

# Wolves in Sheep's Clothing: How Chemically Inert Hydrocarbon Oils Induce Autoimmunity

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## Abstract

Hydrocarbon oils such as pristane (2,6,10,14-tetramethylpentadecane, TMPD) or hexadecane induce arthritis and lupus in rodents sharing clinical and pathological features with the human diseases rheumatoid arthritis and systemic lupus erythematosus, respectively. In pristane-induced lupus in the mouse induction of apoptosis and augmentation of type I Interferon signalling by pristane are supposed to contribute to pathology. Pristane also induces various forms of cell death in rat and human cells and leads to release of inflammatory cytokines such as interleukin (IL)-1 $\alpha$  and IL-1 $\beta$  by both inflammasome-dependent and -independent mechanisms. How exposure to chemically inert oils translates into the establishment of a vicious cycle of autoimmunity is the case of this review.

**Keywords:** Hydrocarbon oils; Autoimmunity; Sheep

## Introduction

For many decades, mineral oils with or without dried mycobacteria, i.e. Complete and Incomplete Freund's Adjuvant, have been the most commonly used immune adjuvants for experimental work. Later on, better defined hydrocarbon oils such as pristane (2,6,10,14-tetramethylpentadecane), squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexane), or hexadecane have emerged and partially replaced them. Nowadays a broad array of hydrocarbon oils is not only used for the induction of experimental diseases but also for human or veterinary vaccines [1].

Dependent on application route and species, adjuvant oils can induce different diseases. Whereas in mice lupus and delayed arthritis are triggered by intraperitoneal injection, susceptible rat strains develop arthritis upon intradermal or subcutaneous application. The reasons for these remarkably differentiated outcomes are still elusive and may be dependent on genetic factors and/or the panel of locally activated types of immune cells. Interestingly, it was also reported that occupational or environmental exposure to hydrocarbon oils is associated with an increased risk for developing rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [2,3]. Hydrocarbon oils such as pristane may thus also play a role in the pathogenesis of human disease employing mechanistic pathways similar to those discovered in experimental animals.

## Autoimmunity Induced by Hydrocarbon Oils in Mice

Intraperitoneal injection of pristane into several inbred mouse strains initially leads to the development of a chronic granulomatous peritonitis involving the formation of ectopic lymphoid tissue (so-called lipogranulomas), which is followed by production of antibodies against nuclear components, such as ssDNA and dsDNA, Sm, snRNPs, and Su autoantigens. Lipogranulomas contain proliferating T- and B- and class-switched B-cells and are an important source of autoantibody production in the peritoneum [4].

Although most mouse strains develop some kind of autoimmunity after pristane-injection, the pattern of autoantibodies and the affected organs vary depending on the genetic background [5,6]. BALB/c and SJL mice gradually develop proteinuria and immune complex-mediated glomerulonephritis which may be followed by the development of arthritis [6-8]. Murine pristane-induced arthritis (PIA) is characterized

by synovial hyperplasia, inflammatory cell infiltrates, and cartilage and bone erosions, resembling the joint inflammation and destruction which occurs in human RA. Rheumatoid factor and autoantibodies with a broad reactivity to potential joint autoantigens like type I and II collagen can be detected [6,7].

In C57BL/6 and BL/10 mice, proinflammatory macrophages, neutrophils, eosinophils, and lymphocytes infiltrate the lungs, leading to hemorrhagic pulmonary capillaritis. Pulmonary hemorrhages emerge early after pristane-injection (within the first 30 days) and resolve over time [9,10]. Comparably weak IgG- and complement-deposits in the glomeruli and mild proteinuria also occur in BL/6 and BL/10 mice [9], whereas arthritis is absent [6].

Similar to SLE, pristane-induced lupus (PIL) is associated with an early upregulation of type I Interferon (IFN) production and an elevated transcription of IFN-stimulated genes in peripheral blood cells [11,12] that correlate with production of autoantibodies and organ involvement. IFN-production is driven by nucleic acid-sensing Toll-like receptors (TLR) and MyD88 signalling. Pristane-induced death of peritoneal cells is believed to provide a constant and abundant source of nuclear antigens presented in an environment of chronic inflammation. Unlike in idiopathic lupus, however, in PIL IFN is produced by immature monocytes infiltrating the peritoneum rather than plasmacytoid dendritic cells [13]. The chronic inflammatory response to pristane also includes neutrophil infiltration which in contrast to monocytes is driven by IL-1 $\alpha$  [14].

