Effects of an asbestos, chrysotile-A, on a human polyclonal T cell line, MT-2

Takemi Otsuki[1)]*, Haruko Sakaguchi[1)], Tamayo Hatayama[1)], Satomi Hatada[1)], Yoshie Miura[1)], Akiko Takata[1)], Hironobu Katsuyama[2)], Fuminori Hyodoh[1)]*

Department of Hygiene[1)], and Public Health[2)], Kawasaki Medical School, Japan

* These two authors equally contributed to this presentation.

INTRODUCTION

Patients with silicosis suffer from not only respiratory disorders but also complication of autoimmune diseases such as systemic sclerosis (SSc), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA).

In addition, many autoantibodies are detected in the sera of silicosis patients as we reported the appearance of ant-topoisomerase I, anti-caspase 8, and anti- desmogleins antoantibodies.

We have been focusing immunological aspects of silicosis patients and found that elevation of serum levels and gene expression in peripheral blood mononuclear cells (PBMC) of soluble Fas (sFas) molecule, which inhibits surface Fas-mediated apoptosis induced by binding with Fas ligand (FasL) by competing with FasL, and overexpression of DcR3 gene, which function as similar to the sFas, in PBMC, highly frequent and larger amounts of other alternative spliced variants of Fas gene, which seemed to act as similar to the sFas molecule, in PBMC.

These findings suggested that alteration of Fas-mediated apoptotic pathways in lymphocytes from silicosis may play certain role in appearance of disruption of autoimmunity in these patients.

In addition, silica (-SiO_2-) and silicate such as asbestos (e.g., chrysotile, crocidolite, and amosite) are known to cause malignant lung cancers and mesotheliomas. Although it is well known that these compounds cause malignant transformation of the alveolar epithelial cells and mesothlial cells, it may be helpful to consider that the effects of silica compounds on lymphocytes, particularly T cells, induce the acceleration of tumor progression by reduction of tumor immunity.

Thus, in this study, we examined the effects of an asbestos, chrysotile-A, on human polyclonal T lymphocytes cell line, MT-2, which present helper T cell surface antigen, CD4.

FIG. 1

Growth Curves

MT-2 cells were cultured with 0, 5, 10, 25, 50 mg/ml of chrysotile-A for 4 days. At day 1 and 4, cells were counted by staining with trypan blue.

The growth of MT-2 cells were markedly reduced by culturing with more than 10 mg/ml of chrysotile-A.

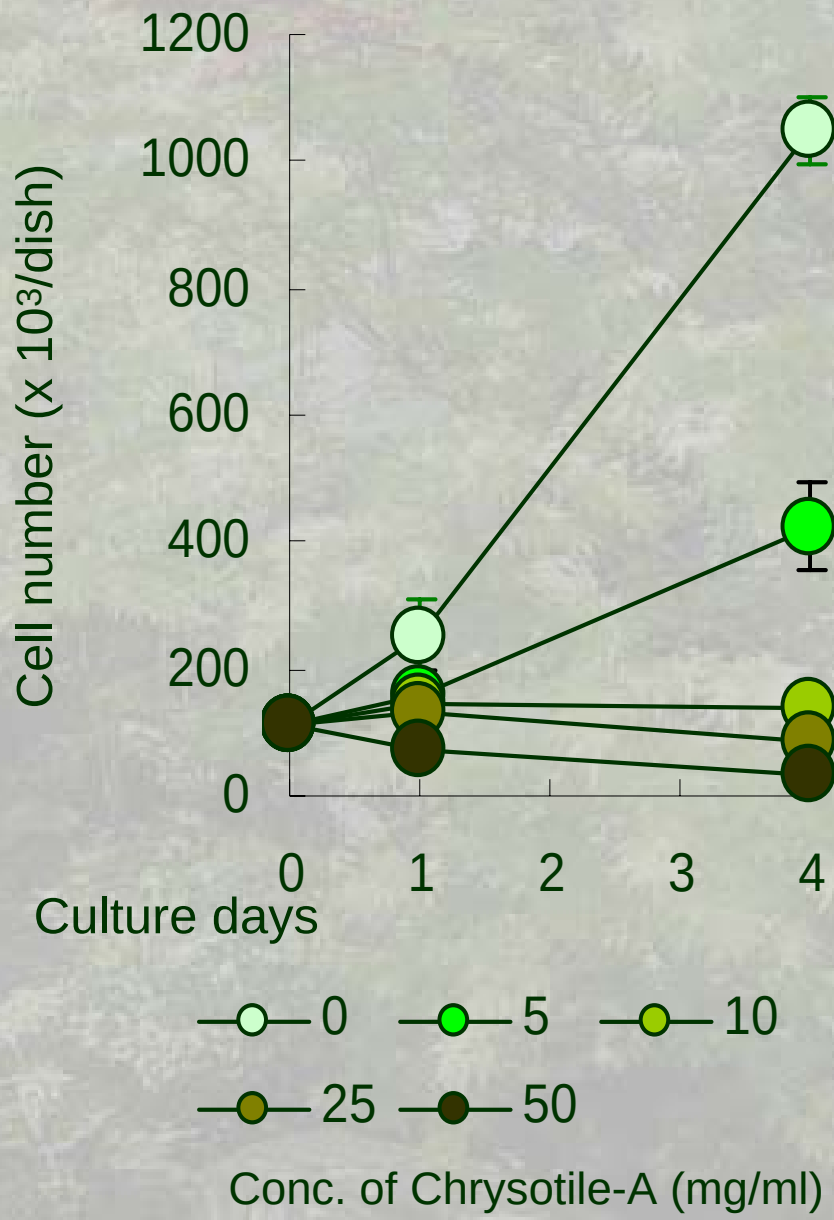


Fig. 2-A: MT-2 cells cultured with 0, 10, 25 and 50 μg/ml of chrysotile-A for 3 days were applied for TUNEL method.

