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Distinctive Patterns of Autoimmune Response Induced by Different Types of Mineral Oil

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Although mineral oils are generally considered nontoxic and have a long history of use in humans, the mineral oil Bayol F (incomplete Freund's adjuvant, IFA) and certain mineral oil components (squalene and n-hexadecane) induce lupus-related anti-nRNP/Sm or –Su autoantibodies in nonautoimmune mice. In the present study, we investigated whether medicinal mineral oils can induce other types of autoantibodies and whether structural features of hydrocarbons influence autoantibody specificity. Female 3-month-old BALB/c (16–45/group) mice each received an i.p. injection of pristane (C19), squalene (C30), IFA, three medicinal mineral oils (MO-F, MO-HT, MO-S), or PBS. Sera were tested for autoantibodies and immunoglobulin levels. Hydrocarbons were analyzed by gas chromatography/mass spectrometry. IFA contained mainly C15–C25 hydrocarbons, whereas MO-HT and MO-S contained C20–C40, and MO-F contained C15–C40. Pristane and n-hexadecane were found in IFA (0.17% and 0.10% w/v, respectively) and MOs (0.0026–0.027%). At 3 months, pristane and IFA induced mainly IgG2a, squalene IgG1, and MOs IgG3 and IgM in sera. Anti-cytoplasmic antibodies were common in mice treated with MO-F, as well as those treated with pristane, squalene, and IFA. Anti-ssDNA and -chromatin antibodies were higher in MO-F and MO-S than in untreated/PBS, squalene-, or IFA-treated mice, suggesting that there is variability in the induction of anti-nRNP/Sm versus –chromatin/DNA antibodies. The preferential induction of anti-chromatin/ssDNA antibodies without anti-nRNP/Sm/Su by MO-S and MO-F is consistent with the idea that different types of autoantibodies are regulated differently. Induction of autoantibodies by mineral oils considered nontoxic also may have pathogenetic implications in human autoimmune diseases.

Key Words: **mineral oil; pristane; autoimmunity; autoantibodies; antinuclear antibodies; lupus.**

Mineral oils are generally considered "nontoxic" and have been used extensively in food, cosmetics, medicines, and other products (Nash *et al.*, 1996). In the food industry, mineral oils are used as a protective coating for fruits and vegetables, for baking, and as defoamers. Contamination of foods with mineral oil is well documented (Grob *et al.*, 1997). Occupational exposure to mineral oil mist also is common (Simpson *et al.*, 2003). There is some evidence that mineral oil exposure may be associated with human disease. Subcutaneous injection of mineral oil induces sclerosing lipogranulomas, a chronic local inflammatory reaction (Di Benedetto *et al.*, 2002), and aspiration causes a severe chronic pneumonitis termed "lipoid pneumonia" (Spickard and Hirschmann, 1994). The oil is absorbed through the intestine and distributes throughout the body, causing lipogranulomas in the lymph nodes, liver, and spleen of healthy individuals (Dincsoy *et al.*, 1982). Oral or intraperitoneal i.p. administration of mineral oil induces similar lesions in laboratory animals (Firriolo *et al.*, 1995; Shaheen *et al.*, 1999). Although it is not known whether chronic oral exposure to mineral oils can induce human disease, the above data have led to proposals to ban its use as a food additive in the United Kingdom (Nash *et al.*, 1996).

Pristane (2,6,10,14-tetramethylpentadecane) and mineral oil induce plasmacytomas in susceptible strains of mice (Anderson and Potter, 1969). Pristane, incomplete Freund's adjuvant (IFA), and squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,-18,22-tetracosahexaene) induce chronic arthritis in mice and rats (Cannon *et al.*, 1993; Carlson *et al.*, 2000; Wooley *et al.*, 1989). We have recently reported that, in addition to pristane (Satoh *et al.*, 1995; Satoh and Reeves, 1994), IFA and squalene, but not medicinal mineral oils, can induce lupus-related anti-nRNP/Sm and –Su autoantibodies in non-autoimmune-prone strains of mice (Satoh *et al.*, 2003a). These data suggest that hydrocarbons can have a variety of immune effects.

In the present study, we have analyzed various types of hydrocarbon by gas chromatography/mass spectrometry (GC/MS) and contrasted the immune response caused by medicinal oils and adjuvant oils. All hydrocarbons, including medicinal oils, induced hypergammaglobulinemia as well as autoantibodies, but the pattern was different. Since humans are exposed to a variety of hydrocarbons in daily life, there may be implications for the pathogenesis of autoimmune disease.

