APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2001; **15**: 252–260
DOI: 10.1002/aoc.145

Cytotoxic evaluation of arsenic compounds in alveolar macrophages in hamsters†

Mitsushi Okada,[1]* Yoko Inoue,[1] Hitomi Karube,[1] Masato Niitsuya,[1] Hideki Tohnori,[1] Yoshiharu Aizawa,[1] Isao Okayasu[2] and Makoto Kotani[3]
[1]Department of Preventive Medicine and Public Health, School of Medicine, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa, 228-8555, Japan
[2]Department of Pathology, School of Medicine, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa, 228-8555, Japan
[3]Department of Electronics, School of Technology, Tokyo Denki University, 2-2 Kanda-Nishikicho, Chiyoda-District, Tokyo, 101-0054, Japan

Gallium arsenide (GaAs) causes various toxic effects in animals, including pulmonary diseases. Although their toxicity is not completely investigated, GaAs is used in workplaces in various semiconductor products. The present report was designed to clarify the toxicity of GaAs suspension and arsenic chloride ($AsCl_3$) solution as additives to the alveolar macrophages in hamsters using magnetometry, enzyme release assays and morphological examinations. Alveolar macrophages obtained from hamsters by tracheobronchial lavage and adhered to disks in the bottom of wells were exposed to ferrosoferric oxide and additives. Ferrosoferric oxide particles were magnetized externally and the remanent magnetic field was measured. Relaxation, a fast decline of remanent magnetic fields radiated from alveolar macrophages, was delayed and decay constants decreased dose-dependently due to exposure to GaAs suspensions. Relaxation was much delayed and decay constants decreased considerably due to exposure to $AsCl_3$ solutions. Because the relaxation is thought to be associated with the cytoskeleton, exposure to GaAs impairs their motor function. Enzyme release assay and morphological findings indicated damage to macrophages. Thus the cytotoxicity caused cytostructural changes and cell death. According to DNA electrophoresis and TUNEL methods, necrotic changes occur more frequently than apoptotic changes. In conclusion, exposure to GaAs suspension and $AsCl_3$ solution induced cytotoxicity of alveolar macrophages.

Keywords: gallium arsenide; arsenic chloride; cytotoxicity; alveolar macrophages; magnetometry

Received 9 December 1999; accepted 11 September 2000

INTRODUCTION

Gallium arsenide (GaAs) is widely used in the microelectronics industry as a semiconductor material for transistors, solar cells and lasers.

Generally, for inorganic elements, differences of atomic valence and chemical structure change the toxicity of elements. It is known that trivalent (arsenite) has greater toxicity than pentavalent (arsenate) and inorganic species are more toxic than organic species.[1] Once trivalent arsenic is absorbed, arsenicals disrupt enzymatic reactions vital to cellular metabolism by interacting with sulfhydryl groups.[2,3] As the affinity of trivalent arsenic for sulfhydryl-containing enzymes is stronger than pentavalent arsenic, the toxicity of trivalent arsenic lasts for a long time until excretion.[4]

Epidemiologic research (IARC) has shown that inorganic arsenic is a human carcinogen for lungs and dermis.[5] Although much evidence has accumulated suggesting that arsenic is cytotoxic for respiratory organs, the precise mechanism of

* Correspondence to: Mitsushi Okada, Department of Preventive Medicine and Public Health, School of Medicine, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa, 228-8555, Japan.
E-mail: okap@med.kitasato-u.ac.jp
†Based on work presented at the Ninth Symposium of the Japanese Arsenic Scientists' Society (JASS-9), held 21–22 November 1999 at Hiroshima, Japan.
Contract/grant sponsor: Ministry of Education, Science, Sports and Culture.

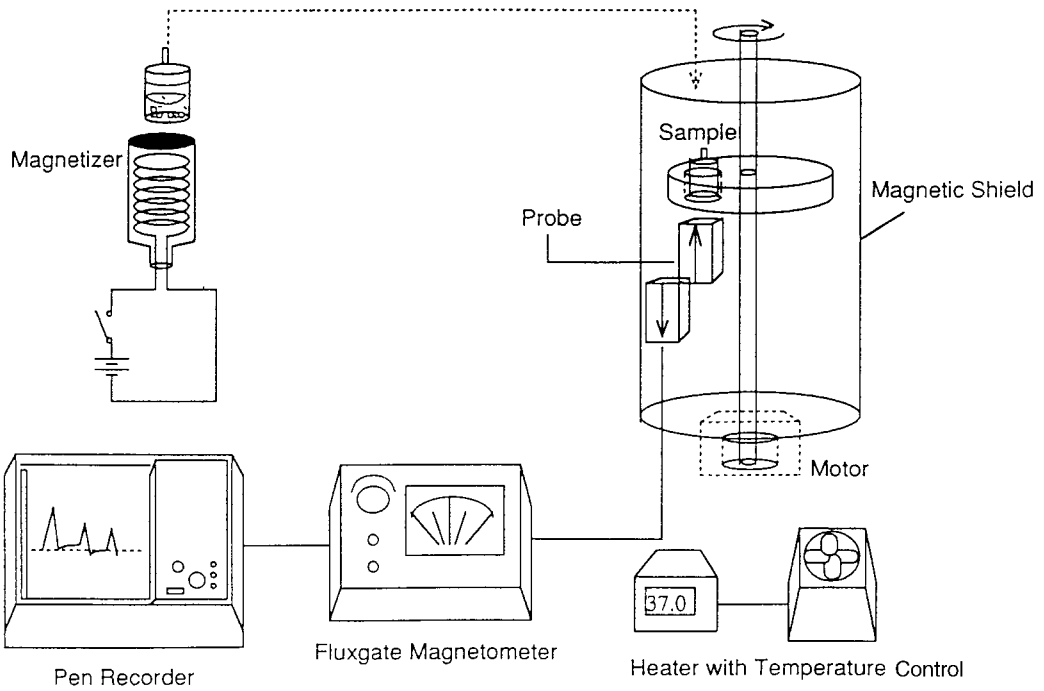

Figure 1 Magnetometric apparatus.

arsenic toxicity for antigen-presenting systems is unknown.[6,7]

The purpose of this study is to determine whether alveolar macrophages can tolerate GaAs particles. We report here an evaluation for the toxicity of GaAs particles to alveolar macrophages and antigen-presenting cells in hamsters. The toxicity of GaAs to alveolar macrophages of hamsters was evaluated not only by cytomagnetometry as a biophysical method, but also by intracellular enzyme release as a biochemical method, and supravital staining, blood cell staining, and electron microscopy as morphological methods. We also performed DNA ladder and terminal deoxynucleotidal transferase (TdT) mediated dUTP–biotin nick end labeling (TUNEL)[8] for DNA fragment detection.

