SCIENTIFIC REPORTS

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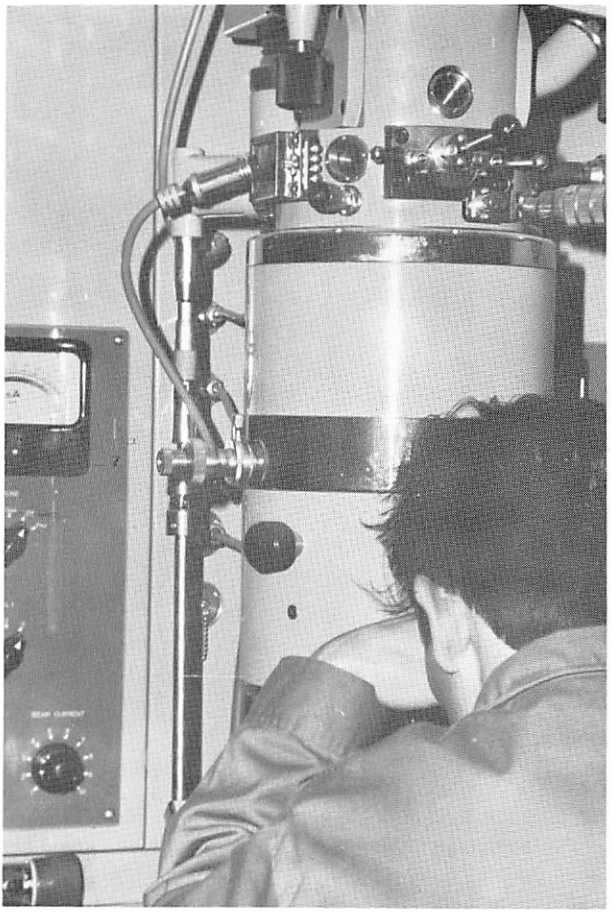
Immunobiology


BEAM CURRENT

DEPARTMENT OF IMMUNOBIOLOGY

GENERAL SUMMARY

In the past few years, the department has gone through a period of transition. Some of these chronological events are as follows. Dr. Toshio Saito resigned from his position as professor and department head in 1976 to participate in the foundation of a new medical college. Dr. Morinobu Takahashi, then Assistant professor of the Department of Molecular Immunology and studying on leave of absence at the New York University School of Medicine was promoted to the position in February 1, 1977. Dr. Shunnosuke Sakai was promoted to an assistant professorship on July 1, 1977. Dr. Masaru Nonaka and Dr. Shigetoyo Amano joined the department as research associates in October and November, respectively. As a whole, the transition was very smooth and the research activity in the department has reached a considerably high level in a relatively short time.

The research activities in this department are directed toward the study of complement. Complement, a collective term for a group of more than 18 serum proteins, plays an essential role in various immunological phenomena as well as in non-immunological inflammatory reactions, serving as a major humoral effector system in body defence mechanisms. With an aim at eventually elucidating an exact role of complement in various diseases including cancer, we are currently devoting our activities to the following three subjects. 1) The mechanism of complement-dependent solubilization of immue complexes and its relevance to immunopathology. 2) Genetic control of complement production. 3) Phylogeny of the complement system.

The first project, being conducted by Dr. M. Takahashi and Ms. M. Nonaka, deals with a recently discovered complement function: solubilization of antigen-antibody complexes. This phenomenon highlights a thus far unrecognized function of complement, namely, the regulation of antigen antibody interaction. Although solubilization of Ag-Ab complexes has been demonstrated only *in vitro*, this phenomenon may be relevant to immunopathology (Reviewed in Transpl. Review *32*, 121, 1976.). Since increasing amounts of evidence show the occurrence of circulating immue complexes in patients sufferring from cancer and other diseases, a tool to characterize immune complexes is very important for the better understanding of the pathogenesis of these complexes in diseases. In the studies carried out in the past year in this laboratory, we found that solubilization occur in the completely homologous system consisting of human antibody and human complement. Therefore, it is highly likely that the solubilization process is taking place also in humans.

The second project is being carried out by a group headed by Dr. Sakai. Dr. S. Amano and Mr. T. Kaido, a graduate student, are actively engaged in this project. We like to emphasize here that this project is made possible only by a close collaboration with Dr. J. Hayakawa, of the institute for Experimental Animals. His excellent background as an animal geneticist is indispensable in the present study. We also wish to express our thanks for continuing advice and generous supply of various strains of mice to Dr. K. Kondo of Nagoya University, Dr. K. Moriwaki of the National Institute for genetics, and Dr. K. Suzuki and Ms. K. Sudo of the Institute for Medical Sciences, Tokyo University.

