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ABSTRACTS

Details of following articles are written in Japanese in following pages.

A STUDY OF TRANSPLANTATION IMMUNITY USING TISSUE CULTURE METHOD

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Transplantation immunity is the most important problem in tissue transplantation, but since it is very difficult to comprehend this by conventional immunological approaches, various points still remain unknown with respect to its cause and mechanism.

Recently, Terasaki, Granger, Vainio, and Wilson have investigated the transplantation immunity reaction using a tissue culture method, and have obtained notable, but not quite satisfactory results.

The author cultured target cells primarily from the donor tissue and made these cause an immunological reaction by adding serum or spleen cells from the recipient. The morphological changes brought about in these target culture cells were then examined.

Part I describes the immunoreaction due to active sensitization between xenogeneic animals, while Parts II and III, discuss xeno- and allo-transplantation.

In studying immunoreactions in cell cultures, one must minimize non-specific reactions. By culturing the donor tissue beforehand in serum from the recipient obtained before sensitization or transplantation, the author tried to observe the transplantation immunoreaction alone in its pure form.

The results thus obtained, interpreted and summarized by the author, are as follows:

1) An almost similar kind and degree of immunoreaction is observed in active sensitization and xeno-transplantation between xenogeneic animals.

That is, when the serum derived from the xeno-sensitized animal or xeno-grafted recipient is added to the target culture cells from the donor, cell division is inhibited, and abnormal chromosomes are observed. The changes observed in the culture cells are diminution, deformity, and overstaining of the nuclei, and turbidity,

over-staining, and deformity of the cytoplasm.

2) The target culture cells from the donor are damaged in various ways by the spleen cells of the xeno-sensitized animals or xeno-grafted recipient, and show decreased frequency of division, deformity and pyknosis of the nuclei and overstaining of the cytoplasm. The changes, however, are slighter than when humoral antibody is added. On the other hand, the added recipient spleen cells aggregate around the target culture cells specifically and are destroyed.

3) In allo-transplantations in rabbits also, the target culture cells are clearly damaged by the recipient serum. That is, the frequency of cell division of the target cells decreases to less than a half; vacuolization, turbidity, deformity are observed in the cytoplasma; and the nuclear membrane and nuclear structures are overstained.

4) In allo-transplantations in rabbits, the damage to the target culture cells from the donor caused by recipient spleen cells is even greater than that caused by serum. The cytoplasm is turbid, but the nuclei suffer more severe changes, and pyknosis is observed. On the other hand, the added recipient spleen cells aggregate around the target culture cells from the donor specifically and are destroyed.

5) In the immunoreaction of xeno-sensitization, xeno-transplantation, and allo-transplantation, cellular damage due to cell-bound antibodies is most severe in the nuclei, appearing mainly as pyknosis. On the other hand, cellular damage due to humoral antibody appears to the same degree in the nucleus and cytoplasm, or is greater in the cytoplasm.

This seems to suggest that the mechanism or site of action of humoral antibody and cellular antibody is different.

6) The location of the antibodies in sensitized lymphoid cells which play an important role in cell-bound antibody reactions is still open to question, but the fact that when spleen cells were added there was an unevenness in the distribution of cells showing cellular damage and the added spleen cells were frequently destroyed specifically, and the fact that there was a difference in the location of the cellular damage caused by the two antibodies suggest that the antibodies are located inside the lymphoid cell. If they are located on the surface of the cell membrane, they must be tightly conbined with it and not easily removed.

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STUDIES ON IMMUNE DEVIATION IN RABBITS
II. THE INHIBITION OF DELAYED HYPERSENSITIVITY BY AN INJECTION OF ALUM-PRECIPITATED PURIFIED PROTEIN ANTIGEN

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Previous investigations have shown that intravenous injection of heat-killed BCG in sensitive rabbits induces apparent but transient suppression of tuberculin sensitivity without impairment of antibody production to tuberculous antigens. This suppression of delayed hypersensitivity is an immunological phenomenon. It was thought that this phenomenon might be one form of the "Immune Deviation" described by Asherson and Stone.

Tubercle bacilli are too complicated to be used as an antigen to analyse this phenomenon, but simple antigens, such as purified serum proteins might be useful for this purpose. Therefore, the writer performed similar desensitization experiments in rabbits using bovine serum albumin along with old tuberculin as antigens. Experiments of Asherson's and Stone's type were also undertaken, using the same antigens as were used in desensitization experiments.

The experimental results were as follows.