The magnetometric system is a unique method for evaluation of the cytotoxicity of chemicals.[9,10] Magnetometric studies show that remanent magnetic fields, which have been generated by external magnetization of magnetic iron oxide particles in alveolar macrophages, decrease rapidly as time passes. This phenomenon of relaxation is principally attributed to the turning of phagocytized magnetic iron particles in alveolar macrophages. We have reported on some cytotoxic chemicals that influenced relaxation.[11–18] Moreover, retardation of the relaxation can be explained by the cytoskeltal changes occuring around the phagosomes containing particles of chemical substances.

MATERIALS AND METHODS

Additives

We used ferrosoferric oxide (Fe_3O_4) as the index of cellular magnetometry (Fig. 1), GaAs suspension and $AsCl_3$ solution as a test material of the cellular toxicity, polystyrene microspheres as a control and PBS as a background. The Fe_3O_4 particles were manufactured by Toda Industry Inc., Hiroshima. The geometric diameter of Fe_3O_4 ranged from 0.08 to 0.57 μm and mean diameter was 0.26 μm (Fig. 6). The GaAs particles (MW = 144.64) were manufactured by Furukawa Electric Inc., Tokyo and kindly provided by Mr M. Kudou. The geometric diameter of GaAs ranged from 0.07 to 1.50 μm and mean diameter was 0.43 μm (Fig. 1). The polystyrene microspheres (Polybead) were

Table 1 Solubility and addition level

$AsCl_3$ sample no.	①	②	③	④
Dose of GaAs (μg)	2	4	10	20
Solubility of arsenic (μg)	1.19	1.76	2.41	2.82
(nmol)	15.9	23.5	32.2	37.6
$AsCl_3$, equivalent to the above arsenic level (μg)	2.88	4.26	5.83	6.82

manufactured by Polysciences Inc., Warrington, PA, USA. The geometric diameter of the polystyrene microspheres was uniformly 1.00 ± 0.01 μm. These materials were suspended in pH 7.4 phosphate-buffered solution with saline (PBS, Takara Shuzo Co., Otsu) and mixed using an ultrasound generator for 20 min just before the exposure.

Collection of alveolar macrophages

Male Syrian golden hamsters (Std: Syrian [Golden]) were anesthetized with intraabdominal pentobarbital sodium (1.5–2.5 ml kg^{-1}) injections (Nembutal Injection, Abbott Laboratories, Chicago, IL, USA). After the median abdominal section, the abdominal aorta was cut and the lungs were collapsed. Then the trachea was revealed and a silicon catheter (Atom Intravenous Catheter 5 French for Cut-Down, Atom Medical Co., Tokyo) was inserted into the trachea. After revealing the trachea, 3.0 ml of pH 7.4 cold PBS with 0.1% ethylenediaminetetraacetic acid (EDTA) was administered and aspirated. This procedure was repeated ten times through a silicon catheter and the lavage fluid was collected. Approximately $(5–8) \times 10^6$ cells were obtained per animal.

Exposure to chemicals and cell culture

1 ml of Eagle minimum essential medium (MEM, Nissui Pharmaceutical Co., Tokyo) with 10% fetal bovine serum (FBS) containing 10^6 cells was poured into each well (Nunclon 4 Wells Multidishes, Japan Inter Med Co., Tokyo) with a cell disk (Cell Disk R1, Sumilon, Tokyo) at the bottom. Cells were divided into four groups.

GaAs-exposed group: the final concentration of Fe_3O_4 was adjusted to 60 μg ml^{-1} and the final concentrations of GaAs were adjusted to 2,4,10 or 20 μg ml^{-1} (0.014, 0.028, 0.069, 0.138 μM).

$AsCl_3$-exposed group: the final concentration of Fe_3O_4 was adjusted to 60 μg ml^{-1} and the final concentrations of $AsCl_3$ were adjusted to 2.88, 4.26, 5.83 or 6.82 μg ml^{-1}. Each solution is equal to the soluble concentration of arsenic trivalent ion for the above GaAs samples (Table 1).

Polystyrene-exposed group: the final concentrations of Fe_3O_4 and polystyrene microspheres were adjusted to 60 μg ml^{-1} and 4.4×10^6 ml^{-1}.

PBS-exposed group: the final concentration of Fe_3O_4 was adjusted to 60 μg ml^{-1} and 40 μl PBS were added to the medium.

Each of the groups was then incubated overnight (18 h) in a 5% CO_2 incubator at 37 °C.

Cytomagnetometry

Cell disks were taken out from wells after overnight (18 h) incubation. A glass tube containing the medium and a cell disk in the bottom was magnetized in 70 mT for 10 ms by the coils. Then the glass tube was installed in the stage, which was rotated at a speed of 10 rpm, above the probe of a fluxgate magnetometer and was kept at 37 °C by a heater with a thermostat. The remanent magnetic field was measured as described previously (Fig. 1).

