ABSTRACTS

IMMUNITY IN CARCINOGENESIS (II) CHEMICAL CARCINOGENESIS

Kimio YASUHIRA

Department of Pathology (Chaiman: Professor Kimio Yasuhira)
Chest Disease Research Institute, Kyoto University

Creech et al. (1939) tried to induce resistance against chemical carcinogenesis by immunizing animals with a carcinogen conjugated with certain heterologous proteins and found that it was very difficult to do so. After that, Miller et al. (1947) showed that a protein fraction of the liver combined with a carcinogenic azo dye in the early stage of treatment with the dye. It is well known that this finding led him only to the "protein or enzyme-deletion theory" of carcinogenesis, but not to pay any attention to the immunological role of conjugated carcinogens.

Pope et al. (1964) and others were successful in demonstrating a new antigen in viral tumors by the use of fluorescent antibodies from tumor-bearing animals. Moreover, Gold et al. (1965) elucidated antigens specific to human stomach carcinomas on gel-diffusion plates by means of adsorbed antisera from heterologous animals neonatally sensitized to normal components of the human body. However, it is doubtful that these humoral antibodies play a role in inhibiting the growth of tumors. Klein et al. (1962) and others called attention to the destructive effect on tumors of lymphoid cells from the tumor-bearing animals, although the antigens and antibodies in this phenomenon have not been described. Takeda and his associates (1963, 65) showed that chemical carcinogenesis seemed to be suppressed in animals treated with a lipoprotein fraction of the tumors induced by the same carcinogen.

This author's experiments investigated the possible inhibition of tumor growth by antibodies against chemically induced autochthonous tumors. An attempt was made to induce tolerance to the carcinogen by injecting newborn mice with the drug or by neonatal thymectomy. The results are open to discussion, but it may be said that specific antigens, if any, differed significantly with the nature of the tumor and with the presence or absence of malignancy. Marked effects on chemical carcinogenesis of heterologous immunizations of rats are also illustrated in the tables.

Bull. Chest Dis. Res. Inst., Kyoto Univ.

CYTOLOGICAL TYPING OF LUNG CANCER CELLS

Yoshio OKADA and Motohiko ITO

Department of Thoracic Sugery, (Chairman: Professor Chuzo Nagaishi), Chest Disease Research Institute, Kyoto University

From the cytological point of view, exfoliated cells from lung cancer can be classified into 4 groups: squamous cell carcinoma cells, adenocarcinoma cells, small cell carcinoma cells, and large cell carcinoma cells.

In this study the relationship between the cytological type as determined by exfoliative cytology and the histological type of the original lesion was investigated. Squamous cell carcinoma cells showed a marked variation in shape and size, and bizarre cells such as "tadpole cells", "fiber cells", and "snake cells", were often seen. Cytoplasmic staining ranged from light green to orange. Irregular nuclear membrans and a marked increase in nuclear chromatin with diffuse hyperchromasia (so called india-ink-like) were often observed.

Adenocarcinoma cells were usually round and often found in clusters. Their cytoplasm was clear and stained light green. The nuclei were also round, and the nuclear membrane was often thin and wrinkled. Large nucleoli were the most significant feature in adenocarcinoma cells. Vacuolization, formation of small acini, and papillary arrangements were also often observed.

Small cell carcinoma cells were as small as lymphocytes, and consisted almost entirely of nuclei with little or no visible cytoplasm. This type of cell could be differentiated from lymphocytes by their anisocytosis, nuclear hyperchromasia or irregular chromatin pattern.

Cells which could not be classified in the above 3 types, were classified as large cell carcinoma cells. These cells had less cytoplasm and a less distinct nuclear membrane.

Squamous cell carcinoma was accurately typed in 94.6% of cases, adenocarcinoma in 78.6%, small cell carcinoma in 100%, and large cell carcinoma in 50%. The over-all accuracy of typing was 80%.

Further studies are needed on the criteria of large cell carcinoma cells, especially on the differentiation between large cell carcinoma cells and noncornified squamous cell carcinoma cells.

