Adverse immunological effects and autoimmunity induced by dental amalgam and alloy in mice

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ABSTRACT **Dental amalgam fillings are the most important source of mercury exposure in the general population, but their potential to cause systemic health consequences is disputed. In this study, inbred mice genetically susceptible to mercury-induced immune aberrations were used to examine whether dental amalgam may interfere with the immune system and cause autoimmunity. Female SJL/N mice were implanted in the peritoneal cavity with 8–100 mg silver amalgam or silver alloy for 10 weeks or 6 months. Chronic hyperimmunoglobulinemia, serum IgG autoantibodies targeting the nucleolar protein fibrillarin, and systemic immune-complex deposits developed in a time- and dose-dependent manner after implantation of amalgam or alloy. Splenocytes from mice implanted with amalgam or alloy showed an increased expression of class II molecules. The functional capacity of splenic T and B cells was affected in a dose-dependent way: 10 weeks of low-dose and 6 months of high-dose amalgam implantation strongly increased mitogen-induced T and B cell proliferation, whereas 10 weeks of high-dose implantation decreased the proliferation. Not only mercury but also silver accumulated in the spleen and kidneys after amalgam implantation. In conclusion, dental amalgam implantation in a physiological body milieu causes chronic stimulation of the immune system with induction of systemic autoimmunity in genetically sensitive mice. Implantation of silver alloy not containing mercury also induced autoimmunity, suggesting that other elements, especially silver, have the potential to induce autoimmunity in genetically susceptible vertebrates. Accumulation of heavy metals, from dental amalgam and other sources, may lower the threshold of an individual metal to elicit immunological aberrations. We hypothesize that under appropriate conditions of genetic susceptibility and adequate body burden, heavy metal exposure from dental amalgam may contribute to immunological aberrations, which could lead to overt autoimmunity.—Hultman, P., Johansson, U., Turley, S. J., Lindh, U., Enestrom, S., Pollard, K. M. Adverse immunological effects and autoimmunity induced by dental amalgam and alloy in mice. *FASEB J.* 8, 1183–1190 (1994)**

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DENTAL AMALGAM FILLINGS, WHICH CONTAIN approximately 50% mercury, are now accepted to be the major source of mercury exposure in the general population (1, 2). This is reflected in the good correlation between the number of amalgam fillings on the one hand and, on the other hand, blood (3) and urinary (4) mercury levels as well as the amount of mercury excreted in the urine after chelation drug challenge (5). Furthermore, mercury levels in the brain (6, 7) as well as in the kidney (7) show a significant correlation with the number of amalgam fillings. Recent studies performed with sheep (8) and monkeys (9) also indicate that a substantial amount of mercury released from insertion of dental amalgam fillings accumulates in the body. Although mercury exposure from dental amalgam fillings stems primarily from mercury vapor inhaled into the respiratory tract, amalgam microparticles, vapor, and dissolved mercuric ions may mix with saliva and food and be swallowed and absorbed in the gastrointestinal tract (8, 10). Furthermore, oral tissues such as gum mucosa, the tooth root, and surrounding bone accumulate mercury from the fillings (8–11).

Despite this overwhelming evidence linking dental silver amalgam fillings to tissue mercury accumulation, controversy exists concerning the possible adverse health effects of the accumulated mercury (12, 13). The chair of WHO's group on environmental health criteria for inorganic mercury has recently concluded that an adverse health effect in 10% or more of the population exposed to mercury from amalgam fillings can be excluded on the basis of the literature (14). However, the available data were deemed insufficient in order to exclude such an effect in 1% or less of the exposed population. Because of the large number of individuals exposed—it is estimated that 100 million amalgam fillings are inserted annually in the United States (15)—such an effect would still indicate a major general health problem.

