SCIENTIFIC REPORTS

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ERRATA

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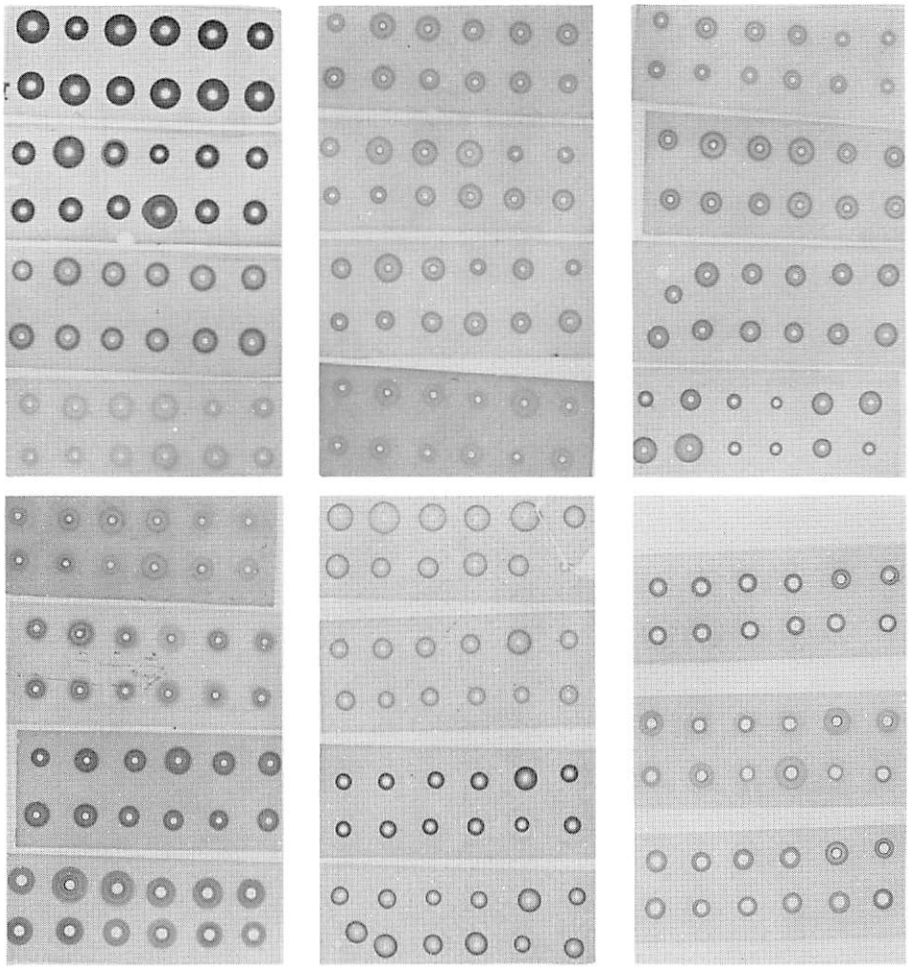
Molecular Immunology



Quantitation of human serum proteins by single radial immunodiffusion in micro-scale deviced by ourself. Specific antiserum plates are for (left) Alb., IgG, α_1AT, α_1AG, C9, ATIII, βL, α_2PI; (middle) α_1B, C4, β_2I, RBP, Hp, Hx, Tf, IgA; (right) C3PA, α_1X, Cp, IgM, Clq, IgD, 9.5Sα_1. Each 10 samples and 2 standard sera have been applied.

DEPARTMENT OF MOLECULAR IMMUNOLOGY

GENERAL SUMMARY

The essential character of the immune mechanism is a multidefence of the living-organism which has been progressively developed over a long period of phylogeny. Our final project, immunity against cancer, involves a wide range of investigation including cellular and humoral immunity.

I. Cellular immunity against cancer.

Characteristic studies of this department are those on plasmacytoma. Plasmacytoma producing different immunoglobulin M components had been induced in BALB/c mice in our laboratory, maintained by serial intraperitoneal transplantation, and kept at −85°C in a freezer. Physicochemical studies on the immunoglobulin M component and oncological studies on plasmacytoma carried out in this department from 1967 to 1975 were changed to mainly cytological studies, some of which had been started from 1973.