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As compared to pristane, other hydrocarbon oils, such as Incomplete Freund's Adjuvant or hexadecane have a lower autoimmunogenic potential [15]. This has been linked to the particular potential of pristane to induce cell death and secretion of type I IFN. Pristane and hexadecane that are contained in IFA at substantial amounts might well be responsible for a large part of IFA's arthritogenic properties.

Recently, also TLR2 has been shown to be involved in the disease process of PIL in BL/6 mice, i.e. required for the production of typical lupus autoantibodies, for ectopic lymphoid neogenesis, and renal disease [16]. TLR2 may recognize endogenous ligands that are locally released during tissue damage and thus participate in the amplification of inflammation and kidney injury.

### Autoimmunity Induced by Hydrocarbon Oils in Rats

In 1998, commercially available cosmetic products containing hydrocarbon oils were tested for their disease-inducing ability in rats. Five out of eight tested products, one of them called "baby oil", induced clinical and pathological features of arthritis when injected intradermally [17]. Many different arthritogenic adjuvants like avridine, squalene, or pristane and other hydrocarbon oils have been used to induce arthritis in different strains of rats (reviewed in [18]). Out of these models, the PIA model that is induced by intradermal or subcutaneous injection of pristane in Dark agouti (DA) rats mimics human RA the closest, especially in terms of chronic relapses. Rats with a DA background show a sudden disease onset 10 to 12 days after pristane injection, followed by a chronic relapsing RA-like disease course with erosive and symmetric affliction of peripheral joints [19]. Animals exhibit pronounced synovial hyperplasia, angiogenesis, inflammatory infiltration, and bone or cartilage erosions mainly in distal joints [20]. In contrast to murine PIA, where microbial stimulation appears to play an important role [21], PIA in the rat can also be induced in a germfree environment [22].

Arthritogenic adjuvant oils are not immunogenic *sensu stricto* since they do not contain major histocompatibility complex (MHC) binding peptides. Nevertheless, PIA is MHC-associated and dependent on the activation of  $\alpha\beta$ T-cell receptor-expressing CD4<sup>+</sup> T cells [23]. So far, the pathological mechanisms behind PIA in rats are not fully known. Injected oil has been shown to disseminate quickly and specifically to local lymph nodes after injection [24] where it induces various forms of cell death [25]. Uptake of autoantigens provided by sustained cell death in the setting of an inflammatory milieu in the draining lymph nodes may lead to enhanced presentation of autoantigens. A break of self-tolerance and the development of arthritis may be the result.

### Autoimmunity Induced by Exposure to Hydrocarbon Oils in Humans

Humans are frequently exposed to hydrocarbon oils by various routes, including oral (dietary, medicine), inhalation (air pollution, oil mist in work environment, diesel or gasoline exhaust, etc.), and cutaneous (cosmetics, contact with mineral oil in work environment) [26]. Hydrocarbon oils are also common food contaminants (e.g., contained at around 320 mg/kg in olive pomace oil, 50-300 mg/kg in coffee, or up to 1000 mg/kg in contaminated sunflower oil) [27]. The health and safety risks in man remain mostly unknown. However, reports point to a strong effect of hydrocarbon oils on the human immune system.

Firstly, individuals occupationally exposed to hydrocarbon oils were observed to have an increased risk of developing RA [3]. Secondly,

the injection of human hydrocarbon oil-containing cosmetics elicits arthritis in rats [17], although it could not be correlated to an increased risk for RA in humans [28]. Thirdly, inadvertent cutaneous injection of hydrocarbon oils triggers a strong inflammatory reaction, often accompanied by skin necrosis and loss of hand function [29].

Taken together, these findings show that exposure to hydrocarbon oils might pose a serious risk for developing autoimmune and chronic inflammatory diseases and warrants thorough investigation and proper control.