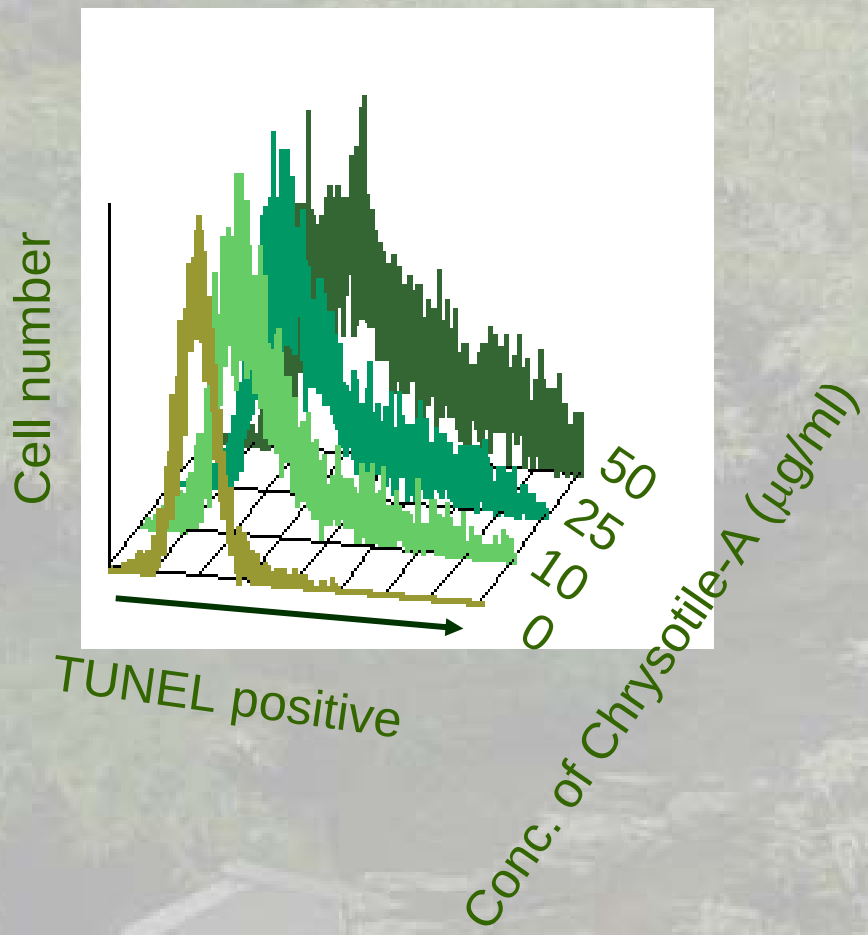
Fig. 2-B: The positive percentage for TUNEL in Fig. 2-A was graphically presented.

The reduction of growth activity of MT-2 cells caused by chrysotile-A was due to the appearance of apoptosis, since TUNEL positive fraction was increased dose-dependently when cells were cultured with 0, 10, 25 and 50 μg/ml of chrysotile-A

