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Lupus 2012 21: 217

DOI: 10.1177/0961203311429818

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SPECIAL ARTICLE

Oily adjuvants and autoimmunity: now time for reconsideration?

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Immunologists have relied heavily on oil-based adjuvants to generate antibodies or induce auto-allergic responses in experimental animals. These are rarely used today for human vaccination because of their persistent irritancies and propensity to cause ulcers at sites of injection. However oily materials with adjuvant properties abound in our modern environment, both personal and extraneous. Their inadvertent impact as cryptotoxins may contribute to the rising incidence of auto-allergic diseases in recent times.

Experimentally, the potential adjuvanticity of various oils, fats and other lipids can be evaluated by their ability (or otherwise) to induce auto-allergic disease(s) in rats and mice with, or even without, the addition of a mycobacterial immunostimulant.

Genetic factors have been recognized that determine an animal's susceptibility or resistance to these oil-induced immunopathies. So it may be profitable to further characterize these factors, first in animals and then perhaps in human populations, to help find ways to enhance natural resistance to those adjuvant-active oils that may be widely distributed in the personal environment, notably mineral oil(s).

(The six tables in this article summarize some relevant facts and a few conjectures.)

A caveat: This review is restricted to the adjuvant properties of some oils in the personal environment. It does not cover the mechanisms of adjuvanticity². *Lupus* (2012) **21**, 217–222.

Key words: arthritis; adjuvants; adverse effects

'Great truths may be very near and yet not be discerned'

James Paget, 1879¹

Introduction

Can auto-allergic diseases be prevented? While there are only two answers; paradoxically both may be correct. The answer is probably no, if the patient's genetic constitution fails to provide a healthy eradicated response to an auto-allergic stimulus. More hopefully, the answer might be yes, if this pathogenic stimulus can be counteracted; either by (i) repairing a non-genetic deficiency in the host (e.g. hormonal, nutritional) or (ii) eliminating a provocative factor from the patient's environment. This latter strategy deserves serious consideration – because it is feasible, cheap and may well be effective, as suggested by experimental studies of autoimmune responses in animals. Essentially it

involves some modification of the human environment, either external or internal.

This discussion focuses on oils and related lipids that are latent toxins if they are capable of inducing/accelerating immune responsiveness. Their harmful character is adduced from two lines of evidence: historical (or anecdotal) and experimental. The historic evidence is haphazard and may be justifiably challenged on various grounds, but it does relate specifically to the human condition.

The experimental evidence is somewhat stronger, being based on ethical – but therefore somewhat restrictive – animal experimentation. It too must be accepted with caution, because of the uncertainties as to how relevant adverse responses in animals might be for modelling disease development in humans. Nevertheless, the combination of these two approaches does provide some insights into possible ways to minimize the development of auto-allergic responses in affluent populations around the globe.

This task has now been much helped by the brilliant conceptualization and explanation of the ASIA syndrome(s);² alerting pathologists, clinicians and public health 'minders' to further harmful consequences from our modern, man-made, personal

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environments – including the development of several anthropogenic diseases besides cancer.

A historical perspective

There seems to be a parallelism in the timing of (i) the comparatively recent evolution of at least one aggressive autoimmune disorder, rheumatoid arthritis (RA), as a global disease (Table 1); and (ii) the discovery and utilization of mineral oil (MO) in products that are now widely distributed in our modern environment (see Table 2). Is this mere coincidence or is there perhaps a causal association? There is no direct way to prove or disprove this, experimentation upon human subjects now being totally unacceptable. So the evidence must be sought indirectly.

To have any significance, this association needs to be explored further in at least two directions: (i) finding out what animal studies might tell us (Table 3) and (ii) considering some of the physical and biochemical mechanisms that could link MO with several adverse clinical responses (Table 4). Some of these are postulated in Table 5.

But looking ahead: what is really required now is some hard thinking about how we might sift and integrate this scattered information, in order to develop realistic measures to prevent/limit those diseases that might be initiated or sustained by

Table 1 Rheumatoid arthritis (RA): a relatively modern disease?

