Common Commercial Cosmetic Products Induce Arthritis in the DA Rat

B. Sverdrup,[1] L. Klareskog,[1] and S. Kleinau[1,2]

[1]Department of Medicine, Rheumatology Unit, Karolinska Institute, Stockholm, Sweden; [2]Department of Pathology, University Hospital, Uppsala, Sweden

Many different agents, including mineral oil and silicone, have the capacity to act as immunological adjuvants, i.e., they can contribute to the activation of the immune system. Some adjuvants, including mineral oil, are known to induce arthritis in certain strains of rats after intradermal injection or percutaneous application. The aim of this study was to determine if common commercial cosmetic products containing mineral oil could induce arthritis in the highly susceptible DA (Dark Agouti) rat. Intradermal injection of five out of eight assayed cosmetic products without further additives resulted in arthritis with synovitis. One of the products induced a very aggressive arthritis, which had declined after 5–9 weeks. When this product was also assayed for arthritogenicity upon percutaneous administration, it induced a mild and transient arthritis in 5 out of 10 DA rats, whereas control animals showed no clinical signs of joint involvement. No arthritic reaction was seen in rats after peroral feeding with the most arthritogenic product or by intravaginal application of Freund's adjuvants. Silicone gel implants in DA rats did not cause arthritis. We conclude that mineral oils included in common commercially available products retain their adjuvant properties and are arthritogenic in the presently investigated arthritis-prone rat strain. There is yet no evidence that mineral oils present in cosmetics may contribute to arthritis in humans, but we suggest that this question should be subject to further investigation. *Key words*: adjuvants, arthritis, cosmetic products, mineral oil, rats, silicone gel.
Environ Health Perspect 106:27–32 (1998). [Online 2 January 1998]
http://ehpnet1.niehs.nih.gov/docs/1998/106p27-32sverdrup/abstract.html

Adjuvants that contain microbial-derived molecules have long been known to have a capacity to induce arthritis not only in experimental animals, mainly rats (*1*), but most probably also in humans in conjunction with administration of adjuvants used in cancer therapy (*2,3*). An interesting extension of these older findings was made relatively recently when it was demonstrated that nonimmunogenic adjuvants, such as mineral oil with no content of microbial products, could also, by themselves, induce arthritis in certain arthritis-susceptible strains of rats after a single intradermal or subcutaneous injection at the root of the tail (*4*).

These findings raised the question of whether substances with adjuvant properties might also contribute to arthritis development when administered in ways that are common in humans, and it was indeed demonstrated that mineral oils, without any additives, could also induce arthritis in Dark Agouti (DA) rats after percutaneous application (*5*).

We have as yet no empirical evidence that mineral oils or other nonimmunogenic adjuvants have any proarthritogenic activity in humans. However, connective tissue disease has been suggested to occur following cosmetic mammary augmentation with silicone or injections of paraffin oil (human adjuvant disease) (*6*), although later epidemiological investigations have failed to support this (*7*). Therefore, we considered it of interest to investigate if pure mineral oils and also some of the many mineral oil-containing commercial products intended for skin care could induce arthritis in the rat experimental system.

In this study we have selected a few commercially available mineral-oil containing skin products and vaginal gel, which are in common use in Sweden, and investigated to what extent these products, as well as two pure mineral oils (constituents of cosmetics and ingredients in food), could induce arthritis in DA rats after intradermal, percutaneous, or peroral administration. To investigate possible arthritogenicity by intravaginal application, we used Freund's adjuvants. The development of arthritis was studied clinically as well as histologically, and the production of autoantibodies was determined. Likewise, we wanted to investigate the arthritogenic potential of a silicone gel used in human mammary implants because silica or related substances such as silicone have been reported to have adjuvant properties (*8*).

Materials and Methods

Animals. DA and Lewis rats of both sexes were used at 2–5 months of age. The animals were maintained in the animal unit at Karolinska Hospital (Stockholm, Sweden). They were obtained from inbred colonies from the Central Institute for Laboratory Animal Breeding (Hannover, Germany) and from Harlan (Zeist, Netherlands). Routine screening for pathogens gave negative results for Sendai virus, coronavirus, pneumonia virus, and Mycoplasma pulmonis. The rats had free access to water and rodent chow (R36; B&K Unviversal Ltd, Hull, England).

Treatments. Freund's complete adjuvant (FCA), composed of mineral oil, mycobacteria, and an emulsifying agent (Arlacel A), and Freund's incomplete adjuvant (FIA), composed of mineral oil and Arlacel A, were obtained from Difco Laboratories (Detroit, MI). Two different mineral oils classified as medicinal white oil for food, pharmaceutical, and cosmetic use, Medicway M 68 (Statoil, Stavanger, Norway) and Kaydol (Witco Corporation, Greenwich, CT), were also studied.