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MATERIALS AND METHODS

Treatment of mice. Female BALB/cJ mice (4 weeks old) were purchased from Jackson Laboratory (Bar Harbor, ME), and housed at a virus-free animal facility in barrier cages. At 3 months of age, 16–45 mice per group each received a single intraperitoneal injection (0.5 ml) of pristane (Sigma, Chemical Co, St. Louis, MO), squalene (Sigma), IFA (Difco Laboratories, Detroit, MI), or one of three medicinal mineral oils (MO-F: C.B. Fleet Co. Inc., Lynchburg, VA, MO-HT: Harris Teeter, Matthews, NC, MO-S: E.R. Squibb and Sons Inc., Princeton, NJ) (Satoh et al., 1995; Satoh and Reeves, 1994; Satoh et al., 2003a). Monthly collected sera from a previous study (Satoh et al., 2003a), as well as additional samples available only at 3 or 6 months (4–21 mice/group in pristane, MO-S, MO-F, and PBS/untreated groups), were included in the analysis. Medicinal mineral oils are complex mixtures consisting mainly of hydrocarbons containing 15–40 carbons (C15–C40, see results). Control mice received phosphate buffered saline (PBS) or were left untreated. Since these two groups showed very similar results in autoantibody analysis, they were analyzed as a single untreated/PBS group. Sera were collected from the tail vein before injection, at 2 weeks, and monthly thereafter for 6 months. This study was approved by IACUC of the University of North Carolina at Chapel Hill (Chapel Hill, NC) and the University of Florida (Gainesville, FL).

Analysis of mineral oils by gas chromatography/mass spectrometry (GC/MS). A 100-microliter sample was diluted to 10 ml in hexane for analysis using an HP6890 gas chromatograph coupled to an HP5973 mass selective detector (Hewlett Packard Company; Wilmington, DE). The components of the injected sample (1 μl) were separated across an HP-5MS column (30 m $\times$ 0.25 mm; 0.250 mm film thickness) under an oven program that ramped from an initial temperature of 60°C at 10°C/min to 270°C (5 min hold); then increased at 5°C/min to 300°C (20 min hold). Each analyte of interest (pristane, n-hexadecane, and squalene) was quantified against a five-point standard curve ($R^2 \geq 0.9999$). Quantification was based on the abundance of the analyte target ion (pristane and n-hexadecane, m/z = 57; squalene, m/z = 341) in ratio with the internal standard pyrene (m/z = 202).

Immunoglobulin ELISA. Serum immunoglobulin levels at 3 months were measured by sandwich ELISA as described (Hamilton et al., 1998). Levels of IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM were determined using 1:200,000 and/or 1:500,000 sera using mouse immunoglobulin isotype controls as standard (mouse immunoglobulin panel, Southern Biotechnology, Birmingham, AL).

Anti-ssDNA antibody and anti-chromatin antibody ELISA. Levels of anti-ssDNA and –chromatin antibodies in sera (1:250 dilution) from BALB/cJ mice 3 and 6 months after treatment were tested by ELISA using calf thymus ssDNA (Sigma) and chicken chromatin, respectively, as antigen (Satoh et al., 2000b). Chicken chromatin was purified from chicken erythrocytes (Pel-Freeze Biologicals, Rogers, AR) as previously described (Sung et al., 1977). Positives were defined as samples showing OD higher than the mean + 3 SD of 10 blank wells.

Immunofluorescent antinuclear antibodies (ANA). The levels of antinuclear antibodies in sera from BALB/cJ mice at 3 and 6 months after treatment were determined by indirect immunofluorescence using L929 (mouse fibroblast) cells as described (Satoh et al., 1996). Sera were screened at a 1:40 dilution, and the titers were estimated using a titration emulation system ImageTiter (RhiGene, Inc., Des Plaines, IL) (Yoshida et al., 2002).

Statistical analysis. Frequencies of autoantibodies were compared by Fisher's exact test. Levels of immunoglobulins and autoantibodies were compared by the Mann-Whitney test.

RESULTS

Serum Immunoglobulin Levels

Following pristane treatment, a dramatic increase in total IgM and IgG3, along with IgM anti-ssDNA antibodies, was generally apparent at 2 weeks, and was followed by increased levels of the T cell-dependent immunoglobulin isotopes IgG1, IgG2a, and IgG2b (Hamilton *et al.*, 1998). The levels of these isotypes at 3 months after treatment were measured by ELISA (Fig. 1). Pristane treatment increased IgG1 ($p < 0.0005$ vs. PBS control, Mann-Whitney test), IgG2a ($p < 0.0001$), and IgG2b ($p < 0.0001$), with predominant effects on IgG2a. IFA increased both IgG1 ($p < 0.0001$ vs. PBS control) and IgG2a ($p < 0.0001$). Squalene dramatically increased IgG1 ($p < 0.0001$ vs. PBS control) and IgG3 ($p < 0.005$), and had less effect on IgG2a ($p < 0.0005$). Medicinal mineral oils all induced hypergammaglobulinemia, but their effects on the IFN dependent isotype IgG2a were small (MO-F and MO-S: not significant vs. PBS control; MO-HT: $p < 0.005$) compared with pristane or IFA. In contrast, IgG3 levels were increased significantly by two of three mineral oils (MO-F: $p < 0.0001$; MO-S: $p < 0.0001$).