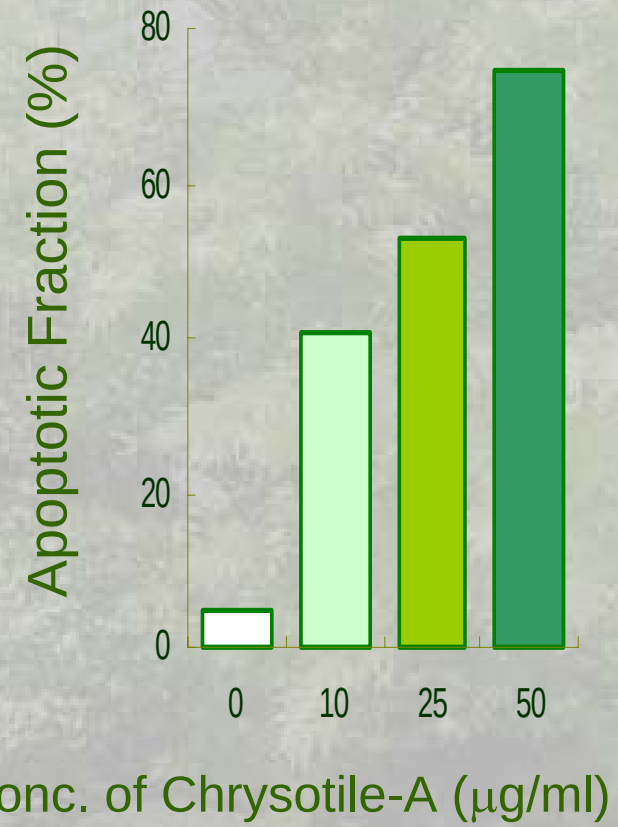
TUNEL analysis

FIG. 2

A

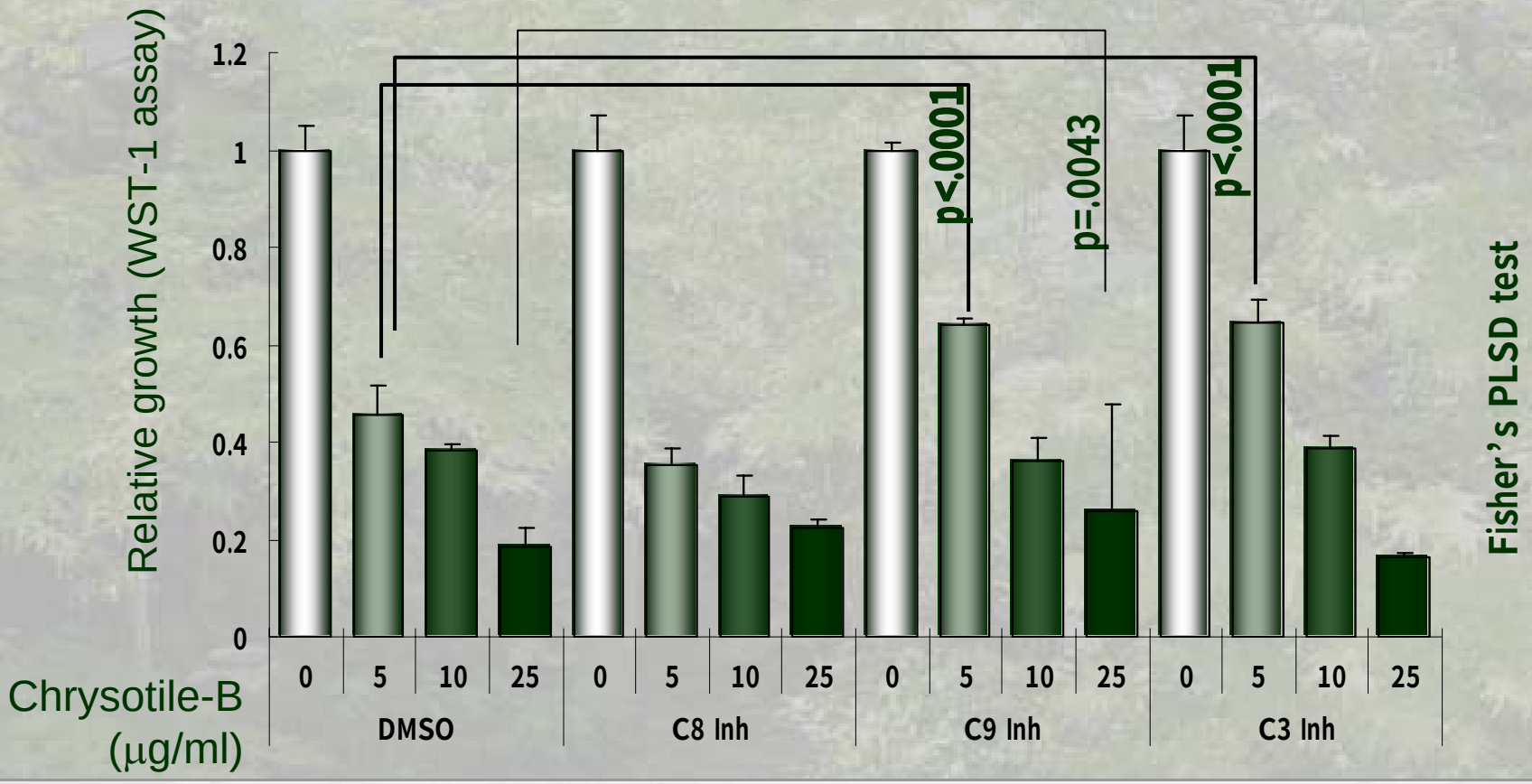


B



Effects of caspase-inhibitors on chrysotile-induced growth inhibition of MT-2 cells

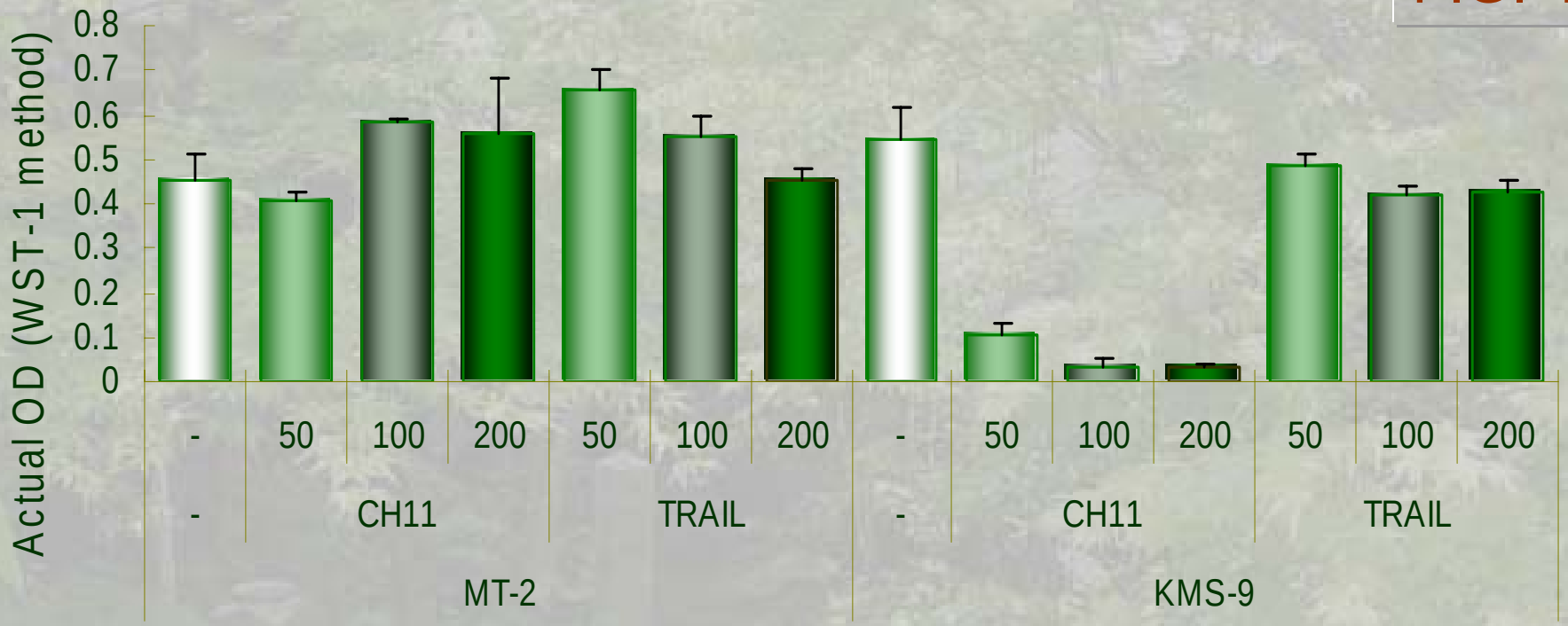
FIG. 3



Casp-9, and -3, but not -8 inhibitors showed recovery of growth when MT-2 cells were cultured with low-concentration (5 μg/ml) of chrysotile fiber.

The apoptotic pathway via death receptors such as Fas and TRAIL-receptors were not utilized in MT-2 cells.

FIG. 4

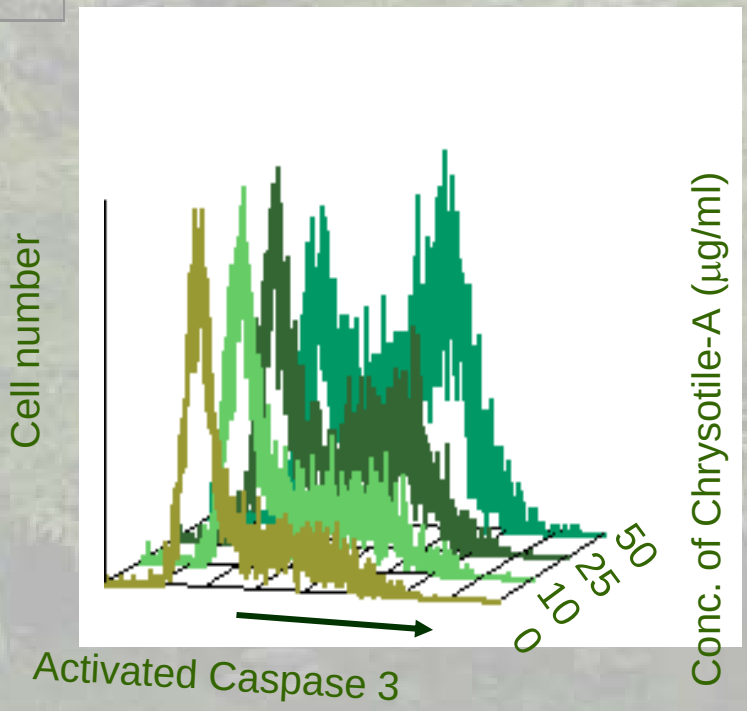


Because CH11 Fas-stimulating antibody, which cause Fas-mediated apoptosis, and recombinant human TRAIL did not affect the growth properties of MT-2 cells, but KMS-9 cells, an EBV-immortalized lymphoblastoid cell line, have Fas, but Trail-Rs.