Seven different commercial skin products containing possible immunological adjuvants (especially mineral oils) were bought in cosmetic shops and pharmacies: three skin creams (ACO, Helosan, and Margaret Astor), two body lotions (Nivea and LdB), and two baby oils (Natusan and Barnängen). The exact composition of the products is not publicly available. However, the fat content (with different shares mineral oil and vegetable oils) is in general about 30–40% for skin cream (ointment) and about 20% for skin lotion. For baby oil, the general mineral oil content is 80%. Compositions of the different products were given on the packages:

- ACO Skin Cream (ACO Läkemedel, Stockholm, Sweden): butylhydroxitoluen, ethanol, glycerol 8%, peanut oil and paraffin, metagin (E218), propagin (E216), monostearin, stearic acid, stearol, trietanolamin, fragrance, water).
- Helosan Ointment (EWOS, Helsingborg, Sweden): paraffin liq, cetanolum, stearolum, POE-stearat glycerylstearat, glycerolum, propagin (E216), polyhexamethylenbiguanid HCL, eucalyptolum, aqua purificata.
- Margaret Astor, Collagen Liposome- Skin Care/Collagen Creme Active (Margaret Astor AG, Mainz, Germany): concentrate with additive of elastine, lecithin, and avocado oil.
- Nivea Milk Emulsion (Beiersdorf AG, Hamburg, Germany): water demin., paraffin oil, isohexadecane, isopropyl palmitate, vaseline, PEG-40 sorbitan

Address correspondence to B. Sverdrup, Department of Medicine, Rheumatology Unit, Karolinska Institute, 171 76 Stockholm, Sweden.
This study was supported by grants from the Swedish Workhealthfund. We thank R.A. Harris for critical reading of the manuscript.
Received 18 November 1996; accepted 17 July 1997.

peroleate, polyglyceryl-3 diisostearate, glycerine, magnesium sulfate, aluminium stearates, cetyl palmitate, glyceryl lanolate (neoceril), lanolin alcohol, phenoxyethanol, methyldibromo glutaronitrile, fragrance.

- LdB Creme Lotion Rich (Elida Farbegé. Malmo, Sweden): water, mineral oil, propylene glycol, glycerinmonostearate, glycerindistearate, alkalistearate, petrolatum, potassium cetyl phosphate, cetearylalcohol, fragrance, carbomer 941, methylparaben, lecithin liposomes with tissue respiratory factor, tocopherol, proline, evening primrose oil, sodium hydroxide, propylparaben, carbomer 940, C.I.21230.
- Natusan Baby Oil (Johnson & Johnson AB, Sollentuna, Sweden): mineral oil, cetearyl octanoate, trilaureth-4 phosphate.
- Barnängen Baby Oil (Barnängen AB, Ekerö, Sweden): paraffin oil, cetyl alcohol, thyme oil.

One vaginal gel rich in mineral oil content was also analyzed. Replens Hormone Free Vaginal Gel (Maropack AG, Zell Switzerland; water, glycerine, mineral oil, polycarbofil, carbopol 934P, and sorbinic acid) was bought in a local pharmacy.

Silicone gel and a silicone elastomer envelope were taken from an unused sterile silicone mammary implant (McGhan Medical Corporation, Santa Barbara, CA).

Routes of Administration

Intradermal injection. One single intradermal (ID) injection of 400 or 500 µl of the different skin products, vaginal gel, or mineral oils was given at the base of the tail. ACO and Helosan skin creams were diluted 1:1 with phosphate-buffered saline (PBS) before injection. As a positive control of arthritis development, one group of rats received 200 µl FIA ID. One group of rats, the negative controls, received no injection. The animals were thereafter examined for clinical manifestations for 1–3 months (Table 1).

Percutaneous application. In the shoulder region of DA rats, a 2-cm^2 area was shaved and lightly abrased with sandpaper during ether anesthesia. Abrasion was performed to accumulate inflammatory cells in the skin and thereby facilitate uptake of the oil. The abrasion was repeated twice more during the treatment period at 3–4 day intervals. Natusan Baby Oil was applied directly onto the skin in a volume of 400 µl, and this treatment was repeated 10 times during 12 days. Control animals were shaved and abrased only. The animals were thereafter examined for clinical manifestations for at least 1 month (Table 2).

Intravaginal application. Under ether anesthesia, each female DA rat received one intravaginal application of 500 µl FCA followed by five applications of 500 µl FIA over 12 days. The animals were thereafter examined for clinical manifestations for 3 months (Table 2).