Intracellular enzyme release

As a method of evaluating intracellular enzyme release, lactate dehydrogenase (LDH) was measured. After collection of alveolar macrophages, 1 ml of serum-free medium (Macrophage-SFM, liquid, Life Technologies, Inc., Rockville, MD, USA) containing 10^6 cells was poured into each well with a cell disk at the bottom and was incubated overnight (18 h) with exposure to chemicals in the 5% CO_2 incubator at 37 °C. Then the medium in the well was taken out and centrifuged at 1800 rpm for 10 min. Then 50 μl of supernatant solution was applied to LDH-UV test kits (Wako Pure Chemical Ind., Osaka) following the Wroblewski–LaDue method[19] and measured to determine the decreasing intensity at 340 nm wavelength during 2 min by U-3000 type autospectrometer (Hitachi Co., Tokyo). For measuring the total LDH activation index, which was derived from both intracellular and extracellular matrices, Triton-X 100 was added to the control group. The LDH release rate (%) was calculated using the following equation: [(LDH activation index from GaAs exposed group) −(LDH activation index from control group)] ×100/[(total LDH activation index) −(LDH activation index from control group)].

Morphological examinations

In light microscopic analysis, we used 0.03% of trypan blue for supravital staining and Giemsa solution[20] for morphological cell staining. In ultramicroscopic analysis, we used a scanning electron microscope (SEM) and a transmission electron microscope (TEM). Macrophages adhered on the polycationics-treated glass were washed with 0.1 M cacodylate buffer (pH 7.4), and prefixed with 1% of glutaraldehyde at 4 °C for 3 h. After removing the buffer, they were postfixed with 1% of osmium tetroxide at 4 °C for 3 h. Ultrathin sections were stained with 3.0% uranyl acetate and Reynolds' lead citrate. For the SEM (S-4500FE, Hitachi Co., Tokyo) observation, the specimens were treated with an iron sputter coating. For the TEM (H-600, Hitachi Co., Tokyo) observation, the specimens were dehehydrated and embedded in resin.

DNA ladder method

The DNA ladder method was performed in two steps. First the DNA extraction, then agarose gel electrophoresis.

After adding the chemicals as described previously, we cultured the macrophages overnight (18 h) in the wells (SUMILON Multi Well Plate, MS-8048R, Sumitomo Bakelite Co., Ltd, Tokyo) that were used for suspending the cell culture. Then, we obtained about 1.0 ml of the cell suspension and centrifuged it at 1800 rpm for 5 min. The supernatunt was removed and 400 μl of DNA extraction buffer was poured into the sediment. After 20 min reaction at room temperature, 1 μl of 20 mg ml^{-1} Proteinase K (Wako Pure Chemical Ind., Osaka) and 20 μl of 10% sodium dodecyl sulfate (SDS, Wako Pure Chemical Ind., Osaka) were added and mixed slowly. Afterwards the solution was incubated for 30 min at 37 °C then 400 μl of PCIA (phenol:chloroform:isoamyalcohol = 25:24:1) (Wako Pure Chemical Ind., Osaka) was poured and reacted for 20 min at room temperature. Then, the solution was centrifuged at 10 000 rpm for 10 min. Approximately 300 μl of supernatant solution was taken out and 30 μl of 3 M (Wako Pure Chemical Ind., Osaka) and 300 μl of isopropyl alcohol (Wako Pure Chemical Ind., Osaka) were added to the supernatunt for preservation at −80 °C.

Prior to electrophoresis, we defrosted specimens and centrifuged them at 12 000 rpm for 10 min, removed the supernatant, added 200 μl of 70% ethanol (Wako Pure Chemical Ind., Osaka) and dried the sample naturally. After that, 10 μl of 10 mM Tris–1 mM EDTA buffer (Tris–HCl, Schwarzy–Mann Biotech Co., Cleveland, OH, USA. EDTA, Wako Pure Chemical Ind., Osaka) and 2 μl of 10 mg ml^{-1} RNase (Wako Pure Chemical Ind., Osaka) were added to the sediment. The solution was then incubated at 37 for 1 h. After the preparation of an agarose gel, specimens with bromophenol blue (Wako Pure Chemical Ind., Osaka) were applied to a mini horizontal electrophoresis unit (MUPID-3, Advance Co., Tokyo), stained for 15 min by ethidium bromide (Wako Pure Chemical Ind., Osaka) and photographed under UV transillumination.

TUNEL method

After adding the chemicals as previously noted we cultured the macrophages overnight (18 h) in the wells (Nunc LAB-TEK 8 Chamber Slide, Nunc Inc., Naperville, IL, USA) that were used for light microscopic stains. Then, we removed the medium and fixed the specimen with 5% paraformaldehyde for over 90 min. The specimens were applied to an *in situ* apoptosis detection kit (Apop Tag Plus-Peroxidase *in situ* apoptosis detection kit, Oncor Co., Gaithersburg, MD, USA) with some modifications.[21] The differences are as follows: in order to inhibit endogenous peroxidase, we substituted Proteinase K (Wako Pure Chemical Ind., Osaka) for hydrogen peroxidase. As a secondary antibody, we used anti-digoxigenin–alkaline phosphatase (Boehringer Mannheim Co., Tokyo) instead of anti-digoxigenin–peroxidase. Then we used 5-bromo-4-chloro-3-indoxyl phosphate and nitro blue tetrazorium chloride (DAKO JAPAN Co., Kyoto) in place of diaminobenzidine substrate for staining. We also substituted Tris buffer saline for PBS. For positive control specimens, 4 μm thick histologic sections of 10% buffered formalin-fixed and paraffin-wax-embedded human tonsils were used.

RESULTS

Cytomagnetometry

Figures 2 and 3 show the characteristics of the relaxation that we evaluated. Relaxation means a fast decline of the remanent magnetic field that was radiated from the alveolar macrophages. The relaxation of the GaAs-exposed group was delayed dose-dependently, whereas neither the polystyrene-

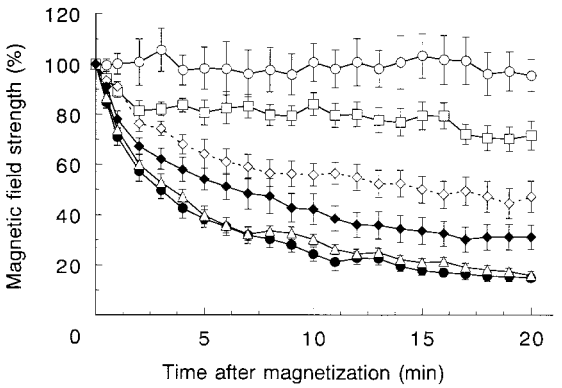

Figure 2 Relaxation curves in alveolar macrophages exposed *in vitro* to various doses of GaAs, polystyrene and PBS as control group. The vertical percentage presents the remanent magnetic field with S.E. from seven hamsters in each group. The initial remanent magnetic field is plotted as 100%. ●, control group; △ polystyrene exposed group; ◆, 2 μg ml^{-1}; ◊, 4 μg ml^{-1}; □, 10 μg ml^{-1}; ○, 20 μg ml^{-1} of GaAs-exposed group.