### Immunogenic Mechanism of Hydrocarbon Oils

After so many decades, the adjuvant action of pure hydrocarbon oils is still not fully elucidated. An important activity that might contribute to pathogenesis is the induction of inflammatory cytokines by hydrocarbon oils. Intraperitoneal injection of hydrocarbon oils reportedly induced expression of the proinflammatory cytokines IL-12, IL-6, and TNF [1]. Furthermore, pristane and hexadecane trigger release of the inflammatory cytokines IL-1 $\alpha$  and IL-1 $\beta$  by inflammasome-independent and -dependent mechanisms, respectively. Increased serum levels of IL-1 $\alpha$ , IL-1 $\beta$  were also described in peripheral blood of rats after stimulation with pristane [25]. IL-1 and the inflammasome are important in the autoimmune diseases typically triggered by hydrocarbon oils. Recent studies have shown that the inflammasome inhibitor bortezomib prevented lupus nephritis in a murine model for lupus [30]. Furthermore, mice deficient for caspase-1, the central enzyme of the inflammasome, are protected against autoantibody formation and glomerulonephritis in pristane-induced lupus [31].

A further mechanism might be the induction of cell death and subsequent accumulation of dead cells. Pristane has dose- and cell type-dependent cytotoxic effects and reportedly induces cell death *in vitro* and *in vivo* [25,32, 33]. In mice, pristane induces apoptosis via the mitochondrial pathway. In addition, Fas appears to be involved, since injection of pristane upregulates expression of Fas and Fas-L in peritoneal cells and cell lines, and Fas or Fas-L deficient mice are protected from PIL [34,35]. Pristane also induces cell death in different ways in rat and human cells. Injection of pristane into rats triggered apoptosis in draining inguinal lymph nodes and increased the number of circulating apoptotic and necrotic cells [25]. There is also evidence that pristane gets incorporated in the cell membrane and therefore could have a detrimental influence on membrane integrity [36]. Furthermore, pristane, and to a lesser extent also hexadecane, induce formation of neutrophil extracellular traps (NETs) in polymorphonuclear neutrophils from the blood of humans, rats, and mice [25] and own unpublished observations). This is an important finding, since NETs have been implicated in the pathogenesis of lupus [37]. Antimicrobial peptides (AMPs) such as cathelicidins are associated with NETs and are able to bind to nucleic acids, protect them from degradation by nucleases, and enhance their uptake into TLR-containing endosomes [38] which might contribute to the pathogenesis of SLE [39,40] and arthritis [41]. In addition, SLE patients frequently express a neutrophil signature, and neutrophils can contribute to SLE and also to arthritis through a variety of mechanisms, e.g., production of inflammatory mediators, release of proteinases that cause vascular damage and tissue injury [42], and production of AMPs that augment the immunogenicity of immune complexes [39-41].

Uptake of autoantigens provided by sustained cell death in the setting of an inflammatory milieu and clearance deficiency at the site of hydrocarbon oil exposure may lead to an enhanced and aberrant

