letvin, A. W. Boyd, M. Hagan, H. M. Brown, M. M. Kornacki, and S. F. Schlossman.** 1985. The isolation and characterization of the human helper inducer T cell subset. J. Immunol. **134:**3762–3769.
23. **Morimoto, C., N. L. Letvin, J. A. Distaso, H. M. Brown, and S. F. Schlossman.** 1986. The cellular basis for the induction of antigen-specific T8 suppressor cells. Eur. J. Immunol. **16:**198–204.
24. **Morimoto, C., P. L. Romain, D. A. Fox, P. Anderson, M. DiMaggio, H. Levine, and S. F. Schlossman.** 1988. Abnormalities in CD4+ T-lymphocyte subsets in inflammatory rheumatic diseases. Am. J. Med. **84:**817–825.
25. **Naim, J. O., R. J. Lanzafae, and C. J. van Oss.** 1993. The adjuvant effect of silicone-gel on antibody formation in rats. Immunol. Invest. **22:**151–161.
26. **Ojo-Amaize, E. A., V. Conte, H.-C. Lin, R. F. Brucker, M. S. Agopian, and J. B. Peter.** 1994. Silicone-specific blood lymphocyte response in women with silicone breast implants. Clin. Diagn. Lab. Immunol. **1:**689–695.
27. **Picha, G. J., and J. A. Goldstein.** 1990. Analysis of the soft-tissue response to components used in the manufacture of breast implants: rat animal model. Plast. Reconstr. Surg. **87:**490–500.
28. **Press, R. I., C. L. Peebles, Y. Kumagai, R. L. Ochs, and E. M. Tan.** 1992. Antinuclear autoantibodies in women with silicone breast implants. Lancet **340:**1304–1307.
29. **Raso, D. S.** 1994. Breast prostheses, the immune response, and B- and T-lymphocytes. Plast. Reconstr. Surg. **93:**649–650.
30. **Raso, D. S., L. W. Crymes, and J. S. Metcalf.** 1994. Histological assessment of fifty breast capsules from smooth and textured augmentation and reconstruction mammoplasty prostheses with emphasis on the role of synovial metaplasia. Mod. Pathol. **7:**310–316.
31. **Rowlands, C., and F. H. Y. Green.** 1994. Tissue responses to silicone breast implants. Mod. Path. **7:**21A. (Abstract.)
32. **Rudolph, R., J. Abraham, T. Vecchione, S. Guber, and M. Woodward.** 1978. Myofibroblasts and free silicon around breast implants. Plast. Reconstr. Surg. **62:**185–196.
33. **Sahn, E. E., P. D. Garen, R. M. Silver, and J. C. Maize.** 1990. Scleroderma following augmentation mammoplasty. Report of a case and review of the literature. Arch. Dermatol. **126:**1198–1202.
34. **Sanchez-Guerrero, J., G. A. Colditz, E. W. Karlson, D. J. Hunter, F. E. Speizer, and M. H. Liang.** 1995. Silicone breast implants and the risk of connective-tissue disease and symptoms. N. Engl. J. Med. **332:**1666–1670.
35. **Sanchez-Guerrero, J., P. H. Schur, J. S. Sergent, and M. H. Liang.** 1994. Silicone breast implants and rheumatic disease: clinical, immunologic, and epidemiologic studies. Arthritis Rheum. **37:**158–168.
36. **Silver, R. M., E. E. Sahn, J. A. Allen, S. Sahn, W. Green, J. C. Maize, and P. D. Garen.** 1993. Demonstration of silicon in sites of connective-tissue disease in patients with silicone-gel breast implants. Arch. Dermatol. **129:** 63–68.
37. **Spiera, H.** 1988. Scleroderma after silicone augmentation mammoplasty. JAMA **260:**236–238.
38. **Thomsen, J. L., L. Christensen, M. Nielsen, B. Brandt, V. B. Breiting, S. Felby, and E. Nielsen.** 1990. Histologic changes and silicone concentration in human breast tissue surrounding silicone breast prostheses. Plast. Reconstr. Surg. **85:**38–41.
39. **Uretsky, B. F., J. J. O'Brien, E. H. Courtiss, and M. D. Beeker.** 1979. Augmentation mammaplasty associated with a severe systemic illness. Ann. Plast. Surg. **3:**445–447.
40. **Van Nunen, S. A., P. A. Gatenby, and A. Basten.** 1982. Post-mammoplasty connective tissue disease. Arthritis Rheum. **25:**694–697.
41. **Varga, J., and S. A. Jimenez.** 1990. Augmentation mammoplasty and scleroderma: is there an association? Arch. Dermatol. **126:**1220–1222.
42. **Varga, J., R. Schumacher, and S. A. Jimenez.** 1989. Systemic sclerosis after augmentation mammoplasty with silicone implants. Ann. Intern. Med. **111:** 377–383.
43. **Vasey, F. B., D. Havice, T. Bocanegra, M. J. Seleznick, P. Bridgeford, and B. F. Germain.** 1992. Clinical manifestations of fifty consecutive women with silicone breast implants. Arthritis Rheum. **35**(Suppl.)**:**S212. (Abstract.)
44. **Vojdani, A., N. Brautbar, and A. Campbell.** 1994. Immunologic and biologic markers for silicone. Toxicol. Ind. Health **10:**25–42.
45. **Ward, C., S. Romero, S. Marlowe, F. B. Vasey, and T. S. Bocanegra.** 1994. Clinical manifestations in 144 patients with breast implants (BI). A comparison with primary fibromyalgia (PFM). Arthritis Rheum. **36**(Suppl.)**:**R28. (Abstract.)
46. **Weiner, S. R., and H. E. Paulus.** 1986. Chronic arthropathy occurring after augmentation mammaplasty. Plast. Reconstr. Surg. **77:**185–187.
47. **Weisman, M. H., T. R. Vecchione, D. Albert, L. T. Moore, and M. R. Mueller.** 1988. Connective-tissue disease following breast augmentation: a preliminary test of the human adjuvant disease hypothesis. Plast. Reconstr. Surg. **82:**626–630.