I. A. When rabbits immunized with Freund's complete adjuvant were challenged by an intravenous injection (Desensitization procedure) of soluble or alum-precipitated old tuberculin (O.T.), the tuberculin reaction was transiently inhibited with no change in circulating antibody titers.

B. When rabbits received an intravenous injection of soluble or alum-precipitated O.T. 3 days before immunization with Freund's complete adjuvant (Asherson type procedure), the tuberculin reaction was inhibited with no change in circulating antibody titers.

II. A. Desensitization procedure with alum-precipitated BSA in rabbits immunized with BSA in Freund's complete adjuvant suppressed transiently the delayed type skin reaction to BSA, but had no effect on the circulating antibody level.

B. The Asherson type procedure with intravenous injection of alum-precipitated BSA 3 days before immunization with BSA in Freund's complete adjuvant suppressed the delayed type skin reaction to BSA for about ten weeks, but had no effect on the circulating antibody level.

The Asherson type procedure with soluble instead of alum-precipitated BSA caused a similar suppression of delayed hypersensitivity.

III. It was shown that the inhibition of delayed hypersensitivity in these experiments was antigen-specific.

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A STUDY ON THE EFFECT OF ARTIFICIALLY INCREASED VISCOUS RESISTANCE WITH SPECIAL REFERENCE TO RESPIRATORY MECHANICS: AN ANALYSIS OF RESPIRATORY PATTERNS

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The changes of tidal volume, minute ventilation and viscous work per tidal volume and unit ventilation were measured in awaking subjects with a spirometer in which artificial viscous resistance was built in. The respiratory frequency of the subjects was paced by a metronome at various preset rates.

The results showed that both V_T and V_E were influenced less by the addition of artificial viscous resistance at low respiratory frequencies. The calculation of viscous work showed that the addition of viscous resistance caused a lowering of mechanical respiratory efficiency in all studies.

Viscous work per unit ventilation was minimum at a respiratory rate of 10, which was therefore considered the "optimal rate of breathing" in human beings. Measurement of viscous work also showed that viscous respiratory efficiency decreased during hyperventilation induced by exercise.

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STUDIES ON DYSPNEA IN PATIENTS WITH PULMONARY DISEASE, WITH SPECIAL REFERENCE TO ELECTROMYOGRAMS OF RESPIRATORY MUSCLES

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Part I.

Electromyographic studies of the respiratory muscles in patients with dyspnea at rest.

Electromyograms of respiratory muscles, arterial blood gas analysis, and ventilatory changes were investigated in nine patients with pulmonary diseases and dyspnea at rest.

1) It was found that the sternocleidomastoid muscles participated in the respiratory movements in all cases. Thus the degree of participation of these muscles can be used as an objective index of the severity of dyspnea.

Part II.

Electromyographic studies on the respiratory muscles in cases of hypoxia, hypercapnia and the artificially increased resistance in the airway.

Electromyograms of the respiratory muscles, arterial gas and ventilatory changes were investigated during induced hypoxia, hypercapnia and artificially increased resistance in the airway in patients with pulmonary diseases and no dyspnea at rest but with slightly disturbed pulmonary function.

1) During moderate anoxemia, the sternocleidomastoid muscles were active in only two of six cases, and even when the anoxemia became severe, subjective dyspnea occurred in only one of six cases.

2) During induced hypercapnia, the activity of the sternocleidomastoid muscles increased in six of seven cases, and subjective dyspnea appeared simultaneously.

3) During breathing through artificially increased resistance, the greater the resistance, the more active were the sternocleidomastoid muscles.

4) These results, indicate that the various types of dyspnea can be graded objectively by electromyograqhy of the sternocleidomastoid muscles.

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STUDIES ON ANTITUBERCULOSIS ACTIVITIES OF δ-HYDROXY-γ-OXO-L-NORVALINE (HON) REPORT 3. *IN VITRO* ANTITUBERCULOSIS ACTIVITIES OF HON COMBINED WITH SOME ANTITUBERCULOSIS AGENTS

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As a sequel to the previous report (Kekken Kiyo, Vol. 12, No. 2, 1967) of fundamental experiment and clinical studies on HON, *in vitro* studies were undertaken on the bacteriostatic and bactericidal effects of HON when used in combination with some other antituberculosis agents such as SM, PAS, INH, KM, CS, TH, VM, PZA, EB, SOM, SI, DAT, TC, and OQ.

The mode of resistance-acquisition by Myc. tuberculosis H37Rv to each of the combined agents; SM, INH, KM and TH was also evaluated by successive transfers to the media containing increasing concentrations of the drug.