Antinuclear Antibodies

Titers of antinuclear (Fig. 2A) and anti-cytoplasmic (Fig. 2B) antibodies in sera from BALB/cJ mice 3 months after treatment were examined by indirect immunofluorescence. Titers of antinuclear antibodies (Fig. 2A) in the pristane- or IFA-treated mice were higher than in the untreated mice ($p < 0.01$ and $p < 0.05$, respectively, Mann-Whitney test). A few MO-treated mice produced antinuclear antibodies, but no difference in mean titers were found versus untreated mice (Fig.

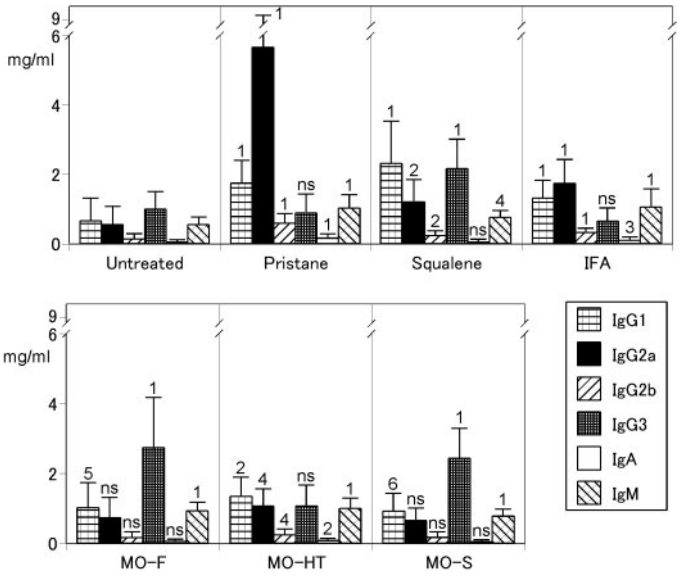

FIG. 1. Serum immunoglobulin levels. Total serum IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM levels were measured (ELISA) 3 months after treatment. Mean values for 16–20 mice per group with standard deviation are shown. Levels of IgG1 and IgG2a were previously shown (Satoh *et al.* 2003a) but were included with data using 4–12/group of additional mice for comparison. (1) $p < 0.0001$; (2) $p < 0.0005$; (3) $p < 0.001$; (4) $p < 0.005$; (5) $p < 0.01$, (6) $p < 0.05$; ns, not significant. All comparisons are versus the PBS/untreated group (Mann-Whitney test).