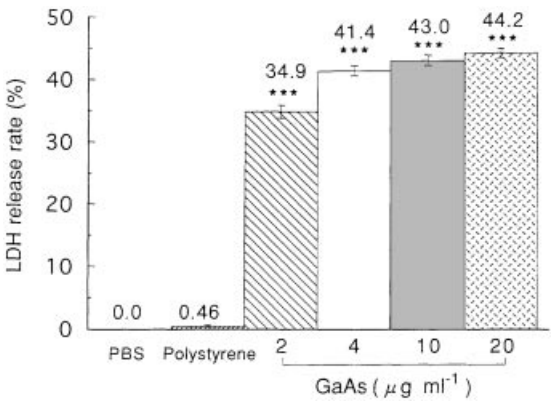

Figure 4 LDH release rate in alveolar macrophages exposed *in vitro* to various doses of GaAs, polystyrene and PBS as control group. The vertical percentage presents the mean ± S.E. obtained from nine hamsters in each group. A significant difference from the polystyrene-exposed group was shown by ***$p < 0.001$.

exposed group nor the PBS-exposed group showed any effect. The relaxation of the $AsCl_3$-exposed group was delayed greatly, whereas the PBS-exposed group showed no effect. The relaxation curve shows a declining curve, which is plotted as the remanent magnetic field normalized to 100% immediately after stopping the magnetization. We performed two-way ANOVA and recognized the significance among groups ($p < 0.001$).

Intracellular enzyme release

The data are summarized in the Figs 4 and 5. A considerable release of LDH from alveolar macrophages exposed to GaAs and $AsCl_3$ was shown. Neither PBS nor polystyrene had any effect on LDH release from alveolar macrophages. As a result of the Scheffe multiple comparison procedure, a significant effect was recognized between

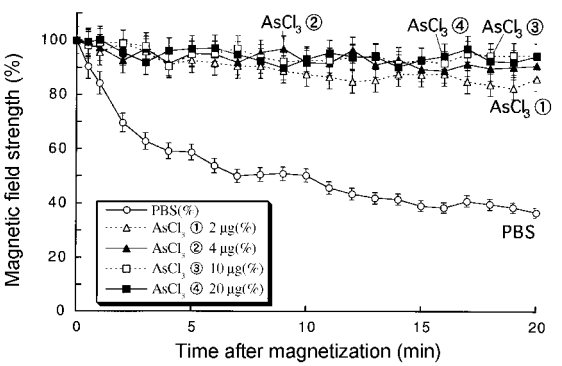

Figure 3 Relaxation curves in alveolar macrophages exposed *in vitro* to various doses of $AsCl_3$, and PBS as control group. The vertical percentage presents the remanent magnetic field with S.E. from seven hamsters in each group. The initial remanent magnetic field is plotted as 100%.

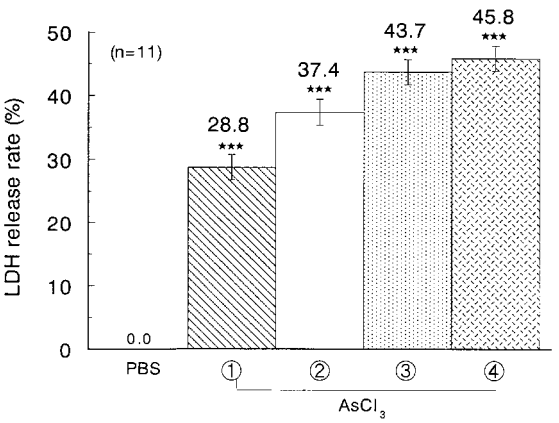

Figure 5 LDH release rate in alveolar macrophages exposed *in vitro* to various doses of $AsCl_3$, and PBS as control group. The vertical percentage presents the mean ± S.E. obtained from 11 hamsters in each group. A significant difference from the PBS-exposed group was shown by ***$p < 0.001$.