- Very little/no evidence for erosive polyarthritis (EP) in skeletons unearthed by archaeological excavations at classical sites (Mesopotamia, Indus Valley, around the Mediterranean and along the Danube River).
- However, EP is present in skeletons of American Indians (first people) residing 400 years ago north of the Ohio River, USA.³ Perhaps significantly, this location is downstream from the Allegheny Mountains, western Pennsylvania, where the Seneca Indians found and used 'rock oil' (i.e. mineral oil), for example as a hair dressing, as noted by French explorers after 1750.
- Further evidence presented that RA is a 'New World disease', subsequently transferred to Europe.⁴
- (Mineral oil [MO] becomes a global commercial product after 1860 [see Box 2]).
- Higher prevalence in females, in common with other autoimmune diseases (SLE, Sjogrens' syndrome, etc.).
- Provocative factors for RA in the environment include cigarette smoke, nutritional deficiencies (e.g. vitamin D, copper, antioxidants) and, somewhat controversially, previous occupational exposure to MO.⁵
- No infectious causes have been unambiguously identified as yet. Circumstantial evidence implicates certain oral/gut bacteria and also immunostimulant *Mycobacteria* from the external environment.⁶ The World Health Organization has estimated that *M. tuberculosis*, a known arthritigen in rats, has now infected approximately one-third of the world's population.

Table 2 Timelines for mineral oil's adoption into the personal environment

- 14th century onwards: MO sourced from terrestrial seepages, used as a liniment, e.g. St. Catherine oil (Modena, Italy), St. Quirinius oil (Tegernsee, Bavaria), Betton's British oil (Pitchford, UK), Seneca oil (Pennsylvania rock Oil).
- 17th century: used for preparation of ointments and plasters.⁷
- 1850s: petroleum = 'rock oil', first mined at Bobrika (Poland), Wietze (Hanover), Oil Springs (Canada) and Titusville (Pennsylvania, USA); later at Baku (Azerbaijan).
- Commercial MO (BP > 300°C) and soft paraffin jelly/waxes (MP > 38°C) subsequently become widely available as by-products from petroleum refining.
- MO then marketed as liquid paraffin, petrolatum, Drakeoil, etc. for medicinal use, e.g. laxative, dermal 'lubricant'; increasingly incorporated into many personal products, e.g. toothpaste, skin and hair creams, skin lotions, e.g. moisturizers, vaginal lubricants, lipsticks, suppositories, barrier creams (skin, vagina), cosmetics and as food additive/preservative; also present in many 20th-century household products, e.g. cooking oils, leather/floor/shoe polishes, (metal) lubricating oils, varnishes, etc.
- MO's widespread use is sustained by its low cost, low volatility and chemical stability, i.e. not readily oxidized and moisture resistant.
- MO is widely accepted as being relatively sterile, odourless, almost tasteless, and unfortunately also presumed to be 'safe'.
- (Cumulative) toxicity, associated with its slow/minimal biodegradation. (This is why petroleum/rock oil is preserved in very long-lasting reservoirs.)
- Human exposure may even begin in the womb(!) and certainly continues post-natally with use of 'baby oils'.
- From the 1930s, growth of oil-seed farming allows increasing replacement of MO in some domestic products (e.g. cooking oils, cosmetics) by bio-labile triglyceride oils.
- From the 1950s, growing perception that the severity of RA may be declining in Western societies but may be increasing in Africa.⁸ Also, declining use of MO in some personal products, e.g. hair creams, body lubricants, etc. and for preparing/preserving food items.
- (Early 21st century: however, newer evidence suggests it's use may now be increasing.)

repeated exposure to MO and other adjuvant-active oils (Table 6).

Evidence from animal studies (Table 3)

In laboratory animals, injections or topical application of MO – or some other oily/waxy materials in our personal environment – can initiate immunopathies after following any of these three experimental procedures:

- a. with the oil alone injected subcutaneously or directly into pre-exposed lymph nodes or even by transdermal administration, inducing an experimental arthritis in rats or SLE-like syndrome in mice.
- b. with the oil plus a second immunostimulant, e.g. components from mycobacterial cell walls; this combination can sensitize an animal to intrinsic, latent immunogens (?slow virus). In rats it can cause systemic chronic inflammation

Table 3 Adjuvant-active oils, experimental polyarthritis and other immunopathies: some notable developments