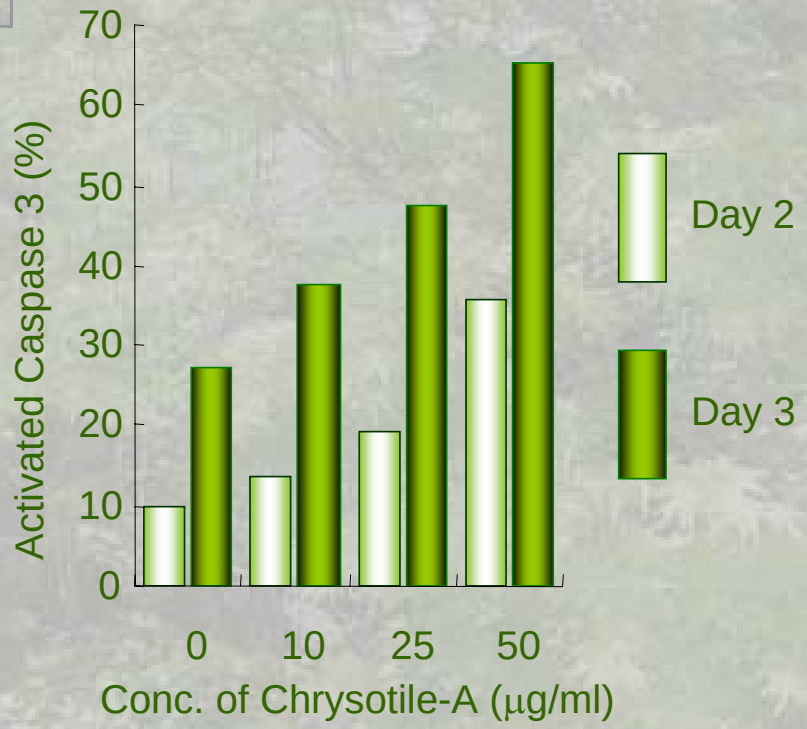
Activation of Caspase-3 in MT-2 cells cultured with chrysotile-A

FIG. 5

A



B



Although the effects of caspase-3 inhibitor was relatively slight (Fig. 3), the activation of caspase 3 was observed in dose- and time-dependent manner when MT-2 cells were cultured with 0, 10, 25 and 50 mg/ml of chrysotile-A.

Fig. 6 showed two typical apoptotic pathways via through death receptors and mitochondria.

To clarify mitochondrial apoptotic pathway was involved in chrysotile-A induced apoptosis of MT-2 cells, alteration of several proteins were examined by Western blotting using whole cell or fractionated lysates from MT-2 cells cultured with 0, 10 and 25 μg/ml of chrysotile-A.

As shown in Fig. 7-E and F, the cleaved form of caspase 3 and 9 were detected dose-dependently by Western blotting when MT-2 cells were cultured with 0, 10 and 25 mg/ml of chrysotile-A for 2 days.

The cytochrome-c was translocated from mitochondria to cytoplasma (Fig. 7-D), the balance of Bcl2/Bax ratio was decreased markedly (Fig. 7-B), BAX protein was also translocated into the cytoplasma (Fig. 7-C), and JNK was phosphorylated dose-dependently (Fig. 7-A)