Per oral feeding. Kaydol mineral oil (500 µl) was given to DA rats by sound feeding under ether anaesthesia daily for 5 days. The animals were thereafter examined for clinical manifestations for 7 months (Table 2).

Subcutaneous implant. A silicone breast implant was cut into 1.6–3.5 g pieces and implanted subcutaneously into a skin pocket in the back of each of 10 DA rats under Brietal anesthesia. In addition, one rat received a piece of the silicone envelope implanted in the same manner. The weight of the implant was adjusted to the rats body weight (6.8–16.4 g/kg body weight, i.e., 0.68–1.64%) to resemble the weight of a silicone breast implant in women (~1.40%). Two control rats were sham operated.

Examination and evaluation of joint inflammation. Animals were examined daily during the second week for clinical manifestations and onset of arthritis and thereafter two to three times every week during the first month. Severity of arthritic joints was scored using a scale of 1–4 for each paw (maximum possible score of 16 per rat) (*9*). In the first series, evaluation of some cosmetic products, positive and negative controls were observed blind (Table 1). The rats were coded and the results were read blind by two independent observers. In the following series, some of the observations were read blind by one independent observer. All rats were followed for at least 1 month after initial treatment.

Skin irritation test. Six DA rats were shaved in the shoulder region under Brietal anesthesia, and 400 µl Natusan Baby oil was administered onto the skin, covered with a cotton compress, and fixed with a bandage for 24 hr. Two control rats were shaved and only covered with a bandage. Macroscopic inspection of the dermis was performed after 24 hr, and skin biopsies

Table 1. Incidence of arthritis in rats after a single intradermal injection of different skin care products, vaginal gel, or mineral oils

Rat strain	Sex	Product	Dose (µl)	Arthritis incidence	Mean day of onset	Maximum arthritic score	Follow-up days
DA		Skin cream					
	M	ACO	400	0/7[a]	–	–	38
	F	Helosan	500	0/10	–	–	84
	2M,8F	Margaret Astor Body lotion	500	2/10	19	8	84
	M	Nivea	500	3/10	21	6	83
	6M,4F	LdB Baby oil	500	2/10	22	12	83
	M	Natusan	400	1/7[a]	15	12	38
	M	Natusan	500	4/10	16	12	29
	M	Barnängen	500	4/10	24	8	83
	3M,4F	Vaginal gel Replens	400	0/7[a]	–	–	38
	F	Mineral oil Medicway	500	6/10	21	12	90
	F	Kaydol	500	9/10	15	13	90
	M	FIA	200	4/4[a]	13	16	38
	M	None	–	0/6[a]	–	–	38
Lewis	F	Kaydol	500	0/10	–	–	50

Abbreviations: DA, Dark Agouti; M, male, F, female.
[a]Observations made blindly.

Table 2. Arthritic incidence in DA rats following different routes of administration of mineral oils or implant of silicone

Sex	Administration	Product	Dose	Arthritis incidence	Mean day of onset	Maximum arthritic score	Follow-up months
M	PC	Natusan	400 µl × 10	5/10	13	4	1
M		Control	–	0/10	–	–	1
2M,8F	PO	Kaydol	500 µl × 5	0/10	–	–	7
F	Invag	FCA/FIA	500 µl × 6	0/10	–	–	3
M	Implant	Silicone gel	–	0/10	–	–	6
		Silicone envelope	–	0/1	–	–	6
		Sham operation	–	0/2	–	–	6

Abbreviations: M, male, F, female; PC, percutaneous; PO, per oral; Invag, intravaginal.

from the treated area were taken and snap frozen. After cryostat sectioning and staining with hematoxylin and eosin (H&E), microscopic evaluation of skin biopsies was performed.

Blood analysis. Retroorbital bleeding was performed under ether anesthesia at different time points after different treatments. Serum and plasma samples were stored at -20°C until assay.

Fibrinogen levels in plasma were determined in rats treated with silicone, measured by the Thrombin method (*10*) with the Stago reagents kit (Diagnostica Stago, Asniéres, France), and read in an autolyzer.

Examination for autoantibodies was performed without knowledge of the treatment given.

Indirect immunofluorescence (IIF) was used to screen for organ-specific autoantibodies (*11*). Briefly, sera diluted 1:10 in PBS from treated and control animals were incubated on cryostat sections of rat kidney and stomach. After rinsing in PBS, sections were incubated with fluorescein isothiocyanate (FITC)-conjugated goat-antirat (GAR) IgG antibodies (Jackson Laboratories, West Grove, PA) at a dilution of 1:40.