- 1942: Freund and McDermott report that an oil-based adjuvant, Aquaphor (petrolatum with lanolin) plus heat-killed tubercle bacilli, stimulated hypersensitivity to co-administered antigens in guinea pigs.
- 1947–1956: Jules Freund (New York) describes two improved adjuvant formulations for amplifying immune responsiveness⁹:
 - (i) the complete (Freund's) adjuvant, CFA = mineral oil (MO), emulsifier and a dead *Mycobacterium* (see text);
 - (ii) an incomplete adjuvant, IFA = MO plus emulsifier but no bacterial component.
- CFA becomes 'adjuvant of choice' to induce cell-mediated autoimmunity in rodents to tissue auto-antigens (extracted from thyroid, liver, CNS, muscle, etc.).
- 1956: Carl Pearson (Los Angeles) describes peri-arthritis induced in rats with a CFA alone, i.e. without any other antigen = adjuvant(-induced) arthritis (AA).¹⁰ Although not readily induced in mice, some brief reports record the occasional incidence of adjuvant disease (arthralgia) in humans after (rare) accidental injection of CFA or after immunization against tuberculosis with some early BCG/MO vaccines.
- 1974: arthritogenic oily adjuvants described containing no mineral oil.¹¹ These 'pseudo-CFAs' can be constituted with pure saturated long-chain hydrocarbons, e.g. hexadecane (C₁₆H₃₂), pristane (TMPD, C₁₉H₄₀), or squalene (C₃₀H₆₂) that are found in MOs; or with the unsaturated natural hydrocarbon squalene (C₃₀H₅₀) or with triglycerides and fatty acyl esters. These can all replace MO as immunostimulants.
- 1977: David Trentham and colleagues (Memphis) describe collagen-induced arthritis (CIA) in rats, initiated by cartilage collagen (type II) emulsified with CFA.¹² Other researchers subsequently induce CIA in mice and monkeys.
- These MO alternatives, when used to prepare 'pseudo-IFAs' (with/without emulsifier), also induce experimental allergic encephalomyelitis (EAE).¹¹
- Olga Bakharevski and her colleagues (Melbourne, Australia) show that CIA can be produced in certain rat strains using IFA, in place of CFA.¹³ 'Pseudo-IFAs', e.g. those containing squalene, can also induce CIA in rats.
- 1994: Sandra Kleinau and colleagues (Stockholm) describe the oil-induced arthritis (OIA) induced by applying MO to the abraded skin of DA rats.¹⁴
- Pristane, squalene or MO can induce lupus-like antibodies in mice.^{15,16}
- Some animal strains are more responsive, or less resistant, to AA, CIA and immunopathic oils. Genes conferring their susceptibility have been identified.^{17–19} Conversely, other strains appear to carry genetically determined resistance factors, suggesting possibilities for perhaps enhancing oil-resistance in humans too.
- 21st century: commercial influenza vaccines are produced containing adjuvant-active squalene, considered safe for human inoculation. BUT injected squalene, by itself, can induce arthritis and experimental neuropathies in animals(!).²⁰ {Can all human populations be equally resistant to the adverse adjuvant activity of exogenous squalene, in contradistinction to experimental animals?}

NOTE: These last four findings all show the (adverse) cell-mediated immunostimulant properties of some adjuvant oils alone, i.e. when used without microbial additives.

that is very damaging to the joints but may also be manifest in other tissues to a lesser degree.

- c. with the oil plus second immunostimulant and also a tissue antigen, e.g. thyroglobulin; this three-part combination is necessary to establish cell-mediated immunity in many targeted tissues, e.g. experimental thyroiditis.

Usually none of these three responses occur in the absence of the oil, indicating that the presence of the second immunostimulant and/or auto-antigen

Table 4 Unhealthy responses following MO exposure: some relevant factors (and a few conjectures)

- A: When presented supradermally.
 - The skin constitutes 12% of the body's weight, has a large surface area (~1.9 m², adult male) and provides a first line of defence against microbes and pollutants, unless compromised.
 - It is protected by a lipid-rich layer of sebum, containing inter alia adjuvant-active squalene and cholesteryl esters,¹² secreted from the sebaceous glands via the hair follicles.
 - The top (horny) layer of the epidermis, the stratum corneum (SC) contains keratinocytes (the 'bricks') embedded in a layer of lipids (the 'mortar') including surfactant phospholipids.
 - MO applied to the skin as lotions, creams or the oil itself (as contaminant or deliberately), being hydrophobic, will not readily wash off – so may:
 - (i) interfere with the antibacterial functions of sebum;
 - (ii) clog the hair follicles, impeding sebum replenishment;
 - (iii) penetrate the skin either via these follicles or by diffusion through the SC lipids or after surface abrasions;
 - (iv) bind to surfactant proteins within the skin,²⁴ disrupting their normal defensive functions (antimicrobial, etc.);
 - (v) be transported after dermal penetration to encounter immunosurveillance macrophages/dendritic cells located within the skin (Langerhans cells), draining lymph nodes or blood-purifying organs (liver, spleen, lung).
 - neonatal skin being more permeable than that of adults, young infants may be more at risk from applications of MO (as 'baby oils').
- B: In contact with mucosal tissues.
 - Buccal epithelia and salivary glands are exposed to MO/soft waxes ingested from lipsticks, food additives or oral laxatives.
 - After bronchial exposure to inhaled MO mists, e.g. from 'cutting oils'.
 - Rectal/vaginal exposure from suppositories/sexual lubricants.
 - A large proportion (> 60%) of the human immune system is in intimate contact with surface epithelia, notably along the GI tract.
- C: Hazardous consequences arising from:
 - Extensive exposure to MO presenting to large surfaces (both external and internal) and over extended time periods.
 - Although internalization may be slow and very low in quantity, the inability of macrophages to rapidly detoxify phagocytosed MO ensures a continual 'grumbling' adjuvant response – hindering, or even ablating, natural tolerance to self-antigens, slow viruses or products of apoptosis.
 - So the most cause for concern is not the actual dose, but the longevity of the toxin in vivo – even after its removal from the external environment.