FIG. 6

Apoptotic pathways

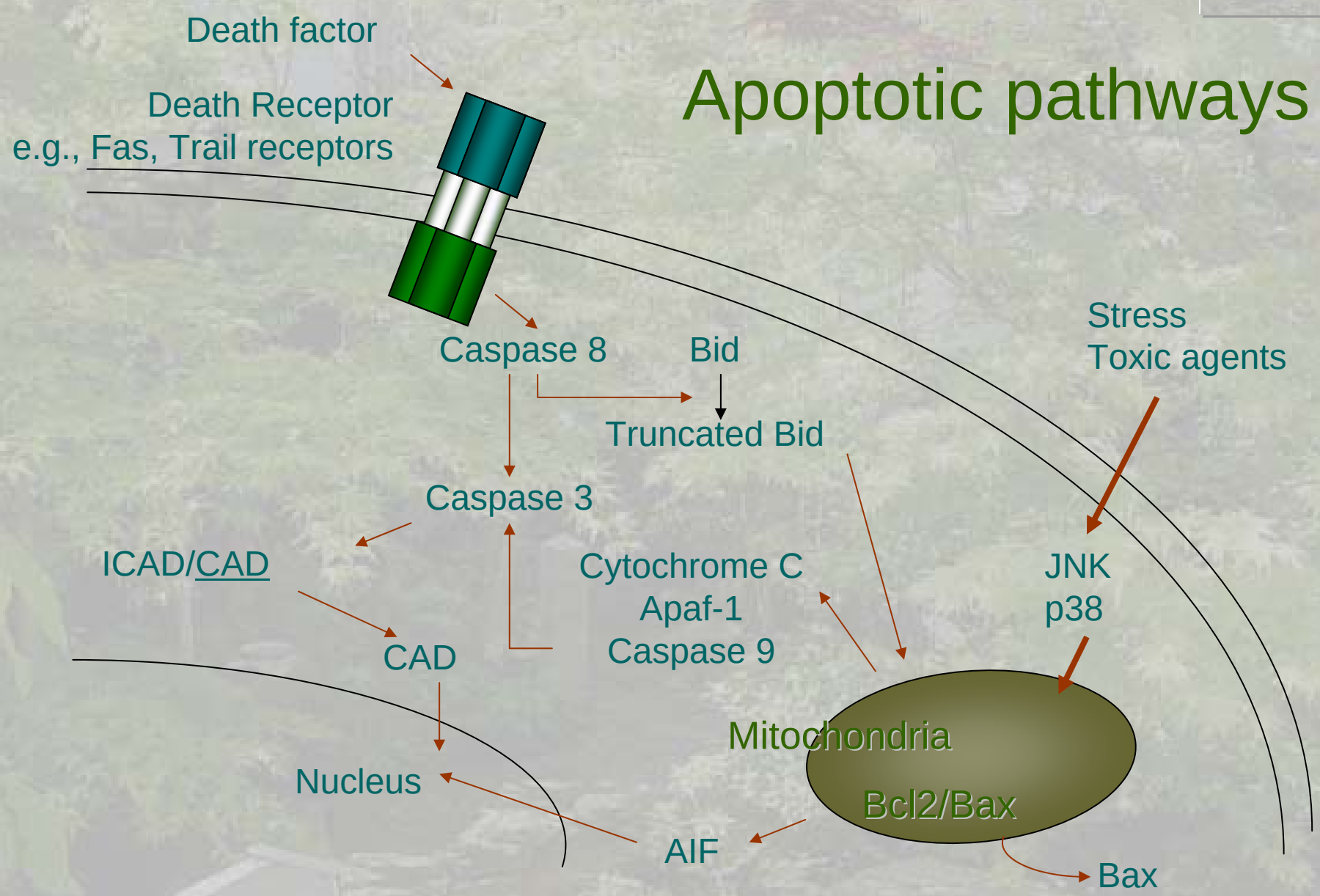
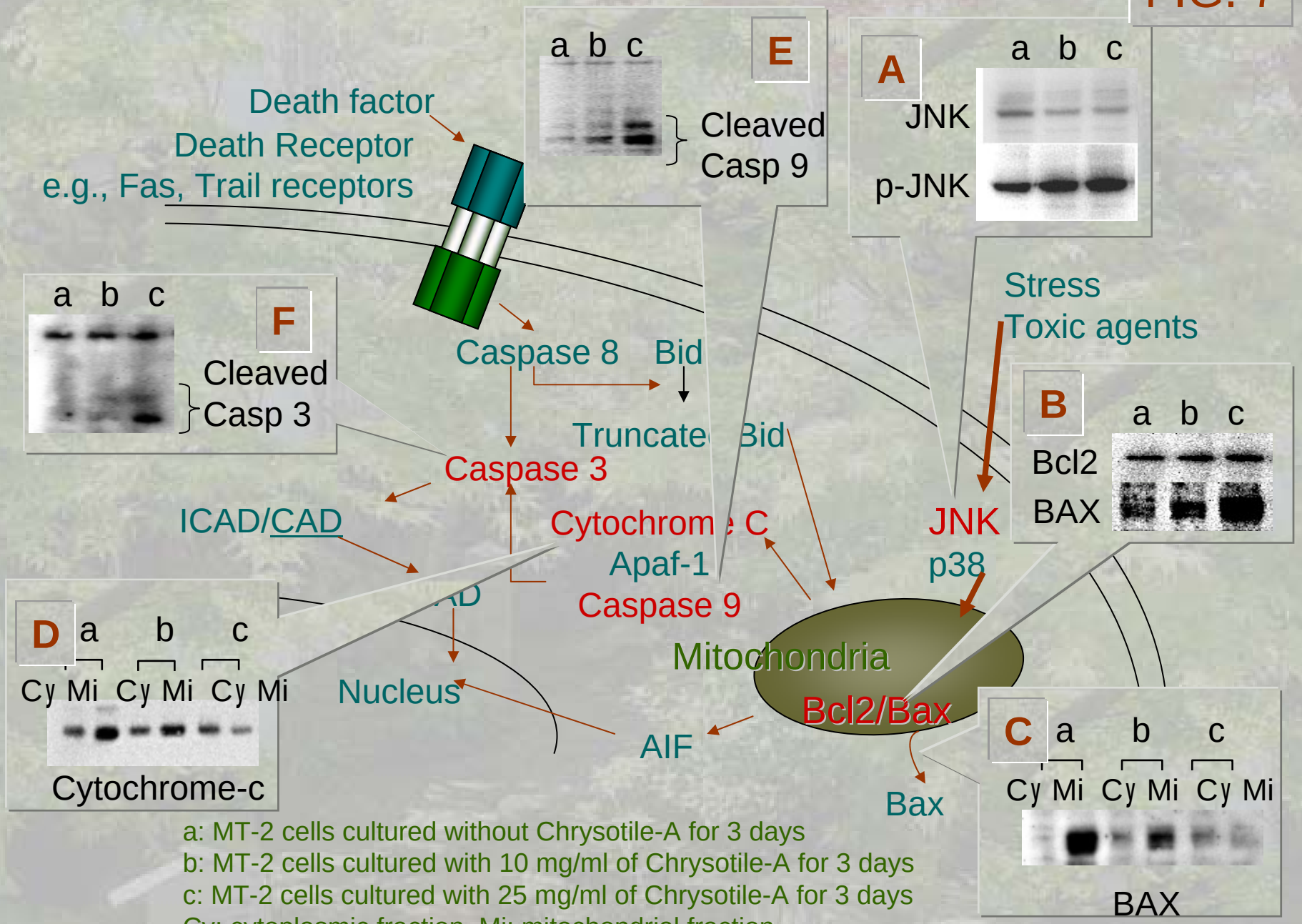


FIG. 7



a: MT-2 cells cultured without Chrysotile-A for 3 days
b: MT-2 cells cultured with 10 mg/ml of Chrysotile-A for 3 days
c: MT-2 cells cultured with 25 mg/ml of Chrysotile-A for 3 days
Cy: cytoplasmic fraction, Mi: mitochondrial fraction

As resulted above, the mitochondrial pathway of apoptosis seemed to be involved in chrysotile-A induced cell death in MT-2 cells.