Analysis of antinuclear antibodies (ANAs) by human epithelial cell (HEp-2, Immunoconcepts, Sacramento, CA) IIF was also performed. Sera diluted 1:40 in PBS were incubated on HEp-2 cells followed by incubation with FITC–GAR IgG. The mounted slides were viewed with a fluorescence microscope.

To detect rheumatoid factor (RF), sera were assayed for IgM antibodies to rat IgG in an enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plates were coated overnight at 4°C with 50 µl/well of 10 µg/ml rat IgG in PBS. Dilutions of sera and washings were made in PBS containing 0.05% Tween-20. All tests were carried out in quadruplicate. Levels of IgM antibodies in a sample were quantified with goat-antirat IgG antibodies conjugated to alkaline phosphatase (Jackson Laboratories). The subsequent quantification of bound enzyme was performed with a *para*-nitrophenol-containing substrate in a spectrophotometer. Samples having an absorbance greater than the mean + 2 standard deviations (SD) of normal rats, read at 405 nm, were defined as positive.

Sera were assayed for antibodies to type II collagen (CII) in an ELISA (*9*). Briefly, microtiter plates were coated overnight at 4°C with 50 µl/well of 10 µg/ml rat CII in PBS. Dilutions of sera and washings were made in PBS containing 0.05% Tween-20. All tests were carried out in duplicate. Levels of anti-CII antibodies in a sample were quantified with GAR IgG antibodies conjugated to alkaline phosphatase (Jackson Laboratories). The subsequent quantification of bound enzyme was performed with a *para*-nitrophenol-containing substrate buffer in a spectrophotometer. Concentrations of anti-CII antibodies were determined by comparison with an affinity-purified rat anti-CII antibody of known activity. Positive samples were defined as having an absorbance greater than the mean + 2 SD of normal DA rats.

Histology. Ankle joints of Natusan-treated animals (ID) were fixed in 4% phosphate-buffered formaldehyde and decalcified with 14% EDTA in 0.36 M NaOH for 4 weeks. After dehydration, clearing, and impregnation, the tissues were embedded in paraffin blocks, sectioned, and stained with H&E.

Lymph nodes and spleens from the same animals were fixed in formaldehyde, embedded in paraffin, sectioned, and stained with H&E.

Results

Effects of intradermal injection. Five out of seven assayed skin products induced polyarthritis in the DA rat (Table 1). The incidence varied from 10 to 40%, and rats of both sexes were affected. Mean day of arthritis onset was between 13 and 24 days after injection. The arthritis often started symmetrically in the hind paws and later also affected the front paws. The severity of arthritis reached its maximum 2–3 weeks after injection and became less severe during the following weeks, without any detectable signs of ankylosis or functional disorders. Duration of the arthritis was from 5 to almost 9 weeks.

Intradermal injection with skin creams with high viscosity or with the vaginal gel (Replens) did not induce any arthritis. However, development of ulcers was observed at the injection site of animals receiving Replens.

A high incidence of arthritis was recorded in DA rats injected with the medicinal mineral oils Medicway and Kaydol (Table 1). Six out of 10 DA rats developed arthritis after injection with Medicway. Kaydol induced very aggressive arthritis in 90% of the DA rats. Lewis rats injected with Kaydol in the same manner did not develop any arthritis. Animals treated with Natusan Baby Oil or Kaydol mineral oil exhibited similar clinical pictures of arthritis as the positive control group receiving FIA (Fig. 1).

Effects of percutaneous application. One of the products, Natusan Baby Oil, which was arthritogenic by intradermal injection, was also tested for its capacity to induce arthritis after repeated percutaneous (PC) application on abrased skin. By this route, the DA rats developed joint inflammations that were, in general, mild and transient and predominantly affected single joints in the forepaws. The mean day of onset of arthritis was 13 days after start of treatment, and the maximum arthritic score was 4. The duration of the arthritis was 4–6 days (Fig. 2). Control rats did not develop any signs of arthritis. Skin irritation tests with Natusan Baby Oil gave no macroscopic changes of the skin, and microscopic evaluation performed on biopsies from Natusan-treated skin did not show any signs of inflammation (data not shown).

Effects of oral sound feeding. One of the medicinal mineral oils, Kaydol, which is permitted as a food additive and which exhibited arthritogenicity in DA rats when injected intradermally, was also investigated for its potential arthritogenicity after oral sound feeding to DA rats. However, no arthritic reaction was detected in these animals during an observation period of 7 months (Table 2).