The immune system is of special interest as a target for low-dose inorganic mercury exposure (2). Case reports of accidental and occupational mercury exposure clearly show that mercury can induce autoimmune reactions and/or systemic immune deposits, and some individuals seem to be especially susceptible (16–18). It is also well documented in rodent models that the susceptibility to mercury-induced systemic autoimmunity is strongly dependent on genetic constitution (19). Very few studies have been designed to ad-

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[2]Abbreviations: ACA, antichromatin antibodies; AFA, antifibrillarin antibodies; AHA, antihistone antibodies; ANoA, antinucleolar antibodies; Con A, concanavalin A; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; I-A*s*, class II molecule encoded by H-2A*s*; IP, immunoprecipitation; LPS, *Escherichia coli* lipopolysaccharide; mAb, monoclonal antibody; RRL, rabbit reticulocyte lysate.

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dress specifically the question of possible adverse effects of dental amalgam fillings on the immune system; some of these have been reported as showing adverse effects (20), others have not (21, 22). Langworth et al. (23) examined 21 persons with alleged disturbances due to dental amalgam fillings. Although several parameters of their immune function did not differ significantly from controls, one individual had antinuclear autoantibodies and markedly augmented lymphocyte proliferation without a detectable explanation. Similarly, Hickel et al. (24) in a study of 50 dental amalgam carriers with symptoms found three patients with distinctly abnormal immunological status, including altered CD4/CD8 (T helper/T suppressor) cell ratios and antinuclear antibodies.

These studies must be interpreted with caution, because mercury and other metals in amalgam, like most chemicals, may cause adverse immunological effects only in individuals with genetically determined susceptibility (25), making it difficult to examine dose-response relationships in a mixed population of susceptible and resistant persons. This experimental study, which aimed at examining the immunological effects of amalgam implantation, was designed to avoid such problems by using an inbred, genetically homogeneous mouse strain susceptible to mercury-induced systemic autoimmunity (26–28).

METHODS

Mice

Inbred female SJL/N mice were obtained from BomMice Breeding and Research Centre, Ry, Denmark. All animals were 8–10 weeks old at the beginning of the experiment. Food and housing conditions of these mice were as described earlier (27).

Treatment

Mice were given mercury treatment either through the drinking water, in the form of $HgCl_2$, or through i.p. implantation of dental amalgam. For the former treatment, mercuric-II-chloride of analytical grade (E. Merck, Darmstadt, Germany) was added to tap water, diluted to obtain a concentration of 5 or 2.5 mg$HgCl_2$/l, and given to groups of seven mice as drinking water ad libitum for 2 weeks and 6 months, respectively. Fresh solutions were made twice a week. For i.p. amalgam implantation, eight groups of mice were given i.p. injections of 90 mg/kg body wt of thiopental sodium (Pentothal sodium; Abbott SpA, Italy) and underwent laparotomy using an aseptic technique. Silver amalgam was obtained by mixing lathe-cut, fine-grain alloy (DAB Fine, Dental AB, Sweden) and elemental mercury in a Dentomat for 40 s in a ratio of 1:1.3 according to the instructions provided by the manufacturer. The alloy consisted of Ag 67.4%, Sn 26.3%, Cu 5.8%, and Zn 0.5%. With the mixing ratios used, the amalgam contained 56% Hg, 29% Ag, 11% Sn, 2.5% Cu, and 0.2% Zn. The amalgam was either modified during the onset of condensation to a weight of approximately 100, 30, or 15 mg (±10%) or condensed for 5 days and then split into smaller pieces. The single pieces or, in the case of the 100 mg group, sets of four–six amalgam pieces with an approximate total weight of 100 mg (±10%) were kept under ultraviolet light for 2 days, put in sterilized gelatine capsules, and implanted in the peritoneal cavity using an aseptic technique. Two groups of mice were implanted with gelatine capsules containing 8.5 and 58 mg alloy. Controls were implanted with empty gelatine capsules. After 10 weeks or 6 months, mice were anesthetized with ether and exsanguinated via the retroorbital plexa.

Serum immunoglobulin concentrations

The serum concentration of IgM, IgG1, IgG2a, IgG2b, and IgG3 was determined by radial immunodiffusion as described before (29) using antisera and standards from Binding Site, Birmingham, U.K.