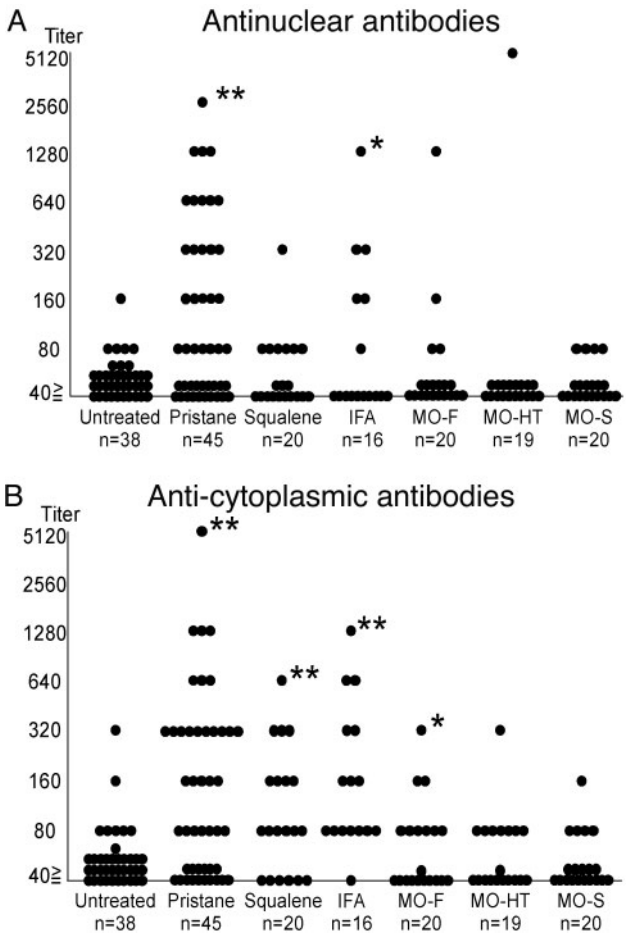

FIG. 2. Titers of antinuclear and anti-cytoplasmic antibodies by immunofluorescence. Titers of antinuclear and anti-cytoplasmic antibodies in sera from BALB/cJ mice 3 months after treatment (PBS/untreated, pristane, squalene, IFA, MO-F, MO-HT, MO-S) were examined by immunofluorescence with titration emulation. (A) Titers of antinuclear antibodies. Titers in pristane and IFA treated groups were higher than untreated group (*$p < 0.05$; **$p < 0.01$ by Mann-Whitney). (B) Titers of anti-cytoplasmic antibodies. Groups treated with pristane, squalene, IFA, or MO-F had higher titers of anti-cytoplasmic antibodies than untreated group (*$p < 0.05$; **$p < 0.01$, Mann-Whitney test).

2A). Anti-cytoplasmic antibodies were commonly found in pristane-, squalene-, or IFA-treated mice ($p < 0.01$), and to a lesser degree in the MO-F group ($p < 0.05$ vs. untreated group; $p < 0.05$ vs. pristane or IFA group, Mann-Whitney test). The

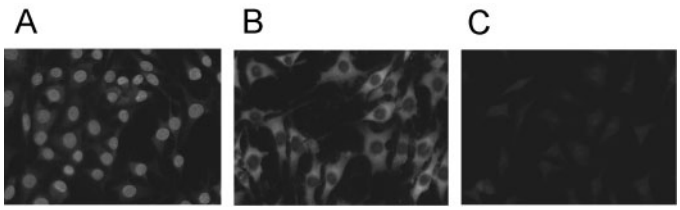

FIG. 3. Antinuclear and anti-cytoplasmic antibodies by immunofluorescence. L929 cells stained with serum from MO-F treated mice 3 months after treatment, showing nuclear (A) and cytoplasmic (B) staining. Negative staining by a serum from an untreated mouse is shown in (C). Serum dilution 1:40, original magnification 200×.

frequency of antinuclear and anti-cytoplasmic antibodies (1:80 or higher) is summarized in Table 1. Although the frequency of antinuclear antibodies was significantly higher than control only in the pristane group, anti-cytoplasmic antibodies were more frequent in mice treated with pristane, squalene, IFA, or MO-F than in controls ($p < 0.01$–0.05 by Fisher's exact test). Representative nuclear and cytoplasmic staining by sera from mice treated with MO-F, and negative staining by serum from an untreated mouse are shown in Figures 3A–3C, respectively. The high frequency of cytoplasmic staining suggests that autoantibodies other than anti-nRNP/Sm, –Su, and –chromatin are produced in response to IFA, squalene, and other hydrocarbons. Some of the cytoplasmic staining probably reflects production of autoantibodies to heat shock proteins located in cytoplasm, as described in pristane-treated mice (Thompson *et al.*, 1990).

Anti-ssDNA and Anti-Chromatin Antibodies

Levels of IgG anti-ssDNA and anti-chromatin antibodies by ELISA are shown in Figures 4A and 4B, respectively. Many sera from mice treated with pristane, MO-F, or MO-S produced low levels (~1–10 units) of anti-ssDNA antibodies (positive control MRL/*lpr* mouse serum at same dilution was 3,125 units). Mice treated with pristane, MO-F, or MO-S had significantly higher levels of anti-ssDNA antibodies than untreated ($p < 0.01$) and squalene- or IFA-treated mice ($p < 0.01$–0.05 by Mann-Whitney test). Few mice in the squalene, IFA, and MO-HT groups produced this specificity. IgG anti-chromatin antibodies were produced by mice treated with pristane, IFA (p

TABLE 1
Frequency of Anti-Nuclear and -Cytoplasmic Antibodies by Immunofluorescence in BALB/cJ Mouse Sera 3 Months After Treatment

	Untreated ($n = 38$)	Pristane ($n = 45$)	Squalene ($n = 20$)	IFA ($n = 16$)	MO-F ($n = 20$)	MO-HT ($n = 19$)	MO-S ($n = 20$)
Nuclear	13%	58%*	35%	38%	20%	5%	20%
Cytoplasmic	18%	64%*	70%*	94%*	45%**	42%	25%

*$p < 0.01$; **$p < 0.05$ (one-sided) by Fisher's exact test versus untreated.