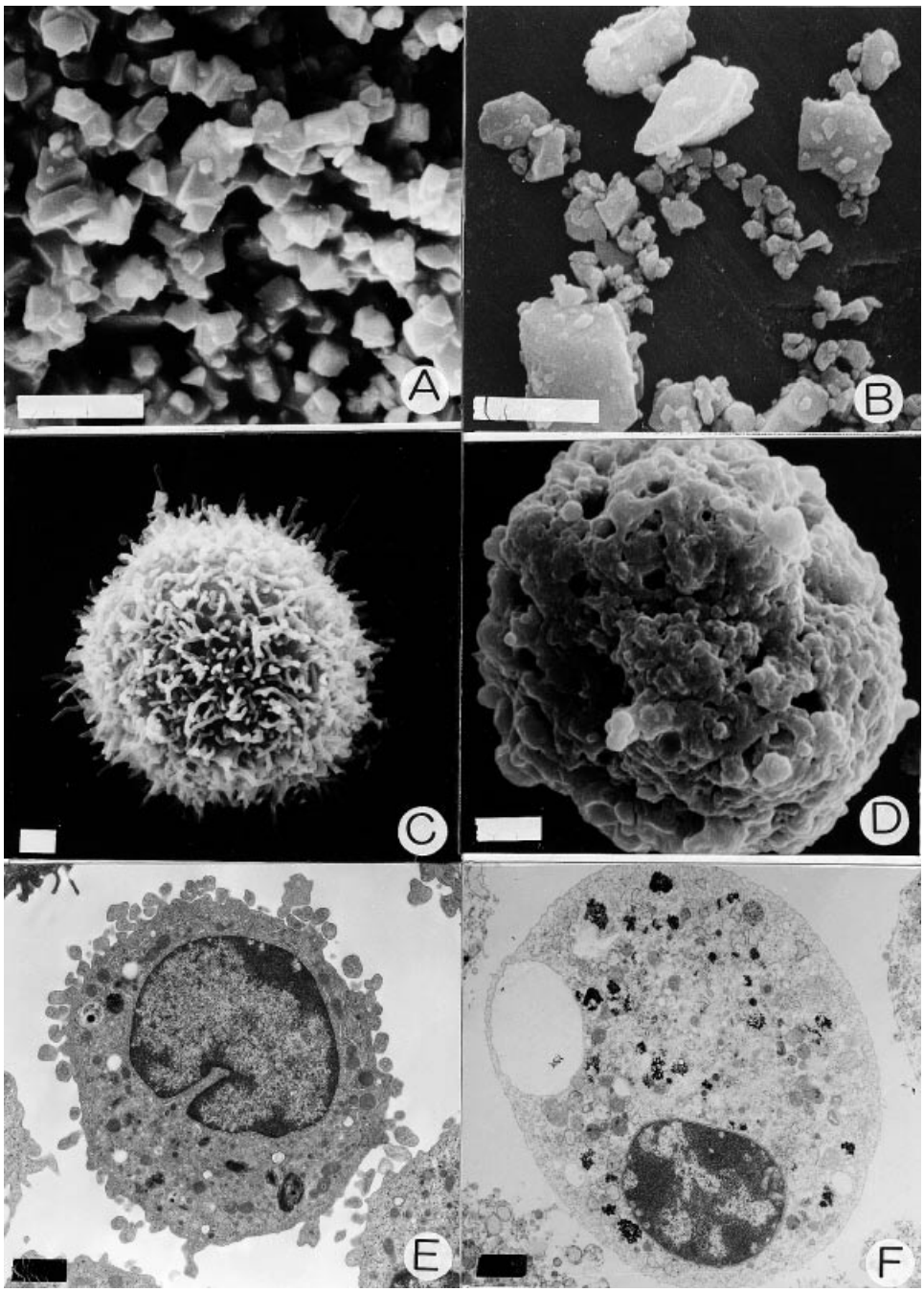

Figure 6 Ultramicroscopic findings in alveolar macrophages. (A) Particles of Fe_3O_4 by SEM. (B) Particles of GaAs by SEM. (C) An alveolar macrophage in control group by SEM. (D) An alveolar macrophage in 20 $\mu g\ ml^{-1}$ of GaAs-exposed group by SEM. (E) An alveolar macrophage in control group by TEM. (F) An alveolar macrophage in 20 $\mu g\ ml^{-1}$ of GaAs-exposed group by TEM. Double stainings with uranyl acetate and Reynolds' lead citrate. Each bar shows 1.0 μm for (A) –(F).

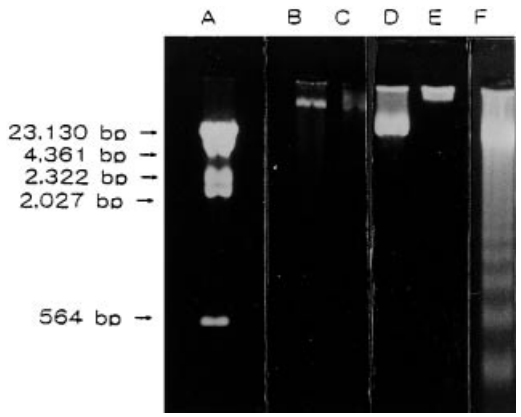

Figure 7 An electrophoretic image by the DNA ladder method. Lane (A): DNA marker (λDNA/Hind III fragments). Indicators with various base pairs (bp) are shown. Lanes (B) and (C): extracted DNA from control group. Lanes (D) and (E): extracted DNA from 20 μg ml^{-1} of GaAs-exposed group. Lane (F): extracted DNA from 60 μg ml^{-1} of TiO_2-exposed group as positive control.

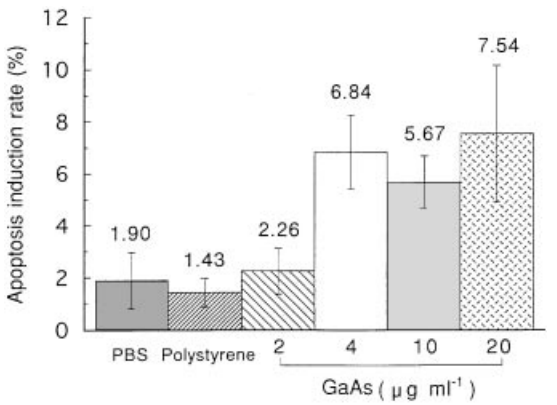

Figure 8 Apoptosis induction rate in alveolar macrophages exposed *in vitro* to various doses of GaAs, polystyrene and PBS as control group. The vertical percentage presents mean ± S.E. obtained from 13 hamsters in each group.

the polystyrene-exposed group and each GaAs-exposed group ($p < 0.001$). The significance was also recognized between the PBS-exposed group and each $AsCl_3$-exposed group ($p < 0.001$).

Morphological methods

The data are summarized in Plate 1 and Fig. 6. Trypan blue staining showed cell injuries in the surface membrane of alveolar macrophages. The positive rates for 2, 4, 10, 20 μg ml^{-1} of GaAs were 12.4, 36.3, 44.6, 46.1% and these increased dose-dependently (Plate 1A–E). Giemsa staining showed enlarged cells, a low nucleus/cytoplasm ratio, and collapsed and poorly stained nuclei of GaAs- and $AsCl_3$-exposed alveolar macrophages (Plate 1F and G). In light microscopic findings, morphological changes of alveolar macrophages were shown in the GaAs- and $AsCl_3$-exposed groups. SEM findings showed the disappearance of cytoplasmic projections (Fig. 6C and D). TEM findings showed the disappearance of cytoplasmic projections, i.e. smoothing and rounding changes of the cell surface, and rounding and indistinct changes of the nucleolus (Fig. 6E and F). In electron microscopic findings, organic degeneration of the cell membrane, cytoplasm and nuclei were shown in the GaAs-exposed group. The control group or polystyrene-exposed group showed almost nomal findings, in contrast with the GaAs-exposed group.