By the energetic work of this group, it was demonstrated, for the first time in the world, that complement C3, the most abundant and most important component of all the complement, is controlled in mice by a single structural locus linked to the major histocompatibility gene complex on chromosome 17.

The third project is a part of the long range study intended to elucidate the origin of the complement system. Dr. M. Nonaka is carrying on this very interesting and at the same time very difficult project. He has already obtained quite convincing evidence that lower vertebrates have an already considerably developed complement system. He is currently purifying from plasma of teleost fish a serum protein possibly related to C3 of higher vertebrates.

Besides extensive collaboration with the scientists cited above, collaboration in many aspects of research activities are under way between this and the department of Molecular Immunology. For example, Dr. S. Sakai has been performing joint experiments with Dr. Y. Kaneko and Dr. S. Migita on the mechanism of immunoresistance in mice against murine plasmacytoma. The results of their experiments have already been published in part in J. Immunology. Furthermore, because of increasing interest in immunology on the side of researchers of other disciplines, members of this department participate in free exchange of information and reagents with many scientists of other departments of this Institute on a day-to-day basis.

In general, the research activities of this department proved to be very fruitful in the past year and some of the results from our research have already been published in international periodicals as shown in the publication Section.

ABSTRACT

(36) Solubilization of immue complexes formed from tetanus toxoid and human antibody IgG by homologous complement.

M. Takahashi, M. Nonaka and S. Takahashi

Immue complexes formed from various soluble antigen and antibody can be solubilized by complement. In order to confirm that a similar phenomenon occurs in humans, we tested human serum for the complex solubilizing activity with immue complexes formed from human Ab. Immune precipitates were prepared from partially purified tetanus toxoid antigen and ^{131}I-labelled human anti-tetanus IgG (both were kindly supplied by Dr. Hirose of Tokyo University School of Medicine) at the equivalent antigen-antibody ratio. Immune precipitates were washed with diluent (phosphate buffered saline containing 15×10^{-5} M $CaCl_2$, 5×10^{-4} M $MgCl_2$ and 0.1% gelatin), resuspended in the diluent and finely dispersed by passage through a tuberculin needle. Precipitates containing 0.1–1 microgram of complexed Ab was incubated with 0.4 ml of dilutions of human serum at 37°C. At various time intervals, 0.05 ml samples were withdrawn and immediately diluted with cold diluent containing 0.025 % sheep erythrocytes and 0.01 M EDTA. The mixtures were centrifuged at 1500 g for 10 min, and supernatant and pellet were assayed for radioactivity in an automated gamma counter.

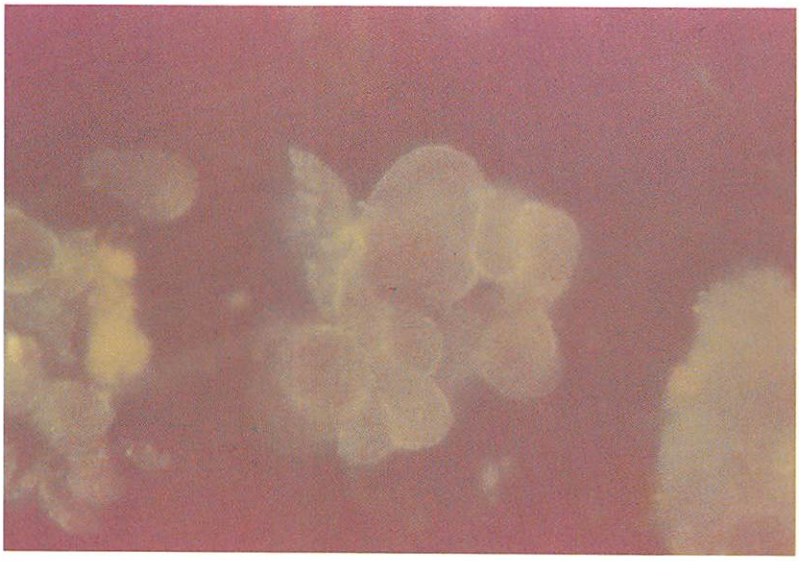
As shown previously with other Ag-Ab systems, immune complexes (tetanus anti-tetanus human IgG) can be readily solubilized by human serum. Controls in which the same amount of immune precipitates were incubated with heated serum or in human serum diluted with EDTA showed little solubilization. In accordance with the previous studies, solubilization of tetanus-anti-tetanus human Ab depends on the alternative pathway, since depletion of factor B from serum completely abrogates solubilizing activity of human serum. Addition of physiological concentrations of purified factor B restored the activity almost completely to the serum. The classical pathway enhances the solubilization of the precipitates. Solubilized complexes cannot combine with the surface receptors of human leukocytes and erythrocytes. The latter finding may explain in part the occurrence of circulating immune complexes in patients suffering from various immunological diseases and cancer (Takahashi, in *Progress in Allergy*, to be published).