COMPARATIVE STUDIES *IN VITRO* ON VALUE OF DAILY AND OF INTERMITTENT ANTITUBERCULOUS REGIMENS

Yoshiko OHTA

First Department of Medicine (Chairman: Professor Masukazu Naito)
Chest Disease Research Institute, Kyoto University

Experimental studies were performed *in vitro* with a silicone-coated slide culture method on minimum inhibitory concentration, minimum sterilizing concentration and development of drug resistance in an attempt to determine the effect of daily and of intermittent (twice weekly) chemotherapeutic regimens with kanamycin (KM), cycloserine (CS), ethambutol (EB) and ethionamide (TH).

The following results were obtained.

(1) In growth-inhibiting activity against H37Rv, for every drug tested in this experiment, the continuous regimen was more effective than the intermittent one.

(2) The bactericidal activities against H37Rv under these experimental conditions showed the inferiority of the intermittent to the continuous regimen. It was very difficult to sterilize tubercle bacilli by the intermittent action of CS, EB or TH even at the concentration as high as 1,000 mcg. per ml.

(3) H37Rv did not develop drug-resistance to CS or EB under either regimen. Slight drug-resistance to KM and TH developed in the range of concentrations of incomplete sterilization. The range of incomplete sterilizating concentration under the intenmittent regimen was higher than that under the continuous regimen and such high concentrations can not be maintained in body fluids clinically. This may be the reason why a drug-resistance is unusual under the intermittent regimens.

(4) The results of these experiments showed that an antituberculous regimen of twice-weekly administration is less effective than daily administration in every respect.

Bull. Chest Dis. Res. Inst., Kyoto Univ.

A STUDY ON PULMONARY VASCULAR RESPONSE TO REGIONAL HYPOXIA

Toshiro TANAKA

Department of Thoracic Surgery (Chairman : Professor Chuzo Nagaishi), Chest Disease Research Institute, Kyoto University

Since Euler and Liljestrand reported their studies on pulmonary vascular response to hypoxia, extensive research has been done on this problem for the purpose of elucidating both the regulatory mechanism of pulmonary circulation and the pathogenesis of cor pulmonale.

Nevertheless complete agreement has not yet been reached on the pulmonary vascular response to hypoxia.

In Chapter 1, we report patho-physiologic studies on the pulmonary vascular response to regional hypoxia and morphological observations of this response with the use of a rapid freezing method with liquid nitrogen in guinea pigs and rabbits.

In Chapter 2, we describe the mechanism of pulmonary vascular response.

The following conclusions were drawn from these experiments:

1) No significant change in P.A. pressure was observed in unilateral regional hypoxia which did not result in general hypoxemia.
2) Marked vasoconstriction and perivascular edema were seen in peripheral muscular arteries in guinea pig bronchioles with diameters of 150-300 μ. No morphological changes were observed in pulmonary capillaries or veins.

   The muscular arterial response observed in guinea pigs was not demonstrated in rabbits.
3) Although the pattern of response of pulmonary arteries to regional hypoxia differs from species to species, it is most likely that morphological changes in peripheral muscular arteries are responsible for the increase of pulmonary vascular resistance induced by hypoxia.
4) Although pulmonary vascular constriction induced by hypoxia is considered to be due to the direct action of hypoxia itself on the vascular wall, a reflex mediated through an Axon reflex or autonomic nerve cannot be completely excluded.

DISTRIBUTION OF ADRENERGIC NERVE FIBERS IN THE LUNGS OF THE RABBIT, GUINEA-PIG AND MAN AS DEMONSTRATED BY FALCK-HILLARP'S FLUORESENCE METHOD

Takayoshi KAI

Department of Thoracic Surgery (Chairman: Professor Chuzo Nagaishi), Chest Disease Research Institute, Kyoto University

By means of Falck-Hillarp's fluorescence method and Staub's rapid freezing method, the distribution of fluorescent, sympathetic postganglionic fibers was studied in the lungs of the rabbit, guinea-pig and man under conditions as physiological as possible.