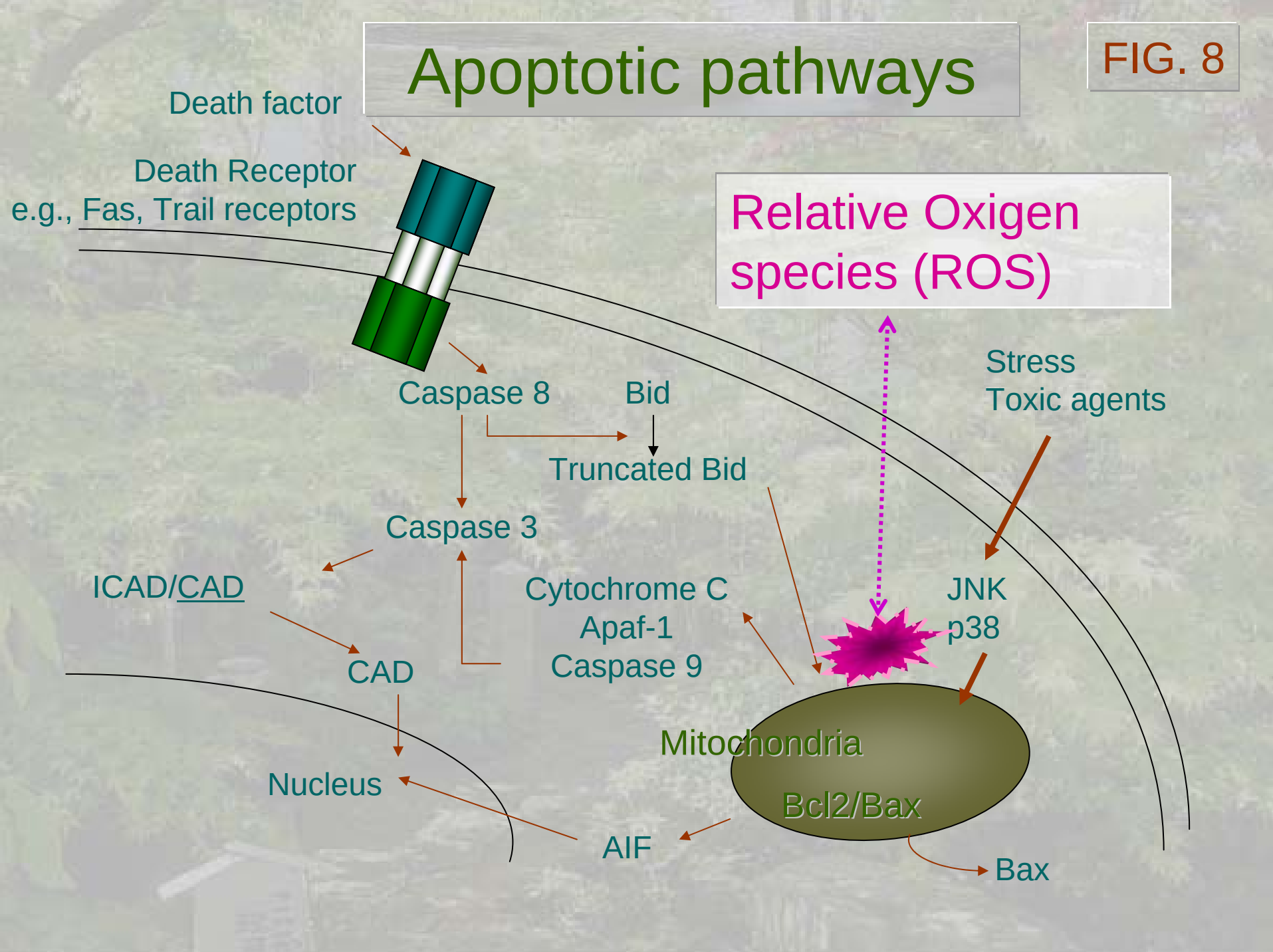
During activation of mitochondrial apoptotic pathway, generating of reactive oxygen species (ROS) in microsomes is considered as one of essential cellular events in this pathway (Fig. 8).

Therefore, the production of superoxides was measured flowcytometrically.

As shown in Fig. 9-A and graphically in Fig. 9-B, the appearance of superoxides was depend on concentrations of chrysotile-A with which MT-2 cells were cultured for 2 days.