(37) Genetic polymorphism of murine complement C3 controlled by a single codominant locus linked to the major histocompatibility complex.

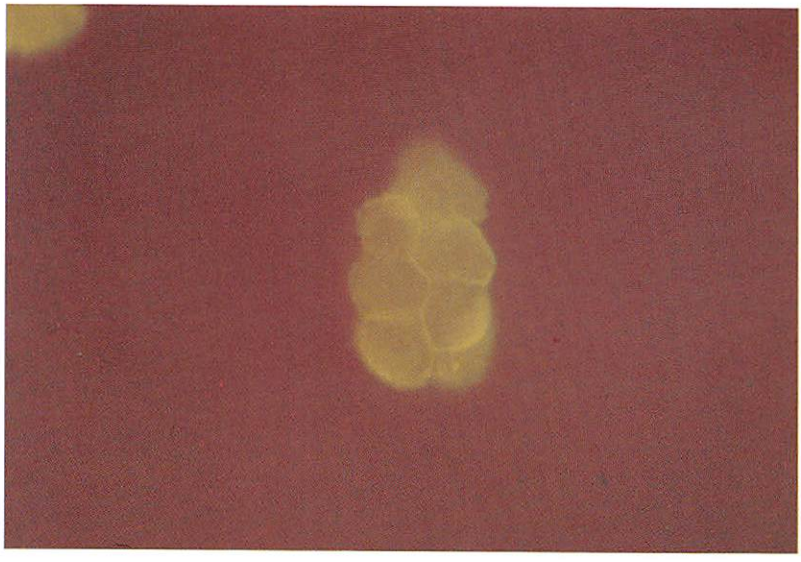
S. Sakai, S. Amano, J. Hayakawa and M. Takahashi

Polymorphism of complement C3 was detected in inbred mice by the combined use of analytical isoelectric focussing (IEF) and immunofixation. Fresh EDTA-mouse plasma from various mice was run on the 5% polyacrylamide gel containing carrier Ampholine (LKB), pH range 3.5–10. C3 was revealed as a single protein band of pI 6.1 (C3 BB type) in all the inbred strains except mice from SWR strain and three strains established originally in Japan. Thus, all of the 18 strains originally derived from the United States showed C3 BB type. On the other hand, mice from three Japanese strains, NC, NBr and MoA, showed a C3 of pI 6.0 (C3 AA type), while SWR mice showed C3 of pI close to but slightly lower than 6.0 (C3 CC type). Subsequent breeding experiments confirmed that observed polymorphism was inherited as a single codominant trait. The locus controlling the structural differences in mice was termed C3-1 and its linkage to other traits with a known chromosomal localization was then studied. A significant linkage was demonstrated between C3-1 and two loci, Ce-2 and S region of H-2. In backcross progeny, the recombination fraction between C3-1 and Ce-2 and between C3-1 and S region was 23 and 11, respectively. These values implicate the location of C3-1 outside H-2 on chromosome 17 (Natsuume-Sakai *et al.* J. Immunol. *121,* no. 2, 1978). In order to confirm that the C3-1 locus is a structural gene, we tried to produce alloantisera which can differentiate allotypic variations of murine C3. By cross immunization of inbred mice with C3 of a different phenotype, alloantisera to C3 allotypes were successfully produced. C3H anti-NC C3 recognizes C3 coded for by C3-1^a allele, while NC anti-BALB/c C3 reacts with C3 coded for by C3-1^b allele. By use of these alloantisera it is now possible to classify C3 of mice from various sources on the basis of their reactivity with the alloantisera (Natsuume-Sakai *et al.* J. Immunol. *121,* no. 5, 1978). The use of the allotype antisera also permitted us to study the origin of C3 detected in fetal and neonatal mice. Allotypic differences between mother and fetuses and neonates provide evidence that C3 is synthesized in late fetuses and neonates according to the C3-1 allele carried by fetuses and neonates (Amano *et al.* J. Immunol. *122*, no. 2, 1979).

Pathophysiology



Living cell membrane immunofluorescence: adenocarcinoma cells of the lung. (Y. Kurata)



Living cell membrane immunofluorescence: epidermoid carcinoma cells of the lung. (Y. Kurata)