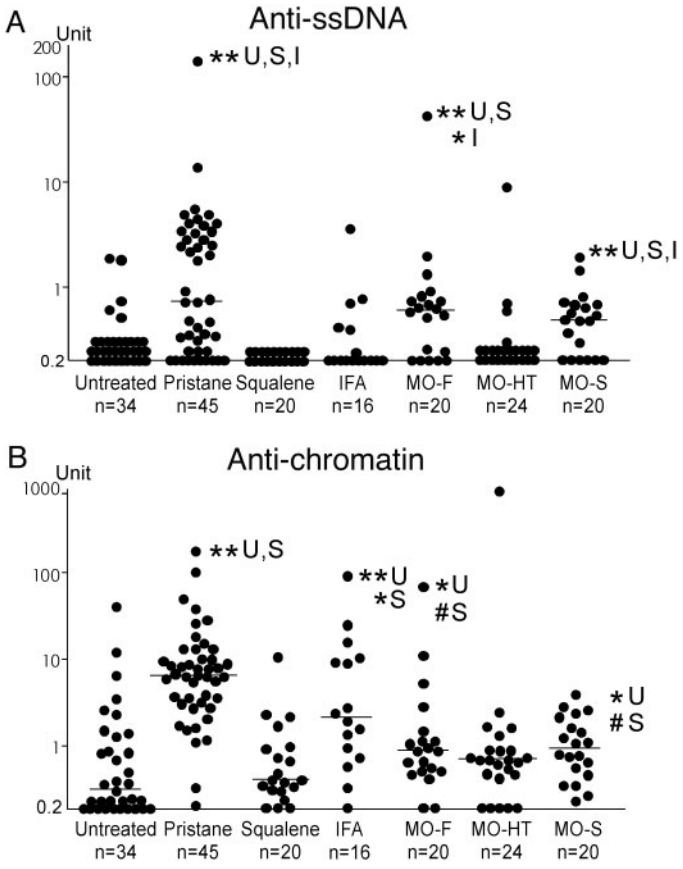

FIG. 4. IgG anti-ssDNA and anti-chromatin antibodies by ELISA. Levels of IgG anti-ssDNA and –chromatin antibodies in sera (1:250 dilution) from BALB/cJ mice 3 months after treatment (PBS/untreated, pristane, squalene, IFA, MO-F, MO-HT, MO-S) were examined by ELISA. (A) IgG anti-ssDNA antibodies. Mice treated with pristane, MO-F, or MO-S had significantly higher levels than untreated, or IFA or squalene-treated group ($p < 0.01$–0.05 by Mann-Whitney test). (B) IgG anti-chromatin antibodies. Groups treated with pristane, IFA, MO-F, or MO-S had significantly higher titers than the untreated group. The MO-F and MO-S groups had higher titers than the squalene group. *$p < 0.01$; **$p < 0.05$; $p = 0.06$; U: versus untreated group; I: versus IFA-treated group; S: versus squalene-treated group. Median in each group is indicated by a horizontal line.

< 0.01 vs. untreated, Mann-Whitney), MO-F ($p < 0.05$), or MO-S ($p < 0.05$). IgG anti-chromatin antibodies appeared to be more common in the MO-F and MO-S groups than in the squalene group ($p = 0.06$, Mann-Whitney). Levels of anti-ssDNA and -chromatin antibodies were higher at 6 months with similar differences between the groups (not shown).

Relationship Between Production of Anti-nRNP/Sm or –Su Antibodies, Anti-Chromatin, and Anti-ssDNA Antibodies

Although medicinal oils did not induce lupus-related anti-nRNP/Sm or –Su autoantibodies (Satoh *et al.*, 2003a), they often were more efficient in inducing anti-ssDNA or –chromatin antibodies than IFA or squalene (Figs. 4A and 4B). Therefore, the relationship between the frequency of anti-nRNP/Sm or –Su versus anti-chromatin or anti-ssDNA antibodies in each group was analyzed (Figs. 5A and 5B). The hydrocarbon oils tested could be classified into two groups (dotted line) based on their ability to induce different types of autoantibodies. One group of oils (MO-F, MO-HT, and MO-S) predominantly induced IgG anti-chromatin and anti-ssDNA antibodies but not anti-nRNP/Sm or –Su antibodies. It is possible that this group of oils may simply enhance the spontaneous production of anti-ssDNA/chromatin antibodies seen in some untreated mice. The other group of oils (pristane, IFA, and squalene) induced both groups of autoantibodies, but the induction of anti-chromatin and -ssDNA antibodies may be less efficient than those by some medicinal oils. In contrast, anti-chromatin and –ssDNA antibody production correlated fairy well ($R = 0.677$, $p = 0.095$) (Fig. 5C).