The results were as follows:

1. No antagonistic effect was demonstrated between HON and the other antituberculosis agents eximaned in both bacteriostatic and bactericidal experiments.

A combined bacteriostatic effect was demonstrated between HON and PZA, DAT or PAS, and a combined bactericidal effect was demonstrated between HON and PZA, PAS or SOM.

2. The development of resistance of H37Rv strain to SM or INH was slightly suppressed when these drugs were combined with HON, but not in case of kanamycin.

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STUDY ON THE TRANSFER FACTOR OF CONTACT HYPERSENSITIVITY
II. ANTIGEN-SPECIFICITY OF THE TRANSFER FACTOR

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Guinea pigs actively sensitized with DNFB did not show any cross reactivity of delayed skin hypersensitivity to NDMA or PCI. The same non-crossreactivity sensitized with NDMA was noted between DNFB or PCl.

The transfer factor obtained from the peritoneal exudate cells or the spleen of highly sensitized donors (the preparation of the transfer factor was described in detail in the first report) was transferred to normal recipients, and 24 hours later the recipients were skin-tested with the three drugs. Successful passived transfer was noted with the chemical which had been used for sensitization, but no reactivity was shown with the other drugs.

Thus, it was demonstrated that passively transferred sensitivity has relatively antigenspecificity.

When donors had been sensitized with the chemicals along with the Freund's complete adjuvant, passively transferred sensitivity with the transfer factor obtained from these donors showed reactivity to other drugs than the one used for sensitization.

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STUDIES ON THE ANTITUBERCULOUS ACTIVITY OF 4, 4'-DIISOAMYLOXY-THIOCARBANILIDE *IN VITRO*

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The antituberculous activity of 4, 4'-diisoamyloxythiocarbanilide (DAT) was studied *in vitro*, including the problem of cross resistance among DAT, thioacetazone and ethionamide.

The results obtained were as follows:

1. The minimum inhibitory concentration of DAT against H37Rv strain was 3.13 mcg. per ml. in Tween-albumin liquid medium and 12.5-25 mcg. per ml. in Kirchner's liquid medium with 10% bovine serum, on 1% Ogawa's egg solid medium and in silicone-coated slide culture.
2. The tuberculostatic activity was lower in of a medium of pH 6.5 than in one of pH 5.5 or 7.5, and was less influenced by the size of the inoculum.
3. The medium-replacing culture method proved that DAT was unstable in Kirchner's medium and its biological activity decreased rapidly.
4. The tuberculostatic effect was examined in Kirchner's liquid medium with 10% bovine serum against 50 strains of *Mycobacterium tuberculosis* isolated from untreated patients. Of these, 20% were inhibited by 6.25 mcg. per ml., 50% by 12.5 mcg. per ml. and 30% by 25 mcg. or more per ml.
5. Resistance to DAT developed very slowly and occurred only twice even after 5 transfers.
6. The development of kanamycin resistance was retarded by combining it with DAT under the conditions of this experiment.
7. The cross resistance berween DAT and other antituberculous drugs was studied in laboratory resistant strains derived from a sensitive strain of H37Rv. There was no cross resistance between DAT and streptomycin, kanamycin, viomycin, isoniazid, or p-aminosalicylic acid.

H37Rv strain resistant to thioacetazone was completely resistant to DAT and *vice versa*. A slight incomplete cross resistance between DAT and ethionamide was observed. On the other hand, H37Rv strain resistant to ethionamide was slightly resistant to thioacetazone although one resistant to thioacetazone remained sensitive to ethionamide.

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A CASE OF PULMONARY EMPYEMA CAUSED BY AN UNCULTURABLE FUNGUS

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A 49-year-old businessman who had had hemoptysis and had been treated with antibiotics, chemotherapeutics, and corticosteroid hormone developed pulmonary empyema. The pus contained numerous soft, yellowish-white grains. When pressed between two slides and observed with a phase-contrast microscope, the grains were consisted of septate hyphae 3 to 4 μ in width. No spores were found. The hyphae were sensitive to drying, and when dried on the slide their morphology was completely destroyed.

Efforts were made to culture the grains on mycological and bacteriological media (87 attempts during 14 months of hospitalization), but the causative fungus could not be grown on any media used.

Inoculation of the grains with mucin into mice and guinea pigs intraperitoneally and rabbits intrathoracically failed to produce any lesions, and no organisms were recovered from the organs of these animals.