is not as critical as the presence of the oil component. This allows us to use the descriptors 'adjuvant-active', pathogenic or 'intrinsically harmful' to describe this adverse/negative property of some slowly metabolized, persistent, oily materials. Some of these may be potentially harmful at one location in/on the body, e.g. triglycerides or hydrophilic esters act upon the skin, but are virtually harmless elsewhere, e.g. within the gut – where they might be rapidly inactivated by normal digestive/detoxicant processes. As in humans, the incidence and magnitude of harmful responses evoked in experimental animals are also genetically determined, the expression of the experimental disease then being the sum of the oil's impact *and* the host's resistance.

A number of non-MO cosmetic oils, e.g. jojoba bean, almond and avocado, are arthritogenic in

Table 5 More consequences from intimate contact with MO: some conjectures

- MO and other lipophilic materials applied liberally on the skin (cosmetics, insect repellents, analgesics, etc.) may disturb the natural segregation of sebum adjuvants, facilitating their resorption from outside the body (probably an unnatural process) and consequent subdermal translocation.
- A combination of residual faecal bacteria on an infant's buttocks with applied 'baby oils' = MO or (more rarely) plant triglyceride oils, i.e. constituting a pseudo-CFA, could predispose infants to allergenic sensitization and subsequent development of asthma, early arthritis or other immunopathies.²
- The preponderance of autoimmune disease in females, versus males, (ranging from 2–3 times for RA and up to 9 times or more for Sjogren's syndrome) may not be solely due to differences in sex hormonal levels. In women their greater exposure to MO in cosmetics, lipsticks, vaginal lubricants (as formulated in previous years) may also be significant here.
- This linkage to Sjogren's syndrome could be partly due to the ingestion of MO and other hydrophobic materials from lipsticks, e.g. after 'lip-smacking', which then becomes smeared over the superficial lacrimal glands (perhaps only in very small quantities – but how low is the intoxicant threshold for these very active secretory organs?).
- Also, the increasing exposure of males to MO following contemporary fashions of using cosmetics (e.g. by ageing homosexuals) or body-gleaming oils (e.g. by 'beach boys') may affect the sex ratios and raise the overall incidence of some autoimmunopathies.
- The confusing stance of private and governmental agencies in protecting animals from being harmed in safety testing of cosmetic products, particularly their oily constituents (+/- surfactants), without regard to possible adverse responses when used (untested) upon humans!

Note: Regrettably, there are few experimental or clinical scientific data that can be cited to validate, or clearly refute, these proposals. They are presented as ALERTS.

rats, their purveyors often proudly proclaiming 'not tested on animals'.

Of particular relevance are some limited studies in rats examining MO-containing cosmetics. In the highly susceptible Dark Agouti (DA) rat, some MO cosmetics induced arthritis after intradermal injection or dermal application but not when fed.²¹ In the less susceptible Wistar rat strain, a mycobacterial immunostimulant is also required to induce polyarthritis, the mycobacterial components (MC) being non-arthritisgenic when administered in saline, i.e. an adjuvant oil is needed to trigger arthritis development. However, these two factors – MC (in saline) and oil – can be administered intralymphatically at separate but contiguous sites (3 cm apart along the tail) and still induce arthritis in Wistar rats. Some MO-containing cosmetics, e.g. Nirvana creams, could replace MO alone in these 'two-hit' experiments.²² These arthritic responses in both DA and Wistar rats to some MO-containing cosmetics would rate the latter as potentially hazardous.

This conclusion may be tempered by a recent report that 'ordinary usage of common cosmetics often containing mineral oil (does not) increase the risk for RA in the population in general'.²³

The authors also state, 'We cannot exclude that these cosmetics can contribute to arthritis in individuals carrying certain genotypes or simultaneously exposed to other arthritis-inducing environmental agents'. So the experimental findings from the 'two-hit' experiments in Wistar rats now become rather more interesting in the light of this last quotation.