Analysis of Mineral Oil by Gas Chromatography/Mass Spectrometry (GC/MS)

To examine the basis for the different autoantibodies induced by IFA versus medicinal mineral oils, we analyzed the composition of the four oils. Total ion chromatograms of IFA and the three medicinal mineral oils (MO-F, MO-HT, and MO-S) are shown in Figure 6. By GC/MS, n-hexadecane (C16), pristane (C19), and squalene (C30) were each determined to have a purity >99.9%. For comparison with the complex oils, the mass spectrum of these three hydrocarbons is

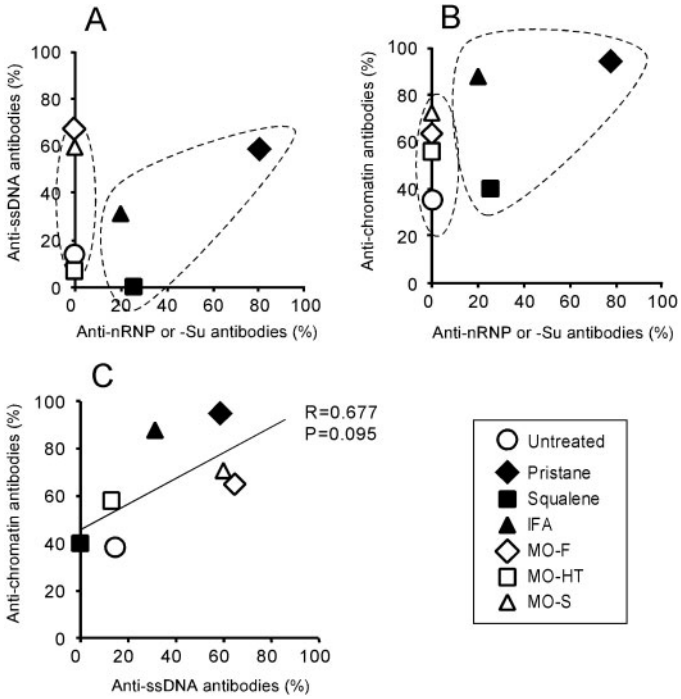

FIG. 5. Relationship between frequencies of different types of autoantibodies. Relationship between the frequency of anti-nRNP/Sm or –Su (immunoprecipitation), anti-chromatin, or anti-ssDNA (ELISA) antibodies in each group was analyzed. (A) Frequency of anti-ssDNA versus anti-nRNP/Sm or –Su. (B) Frequency of anti-chromatin versus anti-nRNP/Sm or –Su. C. Frequency of anti-chromatin versus –ssDNA antibodies.

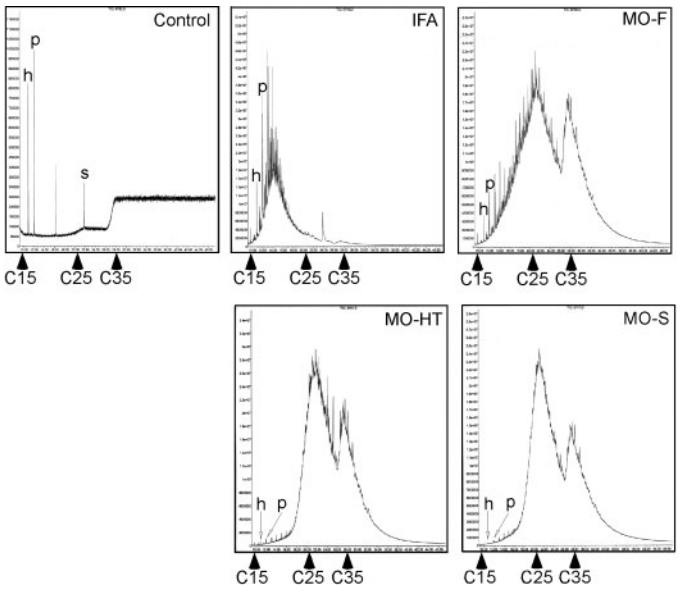

FIG. 6. Analysis of hydrocarbon oils by gas chromatography/mass spectrometry. IFA and three different brands of medicinal mineral oils (MO-F, MO-HT, and MO-S) were analyzed by GC/MS. Since GC/MS analysis showed no indication of contamination in pristane, n-hexadecane, and squalene, these were mixed and used as control. X-axis indicates retention time, which correlates with the carbon number of straight-chain aliphatic hydrocarbons, as indicated. The large bulge in the IFA and MO chromatograms represents an unresolved complex mixture of hydrocarbons. Position of n-hexadecane (h), pristane (p), and squalene (s) in each chromatogram is indicated. Note the different X-axis scale.

also shown (Fig. 6, control). The mineral oil Bayol F (IFA) consists mainly of C15–C25 hydrocarbons with a peak concentration at ~C20, whereas MO-HT and MO-S were mainly C20–C40 hydrocarbons with peaks at ~C28 and C35. The pattern of MO-F was similar to the other medicinal oils, though it contained more C15–C25 hydrocarbons than the others. The concentrations of hydrocarbons that induce lupus-related anti-nRNP/Sm and –Su antibodies in nonautoimmune mice, including pristane, squalene, (Satoh *et al.*, 2003a), and n-hexadecane (Y. Kuroda *et al.*, unpublished) in the various mineral oils are summarized in Table 2. IFA, which induced anti-nRNP/Sm and –Su antibodies (Satoh *et al.*, 2003a), contained 9- to 67-fold more pristane, and 4- to 40-fold more n-hexadecane than medicinal oils; however, the actual concentrations were only 0.17% and 0.10% (w/v), respectively. MO-F contained a larger amount of small molecular weight (C15–25) hydrocarbons, including n-hexadecane and pristane, than the other two medicinal oils.