Effects of intravaginal application. Arthritogenicity was assesed by adminstration

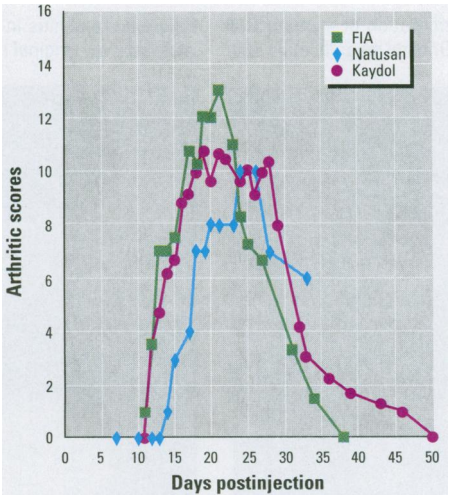

Figure 1. Comparison of mean arthritic scores in groups of arthritic DA rats injected intradermally with Freund's incomplete adjuvant (FIA; 200 µl; *n* = 4), Natusan Baby Oil (500 µl; *n* = 4), and Kaydol mineral oil (500 µl; *n* = 9).

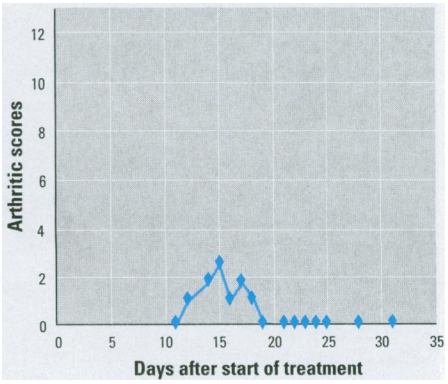

Figure 2. Mean arthritic scores in arthritic rats after repeated percutaneous treatment with Natusan Baby Oil.

via the intravaginal route. DA rats were first treated with one intravaginal application of FCA followed by FIA five times over 12 days. No arthritis was recorded during a follow-up of 3 months.

Effects of silicone implants. Silicone implants or the silicone envelope did not cause any arthritis in DA rats. The animals were observed for a 6-month period (Table 2). Elevated fibrinogen levels were observed in silicone treated animals on days 6 and 17 after implantation of silicone; however, these levels had decreased to those of sham operated animals a month later.

Histopathology. Histology of arthritic joints at days 21, 25, and 33 postinjection from animals injected ID with Natusan Baby Oil verified arthritic reactions with infiltration of mononuclear cells, synovitis, and marginal erosions (Fig. 3). Tendinitis and tendovaginitis were also evident (Fig. 4). Histology of lymph nodes (Fig. 5) from the same animal showed an increased number of cells, diffuse germinal centres, and large round vesicles, probably disseminated oil droplets in the marginal zone (Fig. 6). The spleen was cell rich and vesicles were not evident.

Serological analysis. ANAs assayed by IIF of tissue sections were negative in DA rats treated with Natusan or Barnängen baby oils, Margaret Astor cream, Kaydol, or FCA/FIA by different treatment regimes (Table 3).

However, ANAs analyzed by HEp-2 cells showed positive Golgi or centromere antibodies in 3/10 DA rats administered Kaydol orally. In 4/11 silicone-implanted animals, positive Golgi antibodies could be detected. One sham operated rat also had Golgi antibodies.

RF was positive in 5/10 DA rats orally treated with Kaydol, but was also positive in some animals treated intradermally with Barnängen Baby Oil or Margaret Astor skin cream. In one control rat, positive RF could be detected.

CII antibodies were detected in 4/10 animals previously injected with Margaret Astor skin cream containing collagen, oils, and liposomes. One of the CII-positive animals had arthritis.

Discussion

The aim of this study was to investigate if common commercially available cosmetic products that contain mineral oils or medicinal mineral oils can induce arthritis in the particularly arthritis-susceptible DA rat strain. The results clearly demonstrated that several of these products can induce arthritis with many similarities to the previously described oil-induced arthritis after intradermal administration; in addition, at least one of them can induce arthritis after percutaneous application on skin that had been moderately irritated by mechanical means.

A first conclusion of these results is that mineral oils added to the commercial cosmetic products have retained their arthritogenic

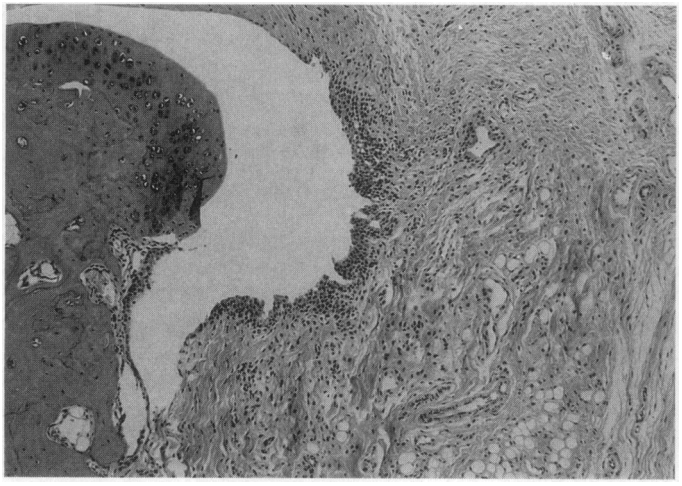

Figure 3. Arthritic hind paw showing synovitis and infiltration of mononuclear cells 33 days after intradermal injection with baby oil. Original magnification ×80.