FACS analysis of splenic lymphocyte subsets

Approximately one-half the spleen was used for aseptic preparation of single-cell suspension, and the cells were stained according to the procedure described before (30) using fluorescein isothiocyanate (FITC)[2] -conjugated monoclonal antibodies to mouse IgM (R6-60.2), mouse I-A^s (class II molecule encoded by H-$2A_s$) (clone 7-16.17) (Pharmingen, San Diego, Calif.), or Thy-1.2 (clone 30-H12) (Becton Dickinson, Mountain View, Calif.). The samples were analyzed using a FACScan cytophotometer and LYSYS II software (Becton Dickinson). The amount of class II molecules on lymphocytes was expressed as the mean channel of fluorescence intensity in the population of splenocytes that showed a fluorescence intensity greater that of the isotype control.

Mitogen-induced lymphoproliferative response

Single-cell spleen suspensions were obtained as described above, and the mitogen-induced proliferation was determined as previously described (31).

Analysis of antinuclear antibodies

The presence, pattern, and titer of serum antinuclear antibodies of the IgG class were determined by indirect immunofluorescence using HEp-2 cells as a substrate (32). IgG and IgM anti-histone antibodies (AHA) were determined by enzyme-linked immunosorbent assay (ELISA) as described by Rubin (33). Antichromatin antibodies (ACA) were measured using the ELISA method of Burlingame and Rubin (34) with goat anti-mouse kappa chain-specific antibodies as detecting reagent. Immunoprecipitation (IP) was performed using in vitro translated mouse fibrillarin as antigen. Mouse fibrillarin labeled with [^{35}S]methionine was produced using a murine cDNA (35) and in vitro transcription and translation were performed using the TnT coupled reticulocyte lysate system kit (Promega, Madison, Wis.) according to the manufacturers directions. In a final volume of 600 µl, 100 µl of protein A Sepharose CL4B (Pharmacia, Uppsala, Sweden) was incubated with 5 µl serum in NET2 + F buffer (0.5% NP40, 150 mM NaCl, 5 mM EDTA, 50 mM Tris, pH 7.4, 0.02% NaN_3, 0.5% sodium deoxycholate, 0.1% sodium dodecylsulfate) at 4°C for 1 h and washed in NET2 + F buffer twice, followed by incubation with 10 µl of murine fibrillarin (35) translation products in 1 ml NET2 + F buffer at 4°C for 1.5 h. Beads were then washed three times with NET2 + F buffer and resuspended in 30 µl 2X LSB + 1 µl rabbit reticulocyte lysate (RRL). After boiling for 10 min, the samples were run on 15% SDS-PAGE, fluorographed, and exposed to film at −70°C overnight. Addition of excess protein from the RRL to the immunoprecipitates before SDS-PAGE was found to reduce smearing of radiolabeled fibrillarin precipitate and to enhance the resulting signal.

Tissue immune deposits

Pieces of the left kidney, myocardium, and spleen were examined by immunofluorescence as described before (27), using FITC-conjugated goat anti-mouse IgG and IgM antibodies (Southern Biotechnology, Birmingham, Ala.) and anti-C3c antibodies (Organon-Technica, West Chester, Pa.). The titer was determined by serial dilution of the antibodies to 1:5120. The end-point titer of the deposits was defined as the highest dilution of antibody at which a specific fluorescence could be detected. When no specific fluorescence was detected at a dilution of 1:40, the result was recorded as zero.

Tissue element concentrations

The right kidney and the spleen were excised from the body using instruments washed regularly in a fresh solution of 65% HNO_3. The kidney and half the spleen were transferred to plastic, conical Eppendorf tubes with a volume of 1.5 ml, which were kept sealed at −70°C until further analyzed. The wet weight of the organs was determined in a test tube of high-quality quartz used for mineralization. Nitric acid (65%) of very high purity (Scan-Pure, Chem Scand AS, Elverum, Norway) was added in a ratio of 1 ml acid to 0.1 g dry weight tissue, and the test tubes were sealed by a Teflon lid and put into a bomb of stainless steel, the capsules of which were tightened by a dynamometric wrench at 20 Nm. The bombs were transferred to an oven and heated at 180°C for 12 h. An indium solution was then added to each sample as an internal standard, and the specimens were analyzed using inductively coupled plasma masspectrometry (VG Plasma Quad). This analytical method has a multielement capacity. In the present work, all elemental concentrations were determined by mass spectrometry. Every fifth sample analyzed was a certified standard reference material. To analyze the kidney, we used BCR no. 186 pig kidney (Community Bureau of Reference, Brussels) and for the spleen samples we used NBS 1577a bovine liver (National Institute of Standards and Technology, Gaithersburg, Md.). The precision of analysis as revealed by BCR was ≤ 4% and by NBS was ≤ 3% for all elements determined. The accuracy of analysis was estimated accordingly to ≤ 5% for elements in the kidney and to ≤ 8% for elements in the spleen. The lower accuracy for the spleens is explained by the small weight of the organs.