DISCUSSION

Mineral oils are generally considered nontoxic and have a long history of use in food, cosmetics, medicine, and other products (Nash *et al.*, 1996). However, it is also well described that mineral oils have significant inflammatory or immunological effects following their injection, ingestion, or inhalation.

Intraperitoneal injection of pristane induces plasmacytomas (Anderson and Potter, 1969), destructive arthritis (Wooley *et al.*, 1989), and a lupus-like autoimmune syndrome (Satoh *et al.*, 1995; Satoh and Reeves, 1994) in nonautoimmune strains of mice. IFA and squalene also induce autoimmune chronic arthritis in mice and rats (Cannon *et al.*, 1993; Carlson *et al.*, 2000). In addition, we recently reported that intraperitoneal injection of IFA or the adjuvant oil squalene (MF59) (O'Hagan *et al.*, 1997), but not medicinal mineral oils, can induce lupus-related autoantibodies to nRNP/Sm and Su, like pristane (Satoh *et al.*, 2003a). GC/MS analysis of hydrocarbon oils revealed that IFA contains 10- to 20-fold more pristane and n-hexadecane than medicinal oils (Table 2), though this represents only ~0.1% (w/v) of IFA. Considering the fact that various hydrocarbons, including pristane, squalene (Satoh *et al.*, 2003a), and n-hexadecane (Y. Kuroda *et al.*, manuscript in preparation), can induce lupus autoantibodies, it is possible that induction of these autoantibodies is not due to the presence of traces of pristane in IFA. Instead, the present data suggest that the induction of autoantibodies may correlate better with the amount of C15–C25 hydrocarbons present in an oil. Consistent with this interpretation, the medicinal oil that most efficiently induced autoantibodies was MO-F, the one with the highest level of C15–C25 hydrocarbons. Interestingly, hydrocarbons with these carbon numbers (C15–C25) represent the optimal size for adjuvanticity, as well (Whitehouse *et al.*, 1974). Thus, pristane and n-hexadecane may be representative of a much larger class of hydrocarbons within the C15–C25 range with the capacity to induce antinuclear or anti-cytoplasmic antibodies. However, it is clear that other factors are involved as well, since squalene (C30) also induces autoantibodies to nRNP/Sm and Su.

The pathogenesis of lupus in human and mice is believed to

TABLE 2
Analysis of Hydrocarbons by Gas Chromatography/Mass Spectrometry

	Distribution	Peak	n-Hexadecane (C16) (μg/ml)	Pristane (C19) (μg/ml)	Squalene (C30) (μg/ml)
IFA	C15–C25	C20	1,009.00	1,703.40	<1
MO-HT	C20–C40	C28, C35	52.00	88.74	<1
MO-S	C20–C40	C28, C35	26.00	25.50	<1
MO-F	C15–C40	C28, C35	274.00	194.82	<1

result from the interaction of genetic and environmental factors (Hess, 2002). Different subsets of lupus are associated with unique symptoms with different autoantibodies (Reeves and Satoh, 2001). However, the mechanisms remain poorly understood. It has been shown that low-affinity polyreactive IgM autoantibodies produced by B-1 cells are regulated differently than high-affinity IgG autoantibodies produced by conventional B-cells (Reap *et al.*, 1993). Data from our laboratory strongly suggest that different pathways exist for different type of autoantibodies. IL-6 plays an essential role for anti-chromatin and -dsDNA antibodies (Richards *et al.*, 1998), whereas IFN is critical for anti-nRNP/Sm and anti–Su (Richards *et al.*, 2001). In NZB/W F1 and CBA/N (*xid*) mice, anti-RNA helicase A (RHA) antibodies are produced spontaneously along with IL-4 and IL-6, but pristane treatment shifts the cytokine balance toward IFN and IL-12, suppresses anti-RHA production, and induces anti-nRNP/Sm and –Su antibodies (Satoh *et al.*, 2003b; Yoshida *et al.*, 2002). The *lpr* gene induces high levels of anti-chromatin/DNA antibodies but not anti-nRNP/Sm or –Su in C57BL/6 mice, whereas pristane treatment induces the latter antibodies with only low levels of the anti-chromatin/DNA antibodies (Satoh *et al.*, 2000a).