DNA ladder method

The photograph of the electrophoresis is shown in the Fig. 7. The photograph shows that the fragmentations of nucleosome units (about 180 bp) are less than the fragmentations of random nucleotide acids. Slight to mild apoptotic changes are observed in the DNA pattern of alveolar macrophages exposed to GaAs particles. The control group or polystyrene-exposed group show negligible fragmentation of nucleosome units.

TUNEL method

The data and the photograph of apoptotic staining are shown in Plate 1H–J and Fig. 8. Even in the group exposed to the highest dose of GaAs, the average positive rate of staining was less than 10%. Therefore, the slight apoptotic changes in the GaAs-exposed alveolar macrophages were shown. The control group or polystyrene-exposed group showed that the average rate of apoptotic change was below 2.0%.

DISCUSSION

Cytomagnetometry is a modified method of pneumomagnetometry, which was invented by Cohen.[9] The magnetometric system is a unique method for evaluation of the cellular toxicity or lung toxicity by chemical substances. The alveolar macrophages

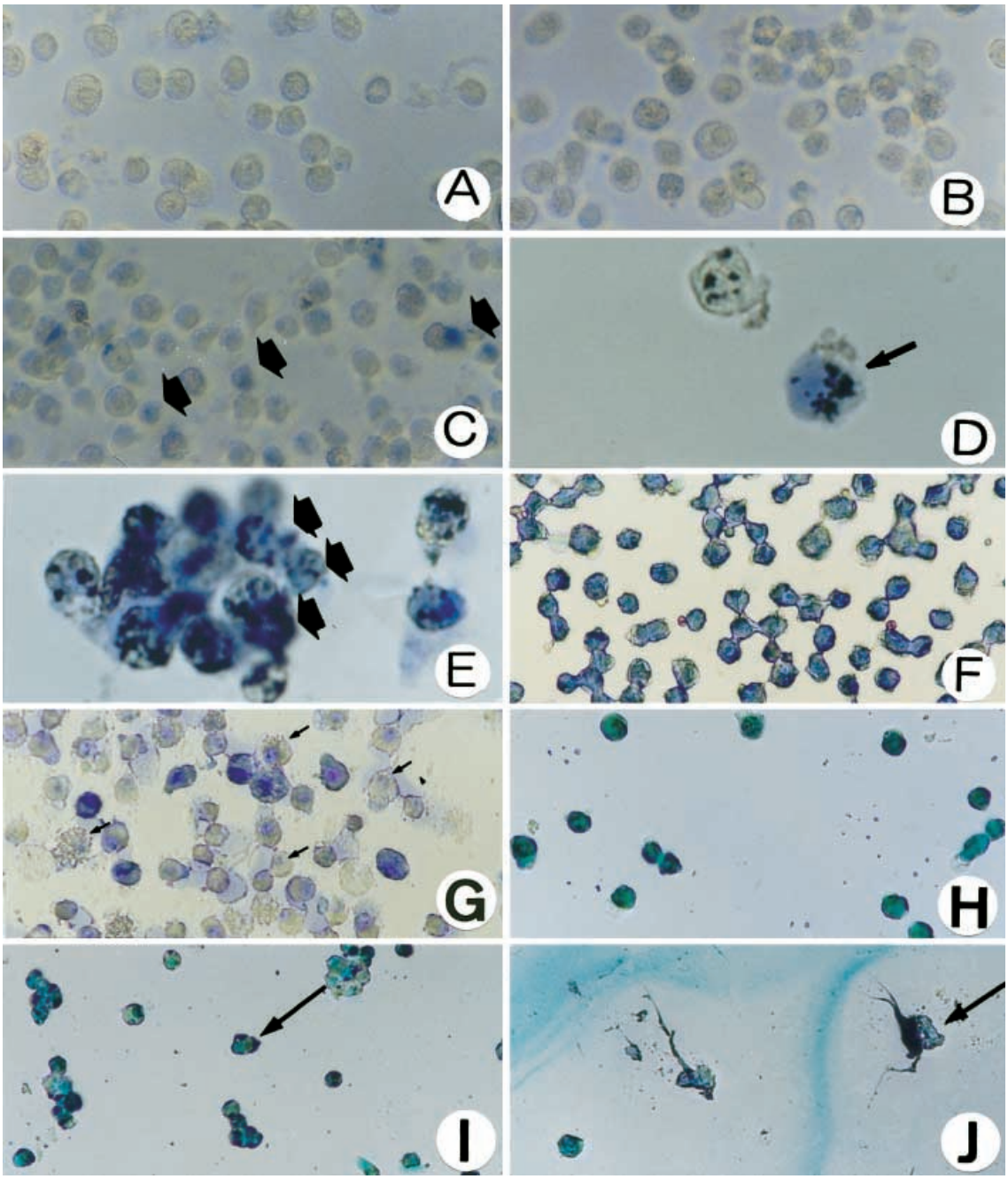

Plate 1 Light microscopic findings in alveolar macrophages. (A) Control group with trypan blue staining. (B) Polystyrene-exposed group with trypan blue staining. (C) 20 μg ml^{-1} of GaAs-exposed group with trypan blue staining. (D) Control group with trypan blue staining. (E) $AsCl_3$-exposed group with trypan blue staining. (F) Control group with Giemsa staining. (G) $AsCl_3$-exposed group with Giemsa staining. (H) Control group with TUNEL staining. (I) 20 μg ml^{-1} of GaAs-exposed group with TUNEL staining. (J) $AsCl_3$-exposed group with TUNEL staining. Arrows present positive cells. Original magnification: (A), (B) and (C): ×200; (D) and (E): ×400; (F)–(J): ×100.