Statistics

Wilcoxon's rank sum test was used to analyze differences in titer of antinucleolar antibodies (ANoA) serum, titer of immune deposits, serum Ig concentration, ELISA titers of AHA and ACA, the number of splenocytes positive for the different monoclonal antibody (mAb) stainings, incorporation of tritiated thymidine, and tissue element concentration. Differences in the number of mice showing ANoA were analyzed with Fisher's exact test.

TABLE 1. *Serum concentration of immunoglobulin isotypes*

Animal group	IgM	IgG1	IgG2a	IgG2b	IgG3
Controls[a,b]	0.3 ± 0.1[c]	1.2 ± 0.2	4.4 ± 0.2	0.9 ± 0.0	1.2 ± 0.1
Amalgam, 30 mg[b,d]	0.7 ± 0.1[e]	2.1 ± 0.3[f]	5.4 ± 0.6	0.9 ± 0.1	1.3 ± 0.8
Amalgam, 100 mg[b,d]	0.9 ± 0.4[e]	1.9 ± 0.1[f]	5.2 ± 0.4	1.0 ± 0.0	1.3 ± 0.2
Amalgam, 100 mg[b,g]	0.9 ± 0.4[e]	2.3 ± 0.3[f]	6.5 ± 0.3[h]	1.0 ± 0.1	1.2 ± 0.8
Alloy, 58 mg[b]	1.0 ± 0.5	2.5 ± 0.5[f]	5.5 ± 0.7	0.9 ± 0.0	1.4 ± 0.5
Controls, 6 months[a,i]	0.4 ± 0.1	2.0 ± 0.4	5.8 ± 0.5	0.9 ± 0.1	1.2 ± 0.8
Amalgam, 15 mg[d,i]	0.6 ± 0.0[f]	3.2 ± 0.2[f]	6.8 ± 1.0	1.0 ± 0.1	1.2 ± 0.1
Amalgam, 100 mg[d,i]	0.6 ± 0.2	3.1 ± 0.3[f]	7.7 ± 0.8	1.0 ± 0.0[f]	1.5 ± 0.2
Alloy, 8.5 mg[i]	0.6 ± 0.0[f]	2.8 ± 0.3	5.6 ± 0.9	0.9 ± 0.1	1.3 ± 0.0
$HgCl_2$, 2.5 ppm[j]	0.6 ± 0.1	3.1 ± 0.2[h]	7.0 ± 0.2[h]	1.0 ± 0.0[f]	1.3 ± 0.2

[a]Implantation of empty gelatin capsules. [b]Implantation for 10 weeks. [c]Values are mean ± SE (mg/ml). [d]Single piece of amalgam implanted in the peritoneal cavity. [e]Significantly different from controls ($P < 0.02$ using Wilcoxon's rank sum test). [f]Significantly different from controls ($P < 0.05$). [g]Multiple pieces of amalgam implanted in the peritoneal cavity. [h]Significantly different from controls ($P < 0.01$). [i]Implantation for 6 months. [j]Given in the drinking water.

RESULTS

Serum immunoglobulin isotype concentrations

Implantation of amalgam or alloy or peroral treatment with 2.5 ppm $HgCl_2$ caused chronic hyperimmunoglobulinemia, which was most pronounced and consistent for the IgM and IgG1 isotypes, although the serum IgG2a and IgG2b was significantly increased in mice with the highest body burden of mercury and in the perorally $HgCl_2$-treated mice (**Table 1**).