Apoptotic pathways

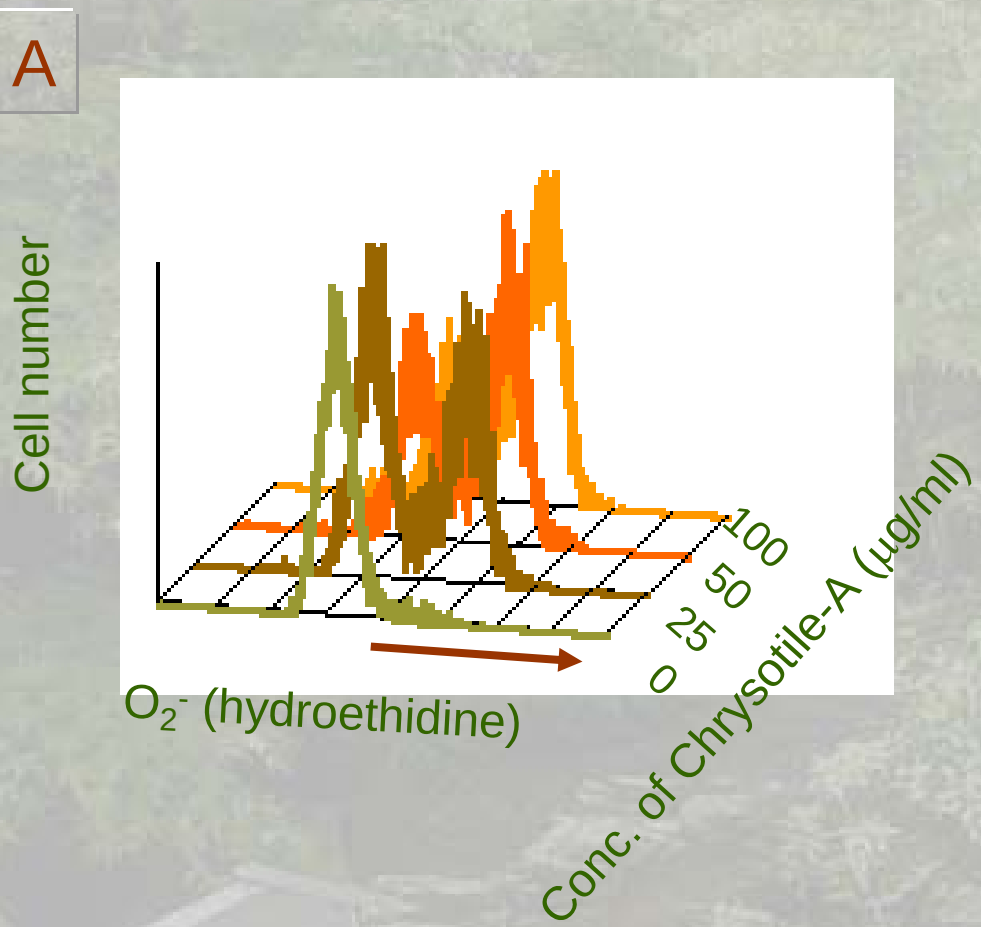
FIG. 8



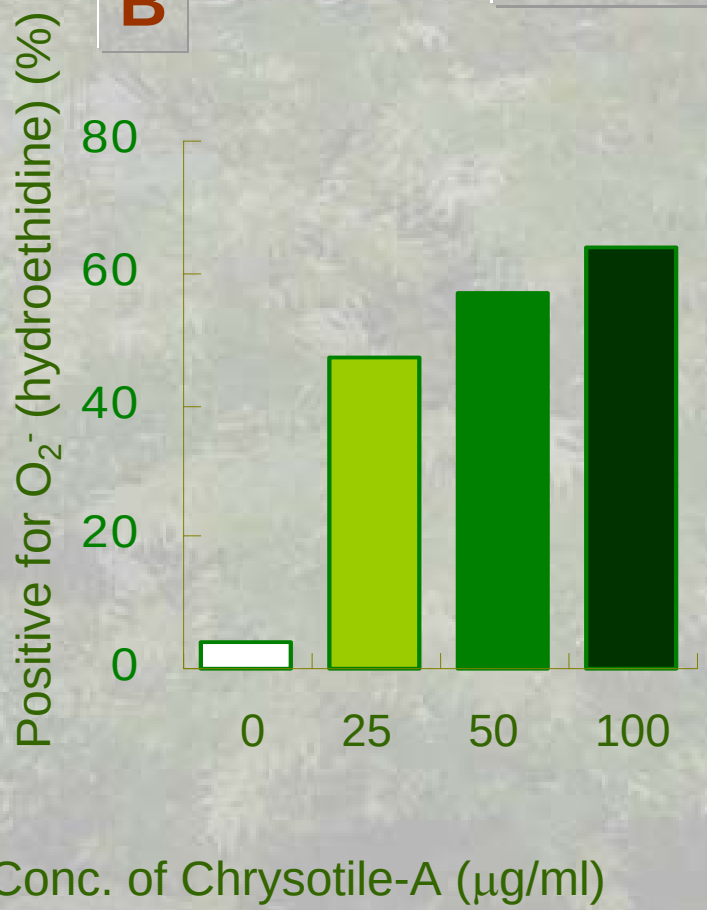
Production of superoxides in MT-2 cells cultured with chrysotile-A

FIG. 9

A

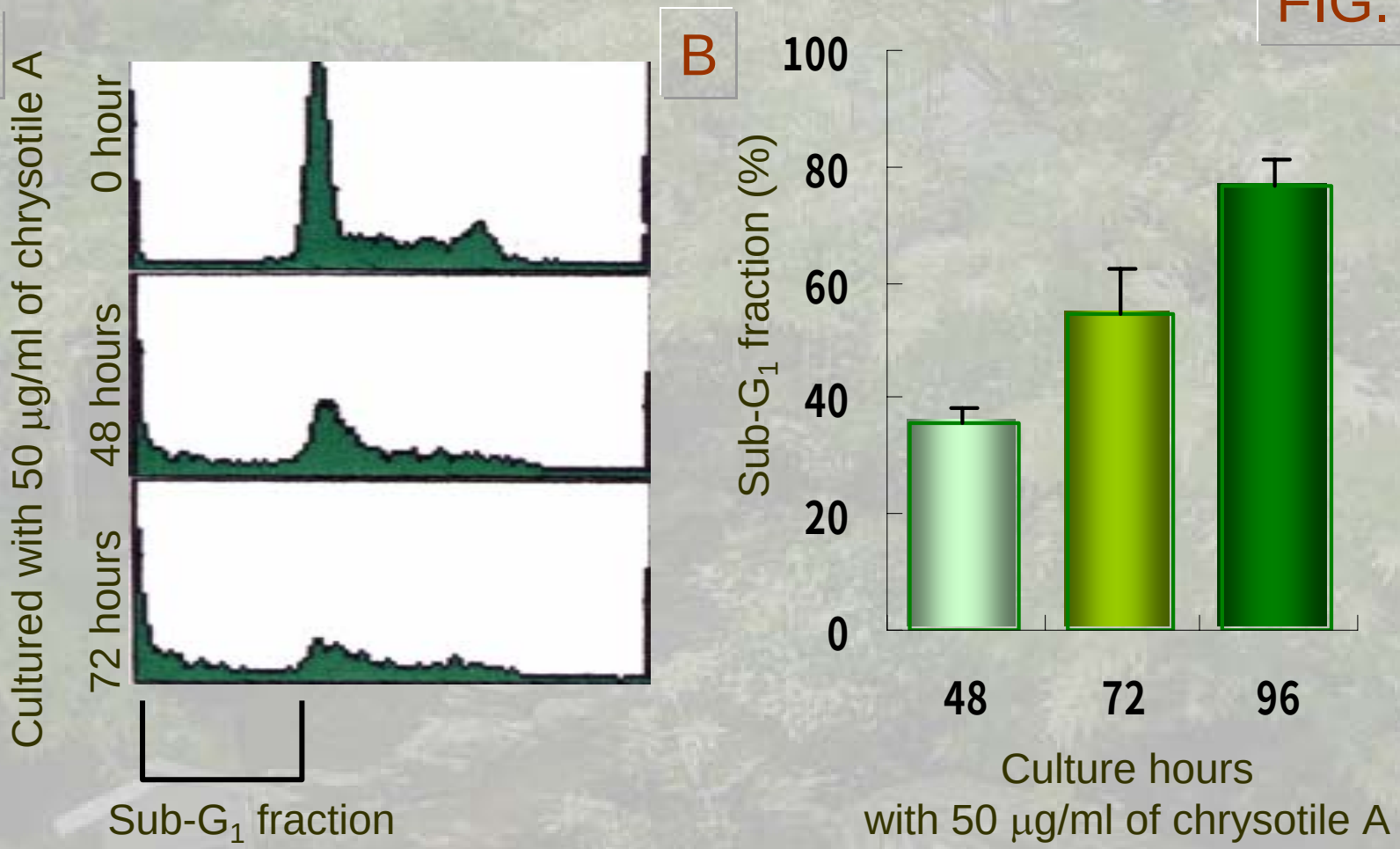


B



To monitor the apoptosis in MT-2 cells caused by chrysotile-A, sub-G1 cell cycle fraction was applied, since the increase of sub-G1 fraction showed also dose- and time-dependency when MT-2 cells were cultured with 50 mg/ml of chrysotile-A for 2 to 4 days (Panel A, and graphically B)

FIG. 10



From the results in Fig. 11-A, lipophobic hydroxyl-radical excluders (DMSO, DU and DMTU) showed effective reduction of apoptosis, although Mannitol, the hydrophobic excluder, did not.