1) Studies of tumor associated antigen defined by antiserum.
Tumor antigen, if present, that had been thought to be the target of immunotherapy, has turned out to be an antigen associated with the major histocompatibility complex. A unique phenomenon, the loss of both tumor associated antigen and major histocompatibility antigen in serially transplanted plasmacytoma, was found by cytotoxicity studies with anti-tumor sera and was extensively studied and published in J. Nat. Cancer Inst. 58 : 229, 1977 and 61 : 203, 1978. Immunotherapy may not be successful if every tumor lost its specific antigen during the generation of tumor. Our study revealed that the surface antigens were not lost but masked with some protein-like material(s). A masking glycoprotein was obtained by elution from the membrane of plasmacytoma and analysed by SDS-disc electrophoresis. Though a part of the character and the origin of this masking protein was clarified, further studies will be needed to reveal this sneaking through mechanism of the tumor cell.
2) Studies of cytotoxic T lymphocytes to plasmacytoma.
The major defence mechanism against tumors was the cytotoxic T lymphocyte (CTL), but not antiserum cytotoxicity. CTL was analysed to find out what is its antigen using immunoglobulin producing and nonproducing plasmacytomas, the later having lost the H-2 antigen. Syngeneic and allogeneic T lymphocyte response revealed that H-2 antigen combined with tumor associated antigen was essential in the induction and effector stages of the cytotoxic T lymphocyte. In this research (J. Immunol. 121 : 427, 1978) major histocompatibility antigens with tumor associated

antigen were confirmed to be lymphocyte defined antigens as well as serum defined antigens. In *in vivo* transplantation, immunoglobulin non -producing plasmacytoma was not rejected even in the allogeneic mouse.

3) Studies on arming macrophage against cancer.

Another effective defence against cancer is carried out by macrophages. The original function of macrophages is nonspecific defence, like phagocytosis. However, macrophages combined with the specific macrophage arming factor (SMAF) that was produced by T lymphocytes after incubation with target cells, changed to specific killer macrophages against the target cell. This field is not well cultivated yet. How specifically do the armed macrophage react with close by preincubated targets? Is SMAF different from the cytophilic antibody or nor? Physico-chemical analysis of ^{3}H-leucine labeled SMAF carried out in this department has revealed basic molecular properties of SMAF (See abstract of Dr. O. Daimaru).

4) Studies on monoclonal antibody induced with *E. coli* lipopolysaccharide.

Oncogenic studies on plasmacytoma in mice done early in this department developed into studies on the monoclonal antibody produced after the injection of lipopolysaccharide of *E. coli.* This phenomenon was first found by Dr. S. Natsuume-Sakai, now in the Dept. Immunobiology of this Institute, and the studies have been followed up by Mr. Y. Ikeda.

5) A new approach to cancer specific changes.

Dr. S. Ohno has been absent from this department and has been studying in the Dept. of Tumor Biology, Karolinska Institute, Stockholm under Dr. George Klein since 1975. There he invented an acid fixed nuclear binding (AFNB) technique for the detection of cells transformed by DNA virus. He has published a lot of work on nuclear antigens, chromosomal changes in mouse preleukemia and so on. When he comes back to Japan in 1979, further progress on research in our department can be expected.

II. Humoral immunity against cancer.

Cancer cells have been completely surrounded by body fluid, except when migrating cells make contact temporarily with cancer cells. Body fluid, with the same composition as plasma, contains many components which affect the growth, the metabolism and the metastasis of cancer cells. Fibronection, a cell surface protein present in sera, is known to control protein through contact inhibition. Proteinase inhibitors and components of complement control the chemotaxis and the phagocytosis of leukocytes, as well as mediate the lysis of target cells. Alpha 2 HS glycoprotein activates cellular immunity of T lymphocytes. However, α_1 acid glycoprotein, α_1 antitrypsin, α_2 macroglobulin and α fetoprotein have more or less function as immune suppressants. The problem is that these components are mixed up with and consist of the body fluid. We did a lot of analytical studies on serum protein fractions for normal, and for cancer patients and

for serial course under treatment. We appreciate the many clinical doctors who sent us such samples that enabled us to accumulate this data.