Splenocyte phenotypes and class II expression

No significant difference was found in the number or fraction of splenic B cells (surface IgM positive) or T cells (Thy-1.2 positive) among controls and the groups implanted with amalgam/alloy or treated with mercuric chloride perorally (data not shown). The mean intensity of class II molecules (I-A*s*) on splenocytes was increased in all mice implanted with amalgam or alloy for 6 months (**Table 2**).

Mitogen-induced lymphoproliferation

Implantation of 100 mg amalgam for 10 weeks resulted in severely reduced concanavalin A (Con A)- and *Escherichia coli* lipopolysaccharide (LPS) -induced proliferation, whereas implantation of 30 mg amalgam substantially increased both Con A- and LPS-induced proliferation (**Fig. 1**). Both the Con A and LPS reactivity was increased after implantation with 15 or 100 mg amalgam for 6 months. Implantation with alloy or peroral treatment with 2.5 ppm $HgCl_2$ failed to significantly influence the mitogen-induced lymphocyte proliferation.

Serum antinuclear antibodies

Serum autoantibodies with a nucleolar pattern (ANoA) (**Fig. 2*A***) developed in a fraction of all mice implanted with amalgam/alloy, except in the 30 mg amalgam/10 weeks group (Table 2). A fraction of mice given 2.5 mg $HgCl_2$/l drinking water for 6 months developed ANoA, but ANoA were not

TABLE 2. *Serum antinucleolar antibodies, tissue immune-complex deposits, and expression of class II molecules on splenocytes*

				Tissue immune-complex deposits[b]						Class II expression[c]	
		ANoA[a]		Renal mesangium			Vessel walls			Mean fluorescent intensity	
Group	No	Pos[d]	Titer[e]	IgM	IgG	C3[f]	Kidney[d]	Heart[d]	Spleen[d]	Absolute[g]	Ratio[h]
Controls, 6 months[i]	6	0	—	147 ± 13[j]	60 ± 23	7 ± 7	0	0	0	414 ± 17	1.00 ± 0.041
Amalgam, 100 mg[k]	6	50	680 ± 360	227 ± 43	240 ± 36[l]	273 ± 208	17	0	17	494 ± 41	1.19 ± 0.10
Amalgam, 15 mg[k]	5	40	400 ± 240	288 ± 93	82 ± 22	24 ± 16	0	0	0	548 ± 42[m]	1.33 ± 0.09
Alloy, 8.5 mg[n]	5	80[o]	780 ± 600	448 ± 18	104 ± 24	264 ± 104[l]	0	0	0	537 ± 79	1.30 ± 0.19
2.5 ppm $HgCl_2$[p]	7	57	1120 ± 530	366 ± 103	109 ± 36	274 ± 169[l]	0	0	0	474 ± 22[l]	1.16 ± 0.05
Controls, 10 weeks[i]	5	0	—	80 ± 0	32 ± 15	48 ± 8	0	0	0	ND	—
Amalgam, 100 mg[k]	5	60	50 ± 10	420 ± 29[m]	896 ± 157[l]	280 ± 147	40	60	80	ND	—
Amalgam, 100 mg[q]	5	20	80 ± 0	208 ± 48[m]	208 ± 48[m]	40 ± 0	0	0	0	ND	—
Amalgam, 30 mg[k]	5	0	—	192 ± 32[l]	88 ± 32	48 ± 8	0	20	20	ND	—
Alloy, 58 mg[n]	4	100[o]	1040 ± 512	140 ± 20	100 ± 38	50 ± 10	25	25	25	ND	—

[a]Serum antinucleolar IgG antibodies assessed by immunofluorescence. [b]Granular deposits. [c]Expression of I-A*s* on splenocytes. [d]Fraction mice shown in percent. [e]Mean reciprocal titer ± SE. [f]Deposits colocalized with IgG and IgM. [g]Mean fluorescence channel ± SE. [h]Mean ± SE using the value of control lymphocytes as 1.00. [i]Intraperitoneal implantation of empty gelatin capsules. [j]Mean ± SE. [k]Implantation of a single piece amalgam. [l]Significantly different from controls ($P < 0.01$ using Wilcoxon's rank sum test). [m]Significantly different from controls ($P < 0.05$). [n]Intraperitoneal implantation. [o]Significantly different from controls ($P < 0.02$ using Fisher's exact test). [p]2.5 ppm $HgCl_2$ in the drinking water. [q]Implantation of multiple amalgam pieces. ND, not determined.

