Asia, and Dr Keicho will show you the data regarding this antigen in DPB later.

The efficacy of erythromycin raises the issue of what is the mechanism of action of the drug at the inflammatory site. Erythromycin is believed to inhibit hypersecretion by inhibiting both mucus and water secretion from epithelial cells. In addition, it is believed to inhibit neutrophil accumulation due to decreased attachment of the cells to the capillary walls and also through decreased production of IL8 by epithelial cells. Thus there would be less neutrophil-derived tissue destructive substances present in the lung, such as elastases and superoxide, following treatment. However more work needs to be done to clarify these actions of neutrophils on airway inflammation.

I would like to discuss the relationship between airway infection and the anti-inflammatory actin of erythromycin. It is believed that erythromycin is important in breaking the vicious cycle of chronic airway infection. Chronic airway infection is accompanied by an inflammatory response that is deleterious and so it is likely that an antibacterial treatment will be limited in effectiveness, whereas an anti-inflammatory agent, such as erythromycin, would be much more beneficial and therefore be more useful as a basic treatment.

Recently it has been published that erythromycin is able to inhibit bromonea induced acute lung injury. We found that erythromycin inhibited neutrophil accumulation in the lung and neutrophil-derived elastase in the lung, which prevented acute lung injury induced by bromophene. These data were published in the March issue of Thorax this year.

The effectiveness of erythromycin therapy in Japan has gone beyond our initial expectations and we believe that the use of this and other 14-MM macrolides may be useful in a variety of diseases in the future.

3. Apoptosis induced by Diesel Exhaust Particles in Human Airway Epithelial Cells *in vitro*

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I am going to talk about apoptosis induced by diesel exhaust particles in human airway epithelial cells in vitro. Our research background is as follows. Several lines of evidence have shown that diesel exhaust particles (DEP) may cause lung cancer, bronchial asthma, and lung fibrosis. The mechanisms of pathogenesis in these diseases remains unknown. We postulated that DEP might induce apoptosis in human airway epithelial cells (AEC). As a first step to investigate the effects of DEP on the human respiratory system, we investigated the effects of DEP on AEC in vitro.

First let me describe the diesel engine we used. The 2369-cc diesel engine manufactured by Hino Motor Company of Japan was operated at a speed of 1050 rpm and the concentration of DEP was in the range of 3 mg/m^3. This is the basic scheme showing the diesel engine system. We connected a small silicon tube to the dilution tunnel B. This small tube was introduced into the cell culture system containing the 5% CO_2 incubator.

This picture shows this culture system. We placed our microcell culture plates and cell chambers into the small container and the container was exposed for different intervals to DEP. Bet-1A, a kind of

SV40 transformed human bronchial epithelial cell line was exposed to DEP at different time intervals; in this case 0 min, 30 min, 60 min and 120 min. Cell survival and apoptosis were evaluated by uptake of tritiated thymidine into the cells, in situ end labeling (TUNEL method), RT-PCR, immunohistochemistry, and electron microscopy.

Now I will show you our results. Survival of cells decreased at 30 min exposure and over. So we used a 30 min exposure time for the following experiments. This slide shows the results by the TUNEL method. We could recognize more apoptotic cells in the experimental group than in the control cells. RT-PCR showed that both Fas and Bax-α mRNA were induced more significantly at 30 min. Again, in this experimental group, Bax was more intensely stained than in control cells. This electron micrograph shows Bet-1 cell exposure to DEP for 30 min; the microvilli are lost.

In summary, 30 min exposure to DEP decreased DNA synthesis in AEC significantly. DEP exposure induced apoptosis as evaluated by the TUNEL method. DEC exposure induced expression of Fas and Bax-α mRNA. Fas, Bcl-2 Bax and Bcl-X were immunostained more strongly in diesel-exposed cells than in unexposed cells.

We are going to clarify the mechanism of cell survival involved in DEP exposure in more detail.

Discussion

Dr Morimoto: Dr Terashima, did you check the viability of the macrophages by charcoal?

Dr Terashima: Yes, with exposure to charcoal most of them survived even at the highest dose.

Dr: I am impressed with the apoptosis caused by DEP. Is apoptosis caused directly by DEP or is there some superoxide induced by DEP or something?

Dr Takizawa: We only have a hypothesis. As far as I know, some of the biological effects of DEP is the effect of superoxide production. Some papers have also reported that some benzopiane, a kind of bioactive aromatic hydrocarbon may bind to the cell surface receptor and send signals into the nucleus. We would like to pursue this in further studies.

Dr Ohta: I have some results from epithelial cells cultured in the presence of DEP. We found that the cells, different from macrophages, can be stimulated to produce GM-CSF with DEP. So I realized that the epithelial cells and macrophages could be different in terms of response to DEP. But our hypothesis is that macrophages may play an important role in induction of airway hyperresponsiveness by producing GM-CSF. So probably epithelial cells could produce GM-CSF initially, then stimulate macrophages to do something additional to induce airway hyperresponsiveness by GM-CSF.

The other thing is when we look at LDH release from cultured epithelial cells in the presence of DEP we were not able to see any increase of LDH in the supernatant. This suggested that the cells were not dying quickly in the presence of DEP. So apoptosis may occur in a later phase as said in the abstract.