1) Studies on cytolysis by complements, especially the late components of complement.
Dr. K. Yamamoto returned from the Dept. of Immunology, Rush Medical College, Chicago to our department in 1978, and started his research on C5b inactivator, which is a new protein, not yet fully identified, but has a distinct function in the alternative pathway.
2) Studies on serum proteins during tumor growth.
We have accumulated data on 40 serum protein profiles for each of 300 normal individuals of different age and 900 patients with different cancers since 1973. Data analysed by the Facom 160 Electronic computer of the Data Processing Center, Kanazawa University, revealed the characteristic changes in normal placenta serum and the serum of aged humans. However, individual variance was rather wide among the sera obtained from normal humans of the same age and sex. This indicated that it is better to measure serially during the development or treatment of cancer instead of measuring only once per patient. A common pattern of cancer serum is the increase in acute phase proteins and complements. We need basic studies on these proteins and so we analysed their increase during and after surgical stress or sex hormone treatment of non-cancer patients. These results may be helpful for indication of immunotherapy and prognosis in cancer patients.
3) Early diagnosis for tumor development.
Another project of this group was a long serial examination of the serum from normal aged persons. The sera have been sent from Tokyo Yoikuin Hospital since 1976 through the cooperation of Director of the Hospital, Dr. M. Murakami, and Dr. K. Okabe. How early we can detect irregularities before the development of cancer or other abnormality by measureing 40 serum protein fractions is the main purpose. Not all serum proteins change in all individuals. Either a few proteins or more than 10 components do change in aged persons in one or two years' observation. Since serum proteins have many different functions, it will be worthwhile to know which changes are related to the cancer that will develop later.
4) Study of a new method, latex photoimmunoassay.
Phototurbidimetry of latex immunoagglutination using an infra red wave length which is almost equivalent with the latex particle size was invented for quantitation of serum components and others by Dr. M. Sawai, Teikoku Hormone Mfg. Co. LTD. This method shortens the time for measurement and increases the sensitivity to the range of radioimmunoassay. It does not need any radioisotope and will be employes widely in the future. Cooperative work is now in progress.

ABSTRACT

(19) Alteration of cell-surface antigenicity of the mouse plasmacytoma. Lack of correlation between synthesis of myeloma protein and alteration of surface antigen.

S. Ohno, S. Natsuume-Sakai and S. Migita

In a previous paper[1], the alteration of membrane antigenicity of IgA -synthesizing plasmacytoma cells (58-8) induced in a BALB/c mouse in our laboratory was revealed, when rabbit antisera against 58-8 plasmacytoma cells that transplanted for 7 to 8 generations (anti-58-8) and mouse antisera against H–2^d antigen were used for cytotoxic test. The results indicated that the antigenic determinants of a transplantable 58-8 to anti-58-8 and anti-H-2^d changed during successive i.p. transplantations into syngeneic BALB/c mice and became nonreactive with antisera after the 13th generation. Although it was conceivable that 58-8 cells seemingly "lost" their surface antigens (plasmacytoma antigen and H–2 antigen), our study revealed that the surface antigens were not lost but "masked" with some protein-like materials(s). During the successive i.p. transplantations of IgA-synthesizing 58-8 plasmacytoma cells, a subline that has no M-component of IgA (nonproducer) was developed. In the present study, correlation between the changes of immunoglobulin synthesis and the surface antigenicity was analysed by cytotoxicity and quantitative antibody-absorption tests with the cells of immunoglobulin-producing and nonproducing mouse plasmacytoma. IgA-synthesizing BALB/c plasmacytoma 58-8 and the non-IgA-synthesizing variant of the 58-8 (nonproducer) were killed with rabbit anti-58-8 plasmacytoma cell antiserum, C3H/He anti-BALB/c spleen cell antiserum, and (C57BL/6 × DBA/2)F_1 anti -BALB/c plasmacytoma cell MOPC-31C antiserum plus complement, only when the cells were pretreated with pronase. Quantitative absorption tests revealed that the nonproducer 58-8 had the same amount of plasmacytoma antigen of 58-8 and PC.1 antigen, and a greater amount of H-2^d antigen, as did producer 58-8. The same analysis was carried out for the C3H mouse plasmacytoma X5563, which has an M-component of IgG_{2a}. The nonproducer X5563 had a greater amount of H-2^k antigen and a smaller amount of the plasmacytoma antigen of X5563 than did the producer X5563. No detectable PC.1 antigen was observed at surfaces of the producer or the nonproducer X5563 cells.