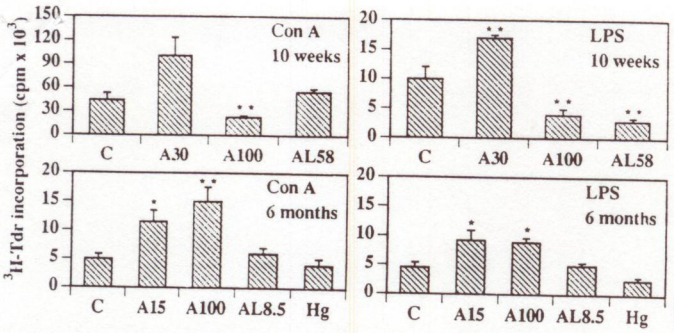

Figure 1. Incorporation of [^{3}H]thymidine in splenocytes from mice implanted intraperitoneally with amalgam or alloy, or given $HgCl_2$ in the drinking water after stimulation with concanavalin A (Con A) or *E. coli* lipopolysaccharide (LPS). Bars denote mean ± SE. C, controls implanted with empty gelatin capsules; A15, implantation of 15 mg amalgam; A30, implantation of 30 mg amalgam; A100, implantation of 100 mg amalgam; AL 58, implantation of 58 mg silver alloy; AL 8.5, implantation of 8.5 mg silver alloy; Hg, 2.5 ppm $HgCl_2$ in the drinking water. $^*P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$ using Wilcoxon's rank sum test.

found in mice treated with 5 mg $HgCl_2$/l drinking water for 2 weeks. Implantation of amalgam for 6 months instead of 10 weeks resulted in an increased fraction of ANoA-positive mice and a higher ANoA titer (compare implantation of 100 mg amalgam), but it also lowered the amount of amalgam needed to induce ANoA (compare 30 mg amalgam implanted for 10 weeks vs. 15 mg implanted for 6 months). All mice implanted with 58 mg alloy for 10 weeks and 80% of mice implanted with 8.5 mg alloy for 6 months developed ANoA. Immunoprecipitation studies showed that high-titered ANoA in both perorally mercury-treated (Fig. 3*A*) and amalgam/alloy-implanted (**Fig. 3*B***) mice reacted with the 34-kDa nucleolar protein fibrillarin. There was no significant difference in AHA or ACA titer between controls and implanted mice (data not shown).

Immune complex deposits

All mice implanted with amalgam/alloy or given $HgCl_2$ showed glomerular mesangial deposits with an increased mean titer of IgG and IgM (Table 2). 100 mg amalgam in multiple pieces caused strong staining for IgG in the mesangium (Fig. 2*B*), with colocalized granular deposits of C3c (Fig. 2*C*). Renal, myocardial, and splenic vessel wall immune deposits were seen in some amalgam- or alloy-implanted mice (Table 2; Fig. 2*D*).

Renal and splenic element concentration

Mice implanted for 10 weeks with 100 mg amalgam in multiple pieces showed the highest renal mercury concentration, whereas implantation of the same amount of amalgam as a single piece caused a 26-fold lower renal mercury concentration (**Table 3**). Implantation of 100 mg amalgam as a single piece for 6 months instead of 10 weeks caused a doubling of the renal mercury concentration, and implantation of a smaller amount (15 or 30 mg) caused lower renal mercury concentration, indicating a dose-response relationship. The spleen showed substantial accumulation of mercury after implantation of amalgam (Table 3). The renal silver concentration was increased 13-fold in mice implanted with 100 mg amalgam in pieces, and an increasing amount of silver was seen in the spleen after prolonged implantation of amalgam. Implantation of alloy caused a large increase in organ silver concentration with a dramatic increase in the 58 mg group (Table 3). The splenic concentration of copper and zinc was not different compared with the controls in either amalgam or alloy-implanted mice (data not shown), whereas the renal selenium concentration was increased in all groups (Table 3).