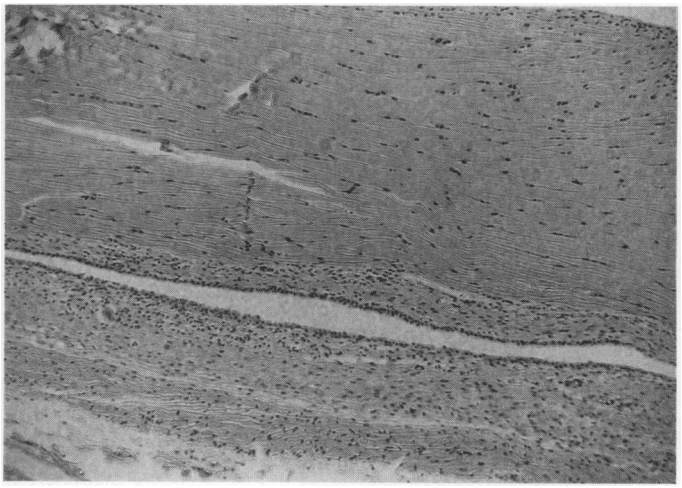

Figure 4. Tendinitis in arthritic hind paw 33 days after intradermal injection with baby oil. Original magnification ×80.

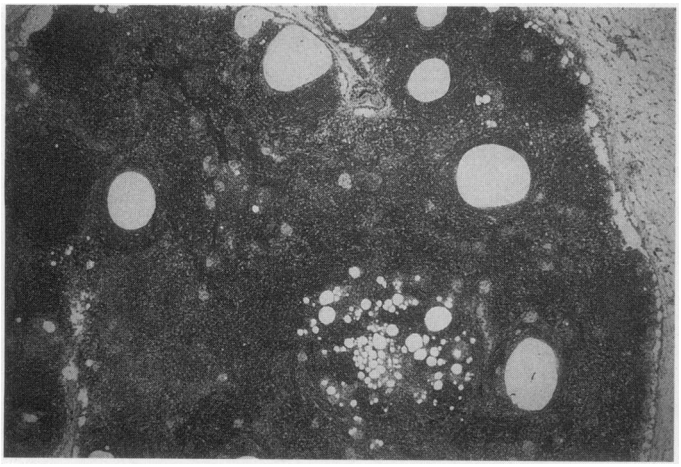

Figure 5. Histology of a lymph node 25 days after intradermal injection with baby oil showing an increased number of cells, diffuse germinal centers, and large, round vesicles. Original magnification ×32

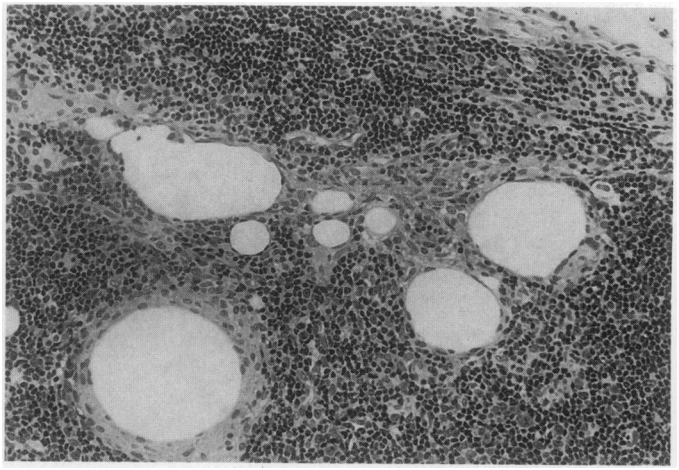

Figure 6. Histology of a lymph node 33 days after intradermal injection with baby oil showing large, round vesicles, probably disseminated oil droplets in the marginal zone. Original magnification ×200.