Some oils that have been identified and which are not arthritisgenic might even be immunologically beneficial; for example, they can induce experimental tolerance to mycobacterial arthritisgens in rats. Oily materials that are quite closely related in their chemical composition may exhibit surprisingly divergent adjuvantcities, either protective/tolerogenic (T) or harmful/arthritisgenic (A). So, for example, phytol, a plant oil (C₂₀H₄₀O, present in chlorophylls) is T but pristane is A; and isopropyl myristate is T but butyl oleate is A.

To conclude this discussion:

1. Animal studies can show which oils are likely to be immunologically hazardous and which are not.
2. Cosmetics (of varying composition, some with MO and some without) are difficult to evaluate in both man and, nowadays, also in rats. (last item Table 5)
3. However, cosmetics containing oily adjuvants may predispose both humans and rats to respond adversely to other arthritisgens – and perhaps also to other triggers for autoimmunity.

Summing up

Amongst adjuvant-active oils MO may be particularly harmful, not being readily metabolized or detoxified. It can only be eliminated by desquamation of the skin when applied in cosmetics or with the faeces when used as a laxative or ingested as a food additive. If and when it enters the body it slowly accumulates in the reticulo-endothelial system (RES), where it may have an almost indefinite half-life. So the adjuvant action may be not just in initiating but also in sustaining a chronic autoimmunity. Not being readily detected in vivo, the latent toxicity of MO is usually disregarded as a potentially serious and long-lasting health hazard.

Some polemics¹

1. Much of the history of medicine is not of progress but of delay, effectively preserving traditional practices and ignoring/sidelining perceived threats that new knowledge might bring.

Table 6 Some alternatives to mineral oils: possibilities and queries (Q)

(AID = autoimmune diseases)

- Avoidance

Use other laxatives.

Abandon cosmetics that contain MO and other adjuvant-active lipids.

Q: What is the incidence of AID in Amish, Quaker and other populations with simpler lifestyles (not seeking glamour or perpetuating youthfulness)?

- Substitute other low-melting/oily lipids as MO substitutes.

e.g. plant and animal triglyceride fats pre-screened in animals for non-pathogenicity (→AID) e.g. phytol, isopropyl myristate.

Q1: Do they need to be rapidly deactivated/degraded in vivo?

Q2: Is failure to induce an auto-allergic polyarthritis or EAE an adequate test? Are there better screening methods to detect (even quantify) harmful adjuvanticity?

Q3: How does one overcome the mindset that components of beauty aids should not be pre-tested in/on animals? (There is legislation actually forbidding this!)

- Further develop non-mined, non-farmed neglected triglyceride food oil resources such as those traditionally used by populations with a low incidence of RA.

e.g. by the ancient Hebrews using an olive oil;²⁵ by Eskimos reliant on fish and seal oils;²⁶ by inland Australian Aboriginals using oils from the fat of the emu (bird) or goanna (reptile); or by coastal Aborigines and Maoris (New Zealand) using mutton bird oil; and by Melanesians (SW Pacific) using ngali nut oil.²⁷

These are all relatively unrefined oils containing natural anti-arthritic/immuno-deactivating components. (Some may be uneconomic for wider use but they do provide 'templates' for finding/constructing similar safer, non-immunopathic oils.)

- Avoid pancreatic lipase inhibitors, e.g. orlistat and promote an adequate bile flow to ensure ingested adjuvant triglycerides are efficiently hydrolysed in the intestine.

- Deliberately adulterate MO with (non-steroid) AID-suppressants.

Q1: Any value beyond use in cosmetics?

Q2: Is this too big a problem for the FDA and other regulatory agencies?

- More lateral thinking about replacing MO, particularly in cosmetics, but still retaining a focus on AID prevention.

2. Bad ideas – such as those espoused by the beauty and tobacco industries – may defeat sensible ideas for recovering/maintaining health. Consequently, progress in knowledge does not always lead to early improvements in well-being.

To which we might add:

3. It is almost 40 years since MO and a number of related oils, e.g. squalene and many triglycerides, were clearly identified as immunopathic toxins in animals.¹¹ It seems rather over-optimistic still to assume they are always safe when used topically or parenterally in humans.
4. Still very much needed are further retrospective studies to relate past exposures to adjuvant oils with the incidence/severity of not only RA – as shown in a landmark survey by Berit Sverdrup and her associates in Sweden⁵ – but for other auto-allergic diseases as well.

5. Then perhaps we might be able to replace a fragile hypothesis about oil-engendered (or oil-assisted) disease(s) with considerably more robust insights into the aetiology and possible regulation of chronic immunopathies.

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