DISCUSSION

This study showed that dental silver amalgam and silver alloy implanted in the physiological milieu of the peritoneal cavity released enough metals to adversely effect the murine immune system. Although metals in general, and mercury in particular, have long been regarded as immunosuppressive agents (36, 37), in this study we demonstrate that prolonged exposure to amalgam and alloy causes a chronic stimulation of the humoral and cellular immune systems and systemic autoimmunity.

The amalgam/alloy-implanted animals showed hyperimmunoglobulinemia characterized by a long-lasting increase of both T cell-independent (IgM) and T cell-dependent (mainly IgG1) isotypes. This is in agreement with studies of peroral (present study) and subcutaneous mercuric chloride treatment of mice with the H-2^s haplotype (38, 39). However, because implantation of alloy also caused hyperimmunoglobulinemia, one or more alloy metal—silver, tin, copper, or zinc—must likewise have the potential to stimulate the humoral immune system. In mercury-treated SJL mice, the hyperimmunoglobulinemia is caused by an increased number of Ig-secreting splenic cells (39). This may result at least partly from activation of T helper cells of the Th_2 type with secretion of Il-4 and Il-10 and subsequent activation and proliferation of B cells; this may be followed by immunoglobulin secretion (40), although B cells may also be directly activated by mercury, bypassing the need for interleukins (41).

The cellular immune system was also affected by implantation of amalgam/alloy. First, splenocytes from mice implanted for 6 months with amalgam showed an increased expression of class II molecules, which was probably caused by the released mercury, as mice treated perorally with mercuric chloride (present study) also showed increased class II expression. An increased expression of class II molecules has been described in murine B cells treated in vitro with mercury (41), in splenocytes (42) and splenic B cells (43) from genetically mercury-sensitive Brown Norway rats treated in vivo with mercury, and in renal interstitial and tubular cells of mice treated with mercury in vivo (44). This could also be due to an activation effect of the Th_2 cell, because Il-4 and Il-10 enhance MHC class II molecule expression on B cells (45, 46). Second, amalgam implantation caused a dose-dependent increase in mitogen-induced T and B cell proliferation. Implantated alloy did not increase lymphocyte proliferation, so the stimulation is likely to be due to mercury. An increased mitogen-induced lymphocyte proliferation occurs after in vivo mercury treatment of SJL (31) and B6C3F1 (47) mice and after in vitro mercury treatment of lymphocytes from SJL (31) and BALB/c mice (48), as well as F344 rats (49). The mechanism underlying this augmented proliferation is unknown, but may be related to release of cytokines as mercury can increase the secretion of γ-interferon (48) and Il-1 (50).

An important finding in this study was the ability of implanted amalgam and alloy to induce autoantibodies against the nucleolus (ANoA). The degree of ANoA induction,