Table 3. Analysis of autoantibody production in rats treated with cosmetic products, mineral oils, or silicone implants

Treatment	Administration	Arthritis incidence	Blood sample day (pi)	CII	ANA in rat tissue	ANA in HEp-2	RF
Natusan	ID	4/10	20	ND	0/7	0/7	0/8
Natusan	PC	5/10	19	ND	0/10	0/10	0/4
PC control	None	0/10	19	ND	0/10	0/10	1/6
Barnängen	ID	4/10	56	ND	0/10	0/10	2[a]/10
M. Astor	ID	2/10	54	4/10	0/10	0/10	1/9
Kaydol	ID	9/10	67	0/10	0/10	0/10	0/10
Kaydol	PO	0/10	80	0/10	0/10	3[b]/10	5/10
FCA/FIA	Invag	0/10	114	ND	0/4	0/4	
Normal	None	0/10		0/10	0/12	0/12	0/5
Silicone	SC implant	0/11	54	0/11	0/11	4[c]/11	0/11
Sham operation	None	0/2	54	0/2	0/2	1[c]/2	0/2

Abbreviations: pi, post injection; ND, not done; CII, collagen II; ANA, antinuclear antibodies; HEp-2, human epithelial cells; RF, rheumatoid factor; ID, intradermal; PC, percutaneous; PO, per oral; Invag, intravaginal; SC, subcutaneous; FCA/FIA, Freund's complete and incomplete adjuvants.

[a]Arthritic.

[b]Contained one centromere and two Golgi.

[c]Contained Golgi.

potentials in the DA rats, even after being mixed with the many additional substances included in the various commercial products. The variation in arthritogenicity evident between various commercial products indicates that such modifications may occur, and we do not know if the quantitative content of mineral oil in a commercial product is the only factor determining arthritogenicity in the present experimental system.

A second conclusion is that at least one of the mineral oil-containing products intended for application on skin can induce arthritis in the DA rat after being applied by a method similar to that recommended for use by humans (percutaneously). We carried out this provocation on mildly abrased skin, as this procedure has previously been shown to enhance the arthritogenic effects of percutaneous exposure of mineral oil present in FIA (*5*). The product that has the capacity to induce polyarthritis after either intradermal or percutaneous exposure still did not induce any irritative reaction measurable by macroscopic or microscopic investigation. This is in line with our previous observation that the effects of these nonimmunogenic adjuvants occur mainly in the regional lymph nodes (*12,13*) in which the adjuvants cause activation of T lymphocytes, which have a capacity to subsequently induce arthritis (*14*). This also means, however, that such substances appear to be able to induce arthritis, at least after being applied to irritated skin, without giving rise to any types of local irritative reaction of the kind that are used to screen commercial cosmetic products for potential irritation or other adverse effects.

The pathogenic mechanisms responsible for arthritis induction after exposure to nonimmunogenic adjuvants, either in the form of Freund's incomplete adjuvant or present in cosmetic products, are still not completely known, but it has been shown that several different genes, both within and outside the major histocompatibility complex, are involved (*15*) and that T lymphocytes are needed for disease development; oil-induced arthritis can be blocked by *in vivo* administration of anti-T-cell antibodies (*16*). Whether the adjuvants trigger activation of T cells with a specificity for certain autoantigens (e.g., from cartilage) or whether the T cells cause arthritis by some more unspecified actions is as yet not known. The relative lack of anti-collagen II antibodies in most of the experiments may indicate that autoimmunity to collagen II is not of major importance in this context.

It is difficult to draw any firm conclusions from the relative lack of antinuclear antibodies and the presence of RF in the serum of two arthritic rats and some orally fed animals. It has previously been demonstrated that the DA rat has a high tendency for production of RF, also after systemic immunizations; this feature may obviously be related to a general high capacity of the DA rat to acquire several different autoimmune diseases, i.e. not only arthritis but also encephalitis, neuritis, and others (*17*).

In the present study, only administration via the skin caused arthritis, whereas peroral or intravaginal applications did not give rise to any disease symptoms. This does not mean that administration of adjuvants via these routes can be excluded as a risk factor for arthritis development in the DA rat; we have not tested the effects of oral administration of adjuvants in conjunction with inflammation of the gut caused by some other agent, for example, thereby paralleling the situation with abrased skin.

Silicone gel from breast implants has been suggested to be an adjuvant (*8*). However, silicone gel alone did not appear to be arthritogenic in our experiments, in agreement with recent reports (*18*).

A major final question is obviously to what extent our present findings in the particularly arthritis-susceptible DA rat has any relevance for the situation in humans as to a potential arthritogenic capacity of any of the presently investigated cosmetic products. So far, there is no epidemiological or clinical evidence favoring a role of adjuvant-containing cosmetic products in the development of arthritis or any other inflammatory diseases. On the other hand, few studies have been performed that would have allowed any such conclusions to be drawn, particularly if a risk for such adjuvant arthritis to occur in humans should be strictly limited to individuals carrying genes which confer high arthritis susceptibility. It should also be noted that current product testing methods for local irritation in animals are probably not relevant in the identification of products with arthritogenic capacities.