phagocitize iron oxide particles during overnight incubation. The iron oxide particles located in the phagosomes of macrophages are magnetized externally.[22] After stopping external magnetization, the remanent magnetic field strength decreases rapidly as time passes. This phenomenon, termed relaxation, is thought to occur due to the random rotation of phagosomes containing magnetized iron oxide particles in the alveolar macrophages.[23] The cytoskeleton is thought to contribute to the random rotation of the phagosomes.[24] Although the detailed mechanism is still to be elucidated, we presume that delayed relaxation is due to cytoskeletal dysfunction induced by exposure to chemical substances. Some experiments that include *in vivo* and *in vitro* work have been reported.[11–18]

In the *in vivo* experiment, the relaxations were measured in the lungs of mammals that were intratracheally instilled with both iron oxide particles and a chemical substance. In rabbits, administration of GaAs showed a dose-dependent delay of relaxation,[17,18] whereas that of calcium carbonate showed normal relaxation.[15] In human beings, cigarette smoke caused delay of relaxation.[25]

There are some reports on *in vitro* experiments using alveolar macrophages of hamsters. The results are as follows: calcium carbonate showed normal relaxation,[16] silicon carbide whiskers caused moderate delay of relaxation,[14] titanium dioxide whiskers caused dose-dependent delay of relaxation[12] and chrysotile fibers indicated moderate delay of relaxation.[13] In this study, the exposure ranged from 2 to 20 $\mu g\,ml^{-1}$ doses of GaAs and indicated a dose-dependent delay of relaxation. The exposure ranged from 2.88 to 6.82 $\mu g\,ml^{-1}$ doses of $AsCl_3$ and indicated a long delay in relaxation. A polystyrene microsphere suspension was added to evaluate the effect of phagocytosis of inert particles on alveolar macrophages. The exposure of this suspension did not influence relaxation.

LDH exists in the cytoplasm of alveolar macrophages. Its release into the external fluid shows an alternation of the cell membrane. Therefore, to measure this LDH activity, which is released from the cytoplasm, is one of several evaluation methods for cell toxicity induced by chemical particles. In the present report, the exposure ranged from 2 to 20 $\mu g\,ml^{-1}$ doses of GaAs and from 2.88 to 6.82 $\mu g\,ml^{-1}$ doses of $AsCl_3$ and indicated a dose-dependent increase of LDH release rate. This result indicates a change of permeability in the cell membrane. We used a serum-free medium for measuring LDH instead of the medium used for magnetometry examinations, because MEM with FBS contains LDH.

Morphological examinations show remarkable changes of alveolar macrophages. In light microscopic findings, the dose-dependent degeneration of alveolar macrophages was shown in the GaAs-exposed group. In electron microscopic examinations, the structural degeneration of the cell membrane and intracytoplasmic organella was prominent in the GaAs-exposed group. We conclude that those changes result from GaAs exposure, because both control groups and polystyrene-exposed groups showed almost normal findings.

In the GaAs-exposed group, DNA electrophoresis showed that the fragmentation of each nucleosome unit (about 180 bp) was less than the fragmentation of random nucleotide acids. Therefore, slight to mild apoptotic changes of alveolar macrophages exposed to GaAs was detected. Both the control group and the polystyrene-exposed group showed negligible fragmentation of nucleosome units.

The result of apoptotic staining by the TUNEL method showed that the average rate of stained macrophages in the GaAs-exposed group was less than 10%. Thus, slight apoptotic changes of alveolar macrophages exposed to GaAs were shown. Both the control group and the polystyrene-exposed group showed that the average rate of apoptotic change was below 2.0%. It is reported that asbestos,[26] silica,[27,28] TNF-α[29,30] and endotoxin[31] induced apoptotic changes in alveolar macrophages. It has been reported that arsenic trioxide induced apoptotic changes of lymphocytes and myelocytes.[32–36] Arsenic trioxide has been used as a medication for adult T-cell leukemia and acute promyelocytic leukemia.[32–36]

Regarding the toxicity of GaAs, it should be elucidated which of the elements of GaAs is the main cause of cytotoxicity and what kind of condition, dissolution or insolution, mainly induces the cytotoxicity. Moreover, the solubility of GaAs is an important factor for evaluating cytotoxicity.[37] According to reports on comparative pulmonary toxicity of GaAs, gallium (trivalent) oxide or arsenic (trivalent) oxide, GaAs had a greater toxicity than arsenic or gallium.[38,39] Dissolved GaAs was more toxic to the cells than the insoluble species.[4,38,39]

In this study, the 18 h exposure of GaAs to alveolar macrophages caused cytoskeletal dysfunction, which was detected by cytomagnetometry, injuries to cell membranes, which were detected by enzyme release and morphological methods, and necrotic changes in most cells, which were detected by the DNA ladder method and the TUNEL

method. For the reasons stated above, cytomagnetometry is thought to be an effective method for evaluating the functional toxicity of GaAs to alveolar macrophages.

Acknowledgements We are grateful to the technical staff, Ms Yoko Inoue Lyons for biochemical analyis, Ms Yumiko Sugiura and Ms Yumi Komatsu for statistical analysis, Ms Kimiyo Mashimo for cytomagnetometric analyis, Mr Shichiro Miyazawa and Ms Noriko Nemoto for electron microscopic analysis, and Ms Yoshiko Numata for morphological analyis. This study was partially supported by the Academic Frontier Project of the Ministry of Education, Science, Sports and Culture.