No great differences were found in the intrapulmonary pulmonary arteries and bronchi of these three species. In pulmonary arteries and bronchi, nerve plexuses appeared, morphologically, to be separate, but physiologically, they seemed unified to form a single "broncho-pulmonary arterial" plexus, because of the abundance of communicating twigs between the two.

In the walls of the pulmonary arteries, almost all fluorescent fibers were found in the border between the adventitia and media. Finer fibers branched off from thicker ones to form a meshwork. The smaller the arteries, the less dense was the fluorescence. No fluorescence was seen in arteries with an inside diameter less than 100 μ, and in precapillaries or capillaries. Very few fluorescent fibers entered the medial smooth muscle layer, and the intima showed no specific fluorescence.

In the intrapulmonary bronchi with cartilaginous tissues, fluorescence was more dense inside this tissue (subchondrial plexus) than outside (extrachondrial plexus). Fluorescence was most marked in the bronchial vessels, which exhibited it when they were even as small as 20 μ in diameter. The bronchial smooth muscle layers and submucosa showed much fluorescence almost equally. However, the submucosal fluorescence appeared to originate from the peribronchial arterial plexus. The submucosal plexus decreased in quantity with the size of the bronchi, and the bronchi with no cartilage had only a peribronchial nerve plexus. In the periphery, the bronchi showed less fluorescence than the pulmonary arteries.

Rabbit lungs showed no fluorescence of intrapulmonary pulmonary veins, while in guinea-pigs and man there was some in the veins but much less than in the pulmonary arteries. Since there were no fluorescence in the lung parenchyma and no communicating branches between the perivenous plexuses and those around the bronchi and pulmonary arteries, the previous plexus seems to be a separate, independent nerve plexus.

Bull. Chest Dis. Res. Inst., Kyoto Univ.

EXPERIMENTAL STUDY ON CROSSCIRCULATION

Nobuaki MIYAMOTO

Department of Thoracic Surgery (Chairman : Professor Chuzo Nagaishi),
Chest Disease Research Institute, Kyoto University

Crosscirculation studies between 50 pairs of rabbits showed the following direct and indirect effects.

(1) For practical purposes the best results in rabbits were obtained when crosscirculation was controlled by a roller-pump for about two hours with a volume of about 2 ml per minute.

(2) With this method the blood of a pair of rabbits can be mixed evenly without having any ill effects on their circulation.

(3) After crosscirculation the pairs were separated and observed for up to one year. Wasting syndrome appeared in only one of the pair.

(4) This wasting syndrome caused by crosscirculation is named "crosscirculation disease".

(5) The incidence of this disease is highest two to three weeks after crosscirculation, and then decreases gradually, but is still about 40% after six months.

(6) In the early stage of this disease swelling of the spleen and lymph nodes and proliferation of lymphocytes in the peripheral blood are observed, and in the late stage atrophy of the spleen and proliferation of neutrophiles in the peripheral blood are observed.

These findings are substantiated pathohistologically, and it seems that this disease resembles parabiosis intoxication.

(7) The views, therefore, of certain European and American scholars who advocate the application of crosscirculation in the treatment of acute icterus and uremia cannot be approved of from the immunological viewpoint.

(8) On the other hand, in several cases both of the pair were in good condition for a long time following crosscirculation. Mutual skin transplantations were performed in such pairs to see if immunological paralysis or tolerance had been induced.

It was found that the survival of the graft was prolonged in most cases, which may show that immunological paralysis or tolerance had been induced to some extent by crosscirculation.

(9) There were differences, however, in the length of time grafts survived depending on the lapse of time between crosscirculation and grafting. When skin transplantation was performed immediately or over five weeks after crosscirculation, it survived for a long time, but when it was performed after two or three weeks, it did not survive so long.

(10) This phenomenon is considered by us to represent "enhancement".

(11) As we have shown, crosscirculation gives rise to various immunological reactions which are of interest, and may be of value in the study of transplantation immunology.