There is consequently a need for well-performed studies, which include genetic techniques for identification of subgroups of potentially arthritis-susceptible individuals, to study the possibility that some commercial cosmetic products may have a capacity to contribute to arthritis induction in humans in some individuals and in certain situations. In the meantime, further mechanistic and genetic studies in the experimental systems may be able to define the pathophysiological mechanisms of this remarkable capacity of both nonimmunogenic adjuvant oils and several commercial products containing such oils to induce a polyarthritis with many similarities to inflammatory joint disease in humans.

References

1. Pearson CM. Development of arthritis, periarthritis, and periostitis in rats given adjuvants. Proc Soc Exp Biol Med 91:95–101 (1956).
2. Goupille P, Poet J, Jattiot F, Mattei J, Védère V, Tonolli-Serabian I, Roux H. Valat J-P. Three cases of arthritis after BCG therapy for bladder cancer. Clin Exp Rheumatol 12:195–197 (1994).
3. Torisu M, Miyahara T, Shinohara N, Ohsato K, Sonosaki H. A new side effect of BCG immunotherapy. BCG induced arthritis in man. Cancer Immunol Immunother 5:77–83 (1978).
4. Kleinau A. Erlandsson H, Holmdahl R, Klareskog L. Adjuvant oils induce arthritis in the DA rat. I: Characterization of the disease and evidence for an immunological involvement. J Autoimmun 4:871–880 (1991).
5. Kleinau A. Erlandsson H, Klareskog L. Percutaneous exposure of adjuvant oil causes arthritis in DA rats. Clin Exp Immunol 96:281–284 (1994).
6. Kumagai Y, Shiokawa Y, Medsger TA, Rodnan GP. Clinical spectrum of connective tissue disease after cosmetic surgery. Observation on eighteen patients

and a review of the Japanese literature. Arthritis Rheum 27:1–11 (1984).
7. Hochberg MC, Perlmutter DL, Medsger TAJ, Nguyen K, Steen V, Weisman MH, White B, Wigley FM. Lack of association between augmentation mammoplasty and systemic sclerosis (scleroderma). Arthritis Rheum 39:1125–1131 (1996).
8. Naim JO, Lanzafame RJ, van Oss CJ. The effect of silicone-gel on antibody formation in rats. Immunol Invest 22:151–161 (1993).
9. Larsson P, Kleinau S, Holmdahl R, Klareskog L. Homologous type II collagen-induced arthritis in rats. Arthritis Rheum 33:693–701 (1990).
10. Vermylen C, de Vreker RA, Verstraete M. A rapid enzymatic method for assay of fibrinogen fibrin polymerization time (FPT test). Clin Chim Acta 8:418–424 (1963).
11. Enestrom S. Immune-mediated glomerulonephritis induced by mercuric chloride in mice. Experientia 40:1234–1240 (1984).
12. Kleinau S, Dencker L, Klareskog L. Oil-induced arthritis in DA rats. Tissue distribution of arthritogenic ^{14}C-labelled hexadecane. Int J Immunopharmacol 17:393–401 (1995).
13. Müssener Å, Klareskog L. Lorentzen JC, Kleinau S. TNF-α dominates cytokine mRNA expression in lymphoid tissues of rats developing collagen- and oil-induced arthritis. Scand J Immunol 42:128–134 (1995).
14. Kleinau S, Klareskog L. Oil-induced arthritis in DA rats. Passive transfer by T cells but not with serum. J Autoimmun 6:449–458 (1993).
15. Lorentzen JC, Olsson T, Klareskog L. Susceptibility to oil-induced arthritis in the DA rat is determined by MHC and non-MHC genes. Transplant Proc 27:1532–1534 (1995).
16. Holmdahl R, Goldschmidt T, Kleinau S, Kvick C, Jonsson R. Arthritis induced in rats with non-immunogenetic adjuvant oil is genetically restricted, α,β T cell dependent autoimmune disease . Immunology 76:197–202 (1992).
17. Lorentzen JC, Issazadeh S, Storch M, Mustafa MI. Protracted, relapsing and demyelinating experimental autoimmune encephalomyelitis in DA rats immunized with syngeneic spinal cord and incomplete Freund's adjuvant. J Neuroimmunol 63:193–205 (1995).
18. Naim JO, Ippolito KML, Lanzafame RJ, van Oss CJ. Induction of Type II Collagen Arthritis in the DA Rat Using Silicone Gel as Adjuvant, vol 210. Berlin, Heidelberg, New York:Springer, 1996.