1) Ohno, S., Natsuume-Sakai, S. and Migita, S.; J. Nat. Cancer Inst., 55, 569-577, (1975).

(20) Cell-surface major glycoprotein of BALB/c mouse plasmacytoma 58-8 cells.

H. Tokuyama and S. Migita

Ohno[1,2] observed a certain alteration of membrane antigenicity of IgA -producing 58-8 plasmacytoma cells during successive intraperitoneal transplantations and concluded that the surface antigens (H-2 antigen and TAA*) of 58-8 cells were not lost but masked with a protein-like meterial that was synthesized by the 58-8 cells themselves. The purpose of this study was to examine and characterize the masking materials. The cell-surface components of 58-8 plasmacytoma were analysed by means of sodium dodecyl sulfate-polyacrylamide gel-electrophoresis (SDS-PAGE). When the whole cells lysed with SDS were directly subjected to SDS -PAGE and periodic acid-Schiff (PAS) staining, a major component with an apparent molecular weight of 170,000 daltons was found. This component increased with increasing transplantation generations, was sensitive to tryptic digestion, and was extracted by 1 M urea. When the cells from various transplantation generations were labeled with radioactive iodine by enzymatic iodination and their SDS-PAGE patterns were compared, a prominent radioactive peak was found that had the same molecular weight as the PAS-positive major component. This membrane component increased with increasing transplantation generations, and the radioactivity reached as much as 20% of the total activity. This component was also sensitive to tryptic digestion and was extracted by 1 M urea. The PAS -positive major component was rich in the crude cell membrane prepared from 58-8 cells and could be partially purified from the 1 M urea extract. With the use of SDS-PAGE, the molecular weight of the partially purified glycoprotein was found to be 120,000 daltons. The glycoprotein contained sialic acid residues. This sialoglycoprotein on the cell surface of 58-8 cells appeared to be the masking material that made the 58-8 cells unreactive with the antibodies directed to their tumor-associated antigens or to H-2 antigen[3].

* TAA: tumor associated antigens.

1) Ohno, S., Natsuume-Sakai, S. and Migita, S., J. Nat. Cancer Inst., 55, 569–577, (1975).
2) Ohno, S., Natsuume-Sakai, S. and Migita, S., J. Nat. Cancer Inst., 58, 229–237, (1977).
3) Tokuyama, H. and Migita, S., J. Nat. Cancer Inst., 61, 203–208, (1978).

(21) Cytotoxic T lymphocytes to murine plasmacytoma cells in allogeneic and syngeneic mice: H-2 antigens are essential in the induction and effector stages of CTL[1].

Y. Kaneko, S. Natsuume-Sakai and S. Migita

It has been shown that cytotoxic T lmphocytes (CTL) could be generated against virus-infected or hapten-modified syngeneic cells in which a homology at the H-2K or H-2D region between stimulater cells and target cells was required for lysis to occur. On the contrary, there is controversy over whether or not H-2 antigens are involved in lysis by syngeneic CTL directed to the tumor-associated antigens (TAA). We, therefore, examined T cell-mediated cytotoxicity and transplantation immunity to immunoglobulin-producing and non-producing mouse plasmacytoma cells in allogeneic and syngeneic mice. By using ^{51}Cr release assay it was found that cytotoxic activity was developed *in vitro* by culturing C57BL/6 spleen cells with immunoglobulin-producing plasmacytoma MOPC 11 (producer) cells of BALB/c origin that were susceptible to lysis by C57BL/6 anti-BALB/c CTL. In contrast, CTL were not induced when C57BL/6 spleen cells were cultured *in vitro* with immunoglobulin-nonproducing variant cells of MOPC 11 (nonproducer) that contained fewer H-2 antigens as revealed by the cytotoxic responses and cold inhibition tests of C57BL/6 anti-BALB/c CTL. MOPC 11 producer cells, when injected i.p. into C57BL/6 mice, were completely rejected, but the tumor developed in C57BL/6 mice that had been previously inoculated with MOPC 11 nonproducer cells. When spleen cells from BALB/c mice that had been sensitized *in vivo* with MOPC 11 producer cells were restimulated *in vitro* with MOPC 11 producer cells, CTL could be generated to the TAA of MOPC 11 producer cells. These anti-TAA CTL lysed MOPC 11 producer cells specifically, but did not kill any other plasmacytoma cells of BALB/c origin and leukemia cells of DBA/2 origin. No syngeneic CTL were induced against MOPC 11 nonproducer cells. Transplantation analyses showed that, although the anti-tumor immunity against MOPC 11 producer cells was observed when BALB/c mice were primed with MOPC 11 producer cells, MOPC 11 nonproducer cells failed to prime BALB/c mice either to MOPC 11 producer or nonproducer cells. The cytotoxic activity of syngeneic CTL generated against MOPC 11 producer cells was completely inhibited by anti-$H\text{-}2^d$ serum, suggesting the involvement of H-2 antigens on the membrane of MOPC 11 producer cells in lysis by anti -TAA CTL. These findings, therefore, indicate:

(1) H-2 antigens and TAA present on plasmacytoma cells are essential in the induction and effector stages of CTL and

(2) H-2 restriction of CTL exists also in plasmacytoma systems.

1) Kaneko, Y., Natsuume-Sakai, S. and Migita, S., J. Immunol. 121 (2); 427-437, (1978).

(22) Activation of macrophage in cell-mediated immunity: Production of specific macrophage arming factor and its role.

O. Daimaru and S. Migita

Three different types of "effector" macrophages have been reported. Activated macrophages which were activated in a non-specific manner such as the use of BCG, kill tumor cells without regard for antigenic specificity. On the other hand, armed and immune macrophages kill the tumor cells with immunological specificity. Concerning the mechanism of armed macrophages, it was found that normal macrophages changes to cytostatic macrophages by specific macrophage arming factor (SMAF). (Evans et al.). This SMAF was produced by immune T cells, but it is not clear how the specificity of SMAF is conducted and whether SMAF is produced by cytotoxic T cells or a different subset of T cells. The exact molecular structure of SMAF and its relationship to immunoglobulin (Ig) has not been determined.

Mice were injected intraperitoneally with 1×10^7 allogeneic spleen cells. After 11 to 14 days, ten million immune spleen cells were mixed with 1×10^5/ml allogeneic spleen cells which were treated with Mitomycin-C in RPMI-1640 medium. The mixture incubated at 37°C in 5% CO_2 for 24hr, and the supernatants recovered were used as SMAF. The activity of SMAF was assayed by inhibition of DNA incorporation to target cells using an effector to target ratio of 10:1.

I. Specificity of SMAF: Normal peritoneal macrophages were incubated with SMAF (BALB/c anti C57BL/6) and after the washing, the armed macrophages were mixed with target cells. They inhibited EL-4 (C57BL/6 origin thymoma: 59.4 ± 4.5%) but didn't inhibit J606 (BALB/c origin plasmacytoma: 4.1 ± 3.8%). On the other hand, when macrophages were armed with other SMAF (C57BL/6 anti BALB/c), the inhibition of J606 was 71.3 ± 13.7%, but that of EL-4 was only 15.1 ± 1.3%. Also, we recognized that there is no H-2 restriction between SMAF and macrophage.

II. Specific binding activity of SMAF: Since the armed macrophages exhibited immunological specificity to target cells, the next experiment attempted was whether SMAF itself might have antigen recognition on sites or not. SMAF were labelled with ^{3}H-leucine. The SMAF (BALB/c anti C57BL/6) bound to EL-4, but not when target cells had different H-2.

These results suggested that normal peritoneal macrophages were elaborated to a specific cytostatic level by SMAF and that the specificity of SMAF were due to the antigen recognition sites on the SMAF molecules. When the target cells which were pre-incubated with radiolabelled SMAF were treated with acid buffer (pH 2.8), the antigen recognition units of SMAF were eluated. The molecular weight of SMAF elucidated was about 52,000 dalton by the analysis of 10% SDS-polyacrylamide gel electrophoresis and the radioautography.

(23) Restricted heterogeneity in antibody response to LPS in mice.