The present data indicate that, whereas some hydrocarbons (squalene, IFA) predominantly induce anti-nRNP/Sm and –Su antibodies, other hydrocarbons (MO-F, MO-S) efficiently induce anti-chromatin/DNA antibodies but not anti-nRNP/Sm or –Su. All hydrocarbons induced hypergammaglobulinemia, but the T-cell-dependent subclasses IgG1 and IgG2a were characteristic of pristane, IFA, and squalene, which induce anti-nRNP/Sm or –Su autoantibodies (Satoh *et al.*, 2003a). The T-independent subclasses IgG3 and IgM were prominent in medicinal oil-treated mice. The predominant increase in IgG3 and IgM along with possible enhancement of spontaneous anti-ssDNA/chromatin antibody production was consistent with what is seen in silicone oil-treated mice (Naim *et al.*, 2000). The former oils are associated with early IL-12 production, whereas the latter group also induces IL-6 and TNF-α, especially at late stages of inflammation (Satoh *et al.*, 2003a). These data suggest that different mechanisms drive the production and regulation of subsets of autoantibodies induced by different type of oils. It is possible that high molecular weight hydrocarbons (>C25) are prone to induce anti-chromatin/DNA antibodies, whereas lower molecular weight hydrocarbons (C15–C25) with adjuvant activity efficiently induce autoantibodies to nonchromatin antigens such as nRNP/Sm and Su, perhaps through the more efficient recruitment of T-cell help, reflected in the shift from IgM/IgG3 to IgG2a or IgG1. We suggest that the production of autoantibodies in this model is not simply the consequence of nonspecific inflammation, but rather it involves mechanisms specific for particular antigens, which maybe differently recognized in mice treated with different hydrocarbons.

The question arises of the significance of these findings for the pathogenesis of human disease. It might be argued that humans are not exposed to hydrocarbons via the intraperitoneal route and that other routes of exposure pose less risk. For instance, the immunotoxicity of aminocarb varies depending on the route of administration, with intraperitoneal exposure resulting in greater toxicity than oral administration (Bernier *et al.*, 1995). Humans are exposed to mineral hydrocarbons via ingestion (foods, medications) (Dincsoy *et al.*, 1982; Grob *et al.*, 1997; Heimbach *et al.*, 2002), inhalation (diesel exhaust, oil mists, aspiration of ingested mineral oil) (Simpson *et al.*, 2003; Spickard and Hirschmann, 1994), skin absorption (cosmetics, skin contact with oils or fuels) (Nash *et al.*, 1996; Riviere *et al.*, 1999), or injection (immunization, accidental inoculation) (Di Benedetto *et al.*, 2002; O'Hagan *et al.*, 1997). It is not known with certainty whether nonperitoneal exposure to hydrocarbons can induce autoimmunity. However, it is clear that such exposure can cause an intense inflammatory reaction (lipogranulomas) in the lungs, liver, and regional lymph nodes (Dincsoy *et al.*, 1982). Moreover, ingested mineral oil is absorbed through the intestine and becomes widely distributed throughout the body (Bollinger, 1970; Ebert *et al.*, 1966). Mouse experiments are in progress to determine whether nonperitoneal exposure to pristane can induce the autoantibodies seen following intraperitoneal injection.

Another issue is whether the hydrocarbon doses given to the mice are ever reached in humans. Although the single 0.5-ml ip dose given to mice is quite large, humans can be exposed to considerable quantities of hydrocarbon oils over the course of a lifetime. It has been estimated that an average person living in a developed country ingests 50 grams of mineral oil per year in food (Heimbach *et al.*, 2002). Individuals using mineral oil chronically as a laxative have much greater exposure. At the recommended dose of 1–3 tablespoons (15–45 ml) per day, exposure may be as high as 0.75 ml/kg/day for a 60-kg person, or the equivalent of 20 μl/day in a mouse weighing 26.7 grams. On a kg-per-kg basis, the intraperitoneal dose given to the mice in these studies is comparable to that of a human ingesting mineral oil as a laxative for 25 days.

In summary, the present data indicate that the lupus-inducing hydrocarbons pristane, squalene, and n-hexadecane were present only in trace amounts in IFA or medicinal mineral oils. Although medicinal mineral oils did not induce anti-nRNP/Sm, or –Su autoantibodies (Satoh *et al.*, 2003a), they promoted anti-chromatin/DNA autoantibody production even more efficiently than squalene or IFA, suggesting that different types of autoantibodies could be generated in response to different hydrocarbons. Since mineral oils are nearly ubiquitous in the environment, whether these substances taken orally have a potential to trigger autoimmunity in human warrants further study.

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