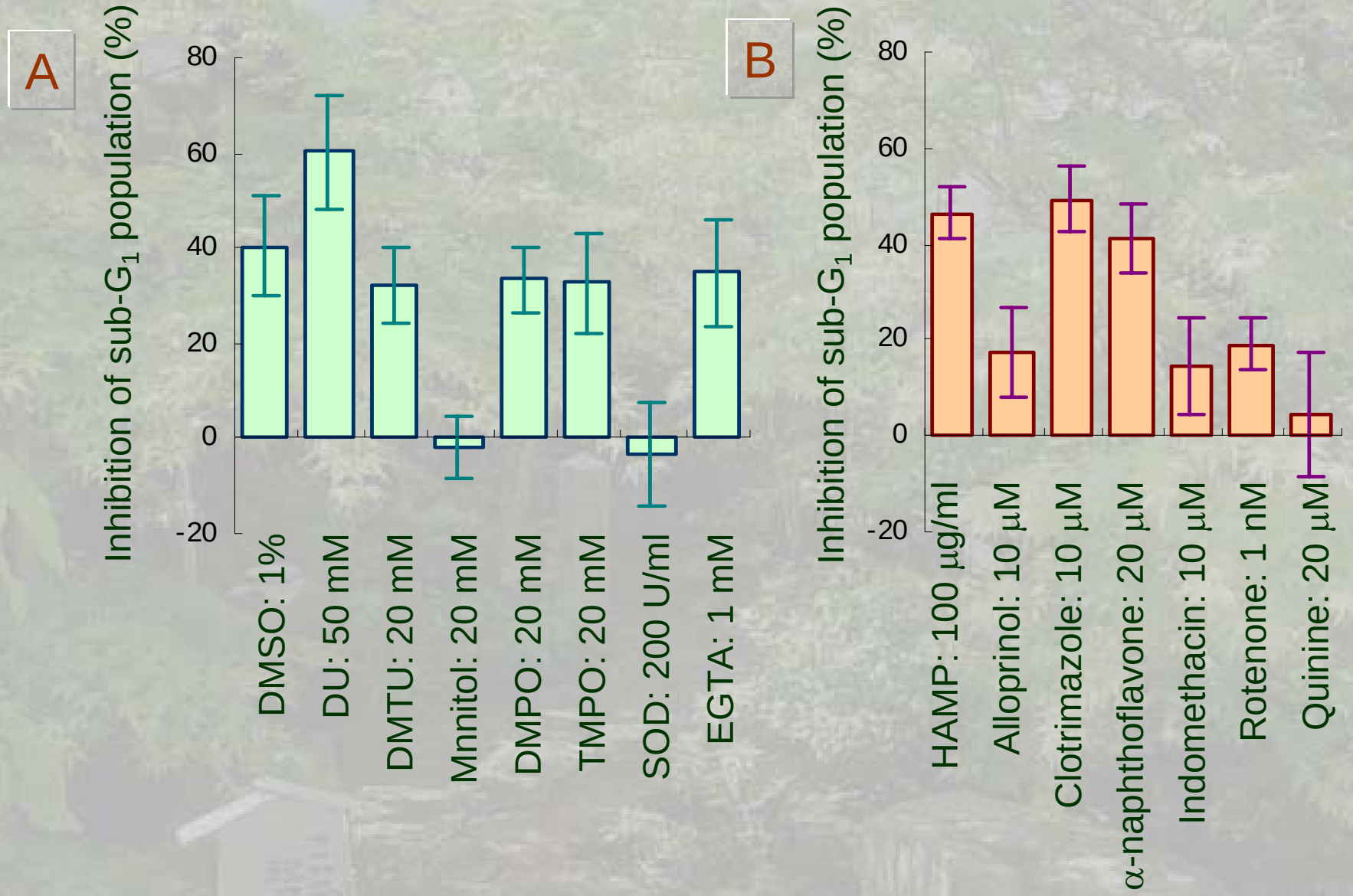
These results indicated that intracellular appearance of hydroxyl-radical played one of the most important roles to cause mitochondria-mediated apoptosis in MT-2 cells. This consideration was also supported that membrane permeable superoxides capturer (DMPO and TMPO) caused similar inhibition of appearance of sug-G1 fractions in MT-2 cells cultured with chrysotile-A, since generation of hydroxyl-radical is depend on production of superoxides in cytoplasmic membrane and microsomes.

In addition, particularly the inhibitors for NADPH-oxidase and cytochrome P450 effectively reduced the population of sub-G1 cell cycle fraction in MT-2 cells cultured with 50 μg/ml of chrysotile-A for 2 days (Fig. 11-B).

These results also strongly suggested that apoptosis of MT-2 cells caused by chrysotile-A was induced by activation of mitochondrial apoptotic pathway due to generation of hydroxyl-radical and production of superoxides in microsomes.

Inhibitory effects of anti-oxidants on apoptosis of MT-2 cells caused by chrysotile-A

FIG. 11



- Silica and silicate cause not only pneumoconiosis but also autoimmune disorders and malignancies such as lung cancers and malignant mesotheliomas.
- To clarify the effects of these substances on cellular features of lymphocytes, human HTLV-1 immortalized polyclonal T cell line. MT-2, was exposed by various concentrations of chrysotile-A, an asbestos classified as silicate.
- MT-2 cells underwent apoptosis in dose-and time- dependent manner.
- The mitochondrial apoptotic pathway was activated during chrysotile-A induced apoptosis of MT-2 cells, because of phosphorylation of JNK, increase and translocation of BAX, and releasing cytochrome-c from mitochondria to cytoplasma.
- In addition, anti-oxidants such as hydroxyl-radical excluder and capturer for superoxide, and inhibitor for superoxides production were effectively reduce the appearance of apoptotic fraction in MT-2 cells cultured with chrysotile-A.
- These results indicate that the activation of reactive oxygen species (ROS) may play central role in asbestos-induced T cell apoptosis and anti-oxidants may be helpful to prevent complications of pneumoconiosis.