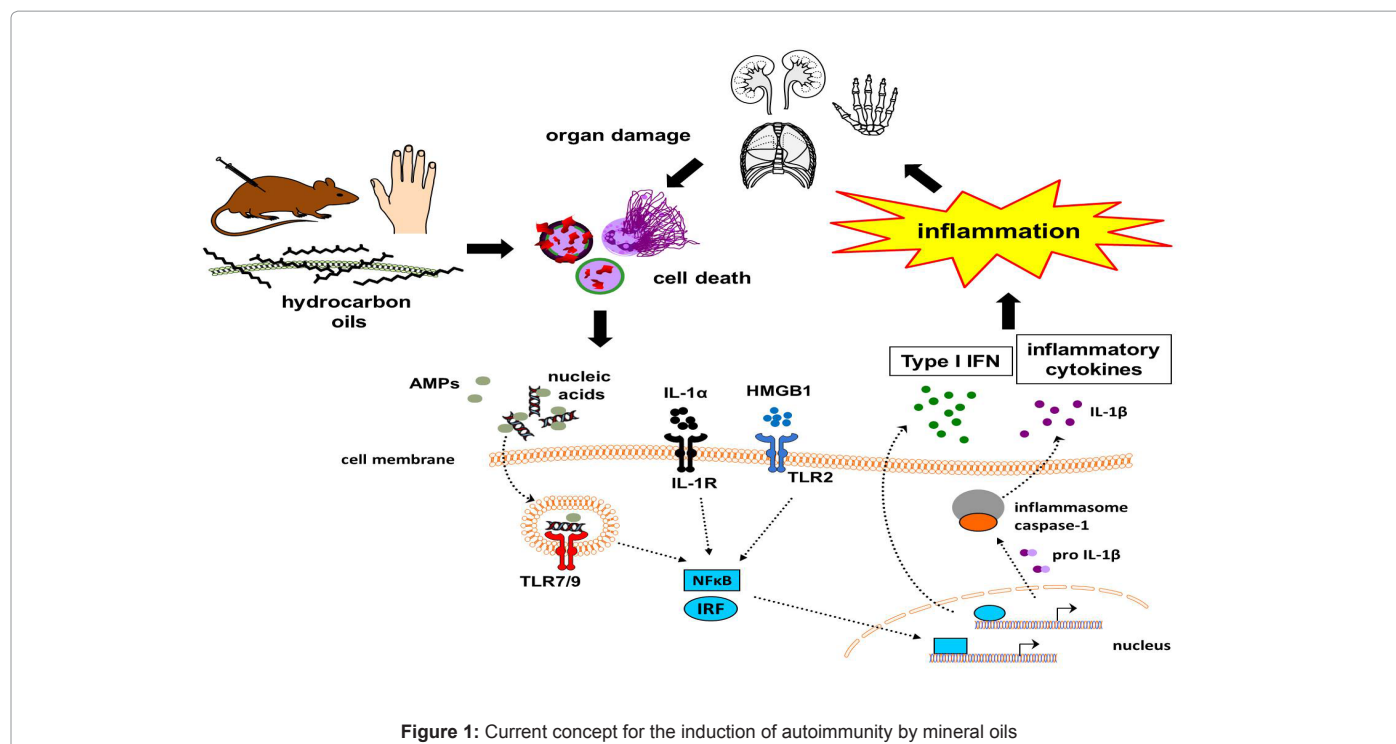


Figure 1: Current concept for the induction of autoimmunity by mineral oils

presentation of autoantigens. Signalling of TLRs and induction of type I IFNs thereby seem to be of critical importance [41,43]. Another factor induced by pristane is the alarmin HMGB1. HMGB1, which is released during cell death and/or from activated immune cells after exposure to pristane, may contribute to induction of antinuclear autoantibodies and perpetuation of lupus-like disease [16]. The vicious cycle established by these processes ultimately results in a break of self-tolerance and the development of lupus-like or arthritic autoimmunity [44] (Figure 1).

At the site of exposure (per example human skin or injection site in rodents), autoimmunogenic oils like pristane or hexadecane interact with the cell membrane and induce extensive and prolonged cell death (apoptosis, necrosis, NETosis). Immunogenic molecules contained in the cell debris are for instance HMGB1, IL-1 $\alpha$ , or nucleic acids that can build ionic complexes with locally released cationic anti-microbial peptides. Upon binding to their respective endogenous receptors, transcription factors like NF $\kappa$ B and members of the Interferon regulatory factor (IRF) family are activated which leads to the production of type I IFN, IL-1 $\beta$ , and other proinflammatory cytokines. For release of active IL-1 $\beta$ , cleavage of pro-IL-1 $\beta$  by the activated caspase-1 is required. The resulting tissue damage in kidneys, lungs, or joints then establishes a vicious cycle that leads to sustained inflammation and development of autoimmune disease.

The biochemical characteristics of different hydrocarbon oils translate into different capacities to induce autoimmune diseases. The length of the carbon chain seems to play an important role on their immunogenic effect. Intermediate length hydrocarbons (C15-20) in general have higher adjuvant and autoimmunogenic properties than high molecular weight hydrocarbons (>C25), the main constituents of mineral oils used in medicine. However, the C30 hydrocarbon squalene has the capacity to promote autoimmunity [45], and the C16 hydrocarbon hexadecane induces arthritis, whereas the closely related

1-hexadecene does not. Other characteristics such as viscosity, density, carbon type or sulfur content might therefore also modulate the effect. The capacity of alkanes to induce oxidative burst also appears to have a strong impact and is often inversely correlated with its autoimmunity promoting effects [46].

Interestingly, hexadecane, an aliphatic oil that has been shown to trigger production of a limited set of lupus-related antibodies upon intraperitoneal injection [47] and arthritis [48], also induces cytokine production and cell death *in vitro*, although to a much lesser extent than pristane. Both oils may, therefore, work engaging similar mechanisms. The increased capacity of pristane, when compared with hexadecane, to induce autoimmunity *in vivo* is mirrored by its higher activity *in vitro* [15,48].

The identification of factors influencing the potency of different hydrocarbon oils in the induction of autoimmune and in chronic inflammatory diseases may provide important clues to identify potentially harmful environmental and food contaminants in the future.

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