REFERENCES

1. Vahter M. In *Biological Monitoring of Toxic Metals*, Clarkson TW (ed.). Plenum Press: New York, 1988; 303–321.
2. Lewis R. In *Occupational & Environmental Medicine*, LaDou J (ed.). Appleton & Lange: Stamford, 1997; 408–410.
3. Williams SR. In *Poisoning & Drug Overdose*, 3rd ed, Olson KR (ed.). Appleton & Lange: Stamford, 1999; 95–97.
4. Rosner MH, Carter DE. *Fund. Appl. Toxicol.* 1987; **9**(4): 730.
5. IARC (ed.). Arsenic & arsenic compound. In *IARC Monographs on Evaluation of Carcinogenic Risks to Man*, IARC: Lyon, 1987; Suppl., 7, 100–106.
6. Harrison RJ. *Occup. Med.* 1986; **1**(1): 49.
7. Edelman P. *Environ. Health Perspect.* 1990; **86**: 291.
8. Sgonc R, Wick G. *Int. Arch. Allergy Immunol.* 1994; **105**: 327.
9. Cohen D. *Science* 1973; **180**: 745.
10. Cohen D, Arai S, Brain JD. *Science* 1979; **204**: 514.
11. Keira T, Okada M, Katagiri H, Aizawa Y, Okayasu I, Kotani M. *Tohoku J. Exp. Med.* 1988; **186**: 87.
12. Okada M, Aizawa Y, Karube H, Keira T, Niitsuya M, Shinohara S, Mashimo K, Kotani M. In *Advances in the Prevention of Occupational Respiratory Diseases*, Chiyotani K, Hosoda Y, Aizawa Y (eds). Elsevier: Amsterdam, 1998; 621–625.
13. Keira T, Aizawa Y, Okada M, Karube H, Niitsuya M, Shinohara S, Mashimo K, Kotani M. In *Advances in the Prevention of Occupational Respiratory Diseases*, Chiyotani K, Hosoda Y, Aizawa Y (eds). Elsevier: Amsterdam, 1998; 664–668.
14. Karube H, Aizawa Y, Ohwada M, Keira T, Okada M, Sugiura Y, Mashimo K, Kotani M. In *Advances in the Prevention of Occupational Respiratory Diseases*, Chiyotani K, Hosoda Y, Aizawa Y (eds). Elsevier: Amsterdam, 1998; 626–630.
15. Mashimo K, Okada M, Aizawa Y, Karube H, Keira T, Watanabe M, Sugiura Y, Kotani M. In *Advances in the Prevention of Occupational Respiratory Diseases*, Chiyotani K, Hosoda Y, Aizawa Y (eds). Elsevier: Amsterdam, 1998; 555–560.
16. Sugiura Y, Keira T, Aizawa Y, Okada M, Watanabe M, Harada H, Mashimo K, Kotani M. In *Advances in the Prevention of Occupational Respiratory Diseases*, Chiyotani K, Hosoda Y, Aizawa Y (eds). Elsevier: Amsterdam, 1998; 550–554.
17. Aizawa Y, Takata T, Karube H, Nakamura K, Kotani M. *Appl. Organ. Chem.* 1994; **8**: 207.
18. Aizawa Y, Takata T, Karube H, Tatsumi H, Inokuchi M, Kotani M, Chiyotani K. *Ind. Health* 1994; **31**(4): 143.
19. Wroblewski F, LaDue J. *Proc. Soc. Exp. Biol. Med.* 1955; **90**: 210.
20. Dacie JV, Lewis SM. *Practical Haematology*, 8th ed. Churchill Livingstone: Edinburgh, 1995.
21. Satoh Y, Tsuchiya B. *Med. Technol.* 1999; **27** (suppl.): 212 (in Japanese).
22. Brain JD, Bloom SB, Valberg PA. In *Biomagnetism '87*, Atsumi K, Kotani M, Ueno S, Katila T, Williamson SJ (eds). Tokyo Denki University Press: Tokyo, 1988; 10–17.
23. Valberg PA, Brain JD. *J. Aeros. Med.* 1988; **1**: 371.
24. Nemoto I, Ogura K, Toyotama H. *IEEE Trans. Biomed. Eng.* 1989; **36**: 598.
25. Meller W, Barth W, Pohlit W, Rust M, Siekmeier R, Stahlhofen W, Heyder J. *Toxicol. Lett.* 1996; **88**: 131.
26. Holian A, Uthman MO, Goltsova T, Brown SD, Hamilton Jr RF. *Environ. Health. Perspect.* 1997; **105**(suppl. 5): 1139.
27. Hamilton RF, Iyer LL, Holian A. *Am. J. Physiol.* 1996; **271**: 813.
28. Iyer R, Hamilton RF, Li L, Holian A. *Toxicol. Appl. Pharmacol.* 1996; **141**(1): 84.
29. Iyer R, Holian A. *Am. J. Physiol.* 1997; **273**: 760.
30. Ortiz LA, Moroz K, Liu JY, Hoyle GW, Hammond T, Hamilton RF, Holian A, Banks W, Brody AR, Friedman M. *Am. J. Physiol.* 1998; **275**: 1208.
31. Bingisser R, Stey C, Weller M, Groscurth P, Russi E, Frei K. *Am. J. Respir. Cell Mol. Biol.* 1996; **15**(1): 64.
32. Bazarbachi A, El-Sabban ME, Nasr R, Quignon F, Awaraji C, Kersual J, Dianoux L, Zermati Y, Haider JH, Hermine O, deThe H. *Blood* 1999; **93**(1): 278.
33. Dai J, Weinberg RS, Waxman S, Jing Y. *Blood* 1999; **93**(1): 268.
34. Koken MH, Daniel MT, Gianni M, Zelent A, Licht J, Buzyn A, Minard P, Degos L, Varet B, deThe H. *Oncogene* 1999; **18**(4): 1113.
35. Rousselot P, Labaume S, Marolleau JP, Larghero J, Noguera MH, Brouet JC, Fermand JP. *Cancer Res.* 1999; **59**(5): 1041.
36. Tamm I, Paternostro G, Zapata JM. *N. Eng. J. Med.* 1999; **340**(13): 1043.
37. Pierson B, VanWagenenn S, Nebesny KW, Fernando Q, Scott N, Carter DE. *Am. Ind. Hyg. Assoc. J.* 1989; **50**(9): 455.
38. Webb DR, Sipes IG, Carter DE. *Toxicol. Appl. Pharmacol.* 1984; **76**(1): 96.
39. Webb DR, Wilson SE, Carter DE. *Toxicol. Appl. Pharmacol.* 1986; **82**(3): 405.