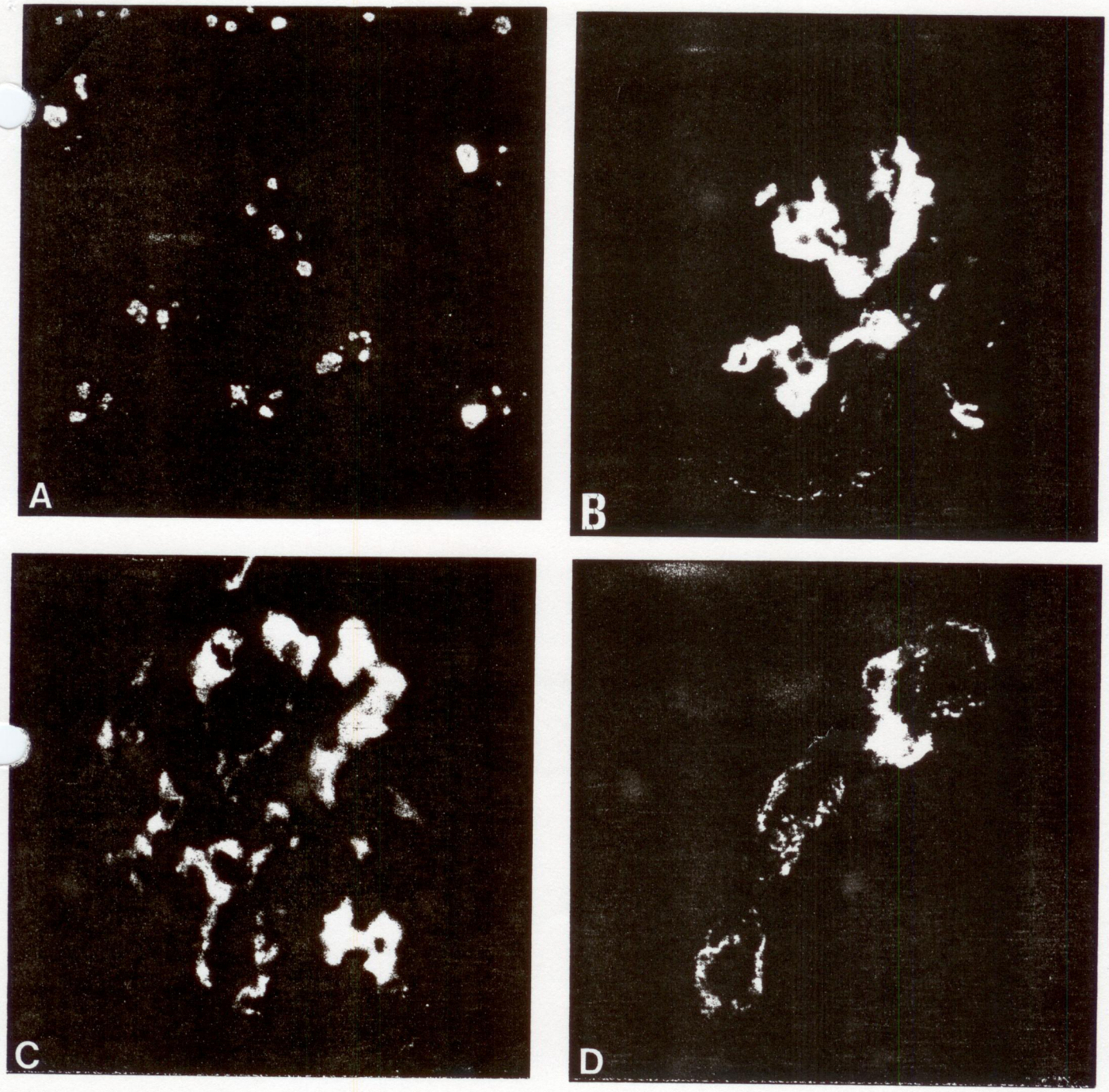

Figure 2. *A)* Antinuclear antibody test. Indirect immunofluorescence using serum from a mouse treated with 100 mg amalgam for 6 months. Bright nucleolar staining. ×1000. **Figure 2.** *B, C, D)* Direct immunofluorescence using FITC-conjugated goat anti-mouse antibodies on cryostate sections. *B)* Bright mesangial and granular arterial wall staining for IgG in a mouse treated with 100 mg amalgam in multiple pieces for 10 weeks. ×750. *C)* Bright mesangial staining for C3c in the same mouse as in *A*. ×800. *D)* Granular IgG deposits in the walls of small splenic arteries from a mouse treated as in *A*. ×1100.

measured as the fraction of ANoA-positive mice and the titer attained, correlated with the body burden of mercury, which in turn was dependent on the amount and form of the implanted amalgam and the length of implantation. ANoA develop in mice treated with mercuric chloride subcutaneously or perorally in a dose-dependent fashion (32, 39), which was confirmed in this study using low-dose mercury exposure in the drinking water. We (26–28) and others (51) have shown by immunoblotting that the target for these ANoA is the 34-kDa nucleolar protein fibrillarin, which is part of the small nucleolar ribonucleoprotein particles U3, U8, and U13. In this study, the more sensitive and specific immunoprecipitation technique, which has recently been made available by the cloning of mouse fibrillarin (35), confirmed that the ANoA in both mercuric chloride-treated and amalgam/alloy-implanted SJL mice are directed against fibrillarin. Mercury-induced antifibrillarin antibodies (AFA) are of interest in human medicine because the ANoA in approximately half the patients with systemic scleroderma target fibrillarin (52), and on the molecular level, appear to recog-