Y. Ikeda, S. Natsuume-Sakai and S. Migita

Previous reports showed that the antibody to *E. coli* 055 : B5 LPS antigen (lipopolysaccharide extracted by trichloracetic acid) (Difco, USA) produced in BALB/c, DBA/2 and A/J mice was mainly IgG_3 and had a restricted heterogeneity as revealed by agarelectrophoresis and isoelectrofocusing. However, C57BL/6 mice did not produce an M component-like antibody. C57BL/6 mice produce less anti-LPS IgG_3 antibody than BALB/c, DBA/2 and A/J mice because anti-LPS IgG_3 antibody producing cells in C57BL/6 mice may not be well triggered to produce antibody. When B10.A, B10.A (2R) and B10.BR mice, which are H-2 (histocompatibility-2) congeneic mice of C57BL/10, were immunized with the LPS, some mice produced an M component-like antibody. This fact suggests an M component-like anti-LPS IgG_3 antibody response is more or less controlled by a gene or genes in the H-2 complex, but control by this gene is not absolute (Fig. 1). We have tried to investigate an idiotype of anti-LPS antibodies and idiotypic distribution among these mouse strains. As an immunizing antigen for preparing anti-allotypic and anti-idiotypic antibodies, antigen-antibody compexes were employed. LPS is a potent adjuvant to antibody formation, and antigen-antibody complexes may effectively elicit anti-allotypic antibody and anti-idiotypic antibody production. The BALB/c mice sera immunized with the LPS was mixed with LPS and the precipitate was washed with saline. LPS and anti-LPS BALB/c antibody complexes were injected into C57BL/6 mice. Some of these hyperimmunized C57BL/6 mice could produce anti-allotypic antibody as well as anti-idiotypic antibody, the later was confirmed by the Ouchterlony test.

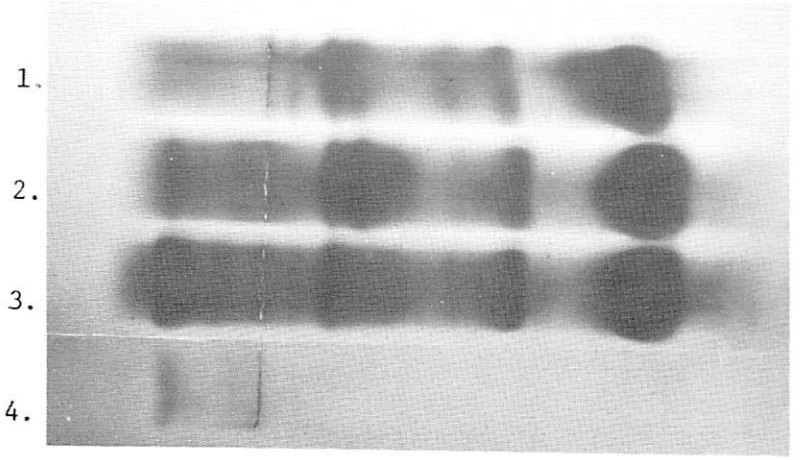


Fig. 1. Agarelectrophoresis of *E. coli.* 055 : B5 LPS immunized mouse sera. No. 1. Normal B10.A mouse serum. No.2–3. LPS immunized B10.A mouse serum. No. 4. LPS -sepharose 4B eluted A/J anti-LPS antibody.

(24) Age dependent level of 35 serum protein fractions in normal individuals from new born to 90 years old.

S. Migita, K. Motonishi, K. Okabe[1], K. Hirohashi[2], N. Okuda[3] and K. Taniguchi[3]

The normal level of 35 serum protein fractions was measured for 300 individuals from new born to 90 years old by the micro single radial immunodiffusion method.

Changing patterns according to age could be classified into 5 types; (1) decreasing type, (2) mountain type, (3) increasing type, (4) U-type, and (5) M-type. The decreasing type, those levels that decreased with age, were albumin, transferrin, α-lipoprotein, α_2HS-glycoprotein and 8Sα_3-glycoprotein. These proteins increased early in ontogeny and were maintained relatively constant with a gradual decrease with age. They transport basic metabolites and looks essential for life, though functions of α_2HS and 8Sα_3 are unknown at present. Mountain type has its peak located between 20 and 50 years of age, and those levels which decreased both in young and in old age were prealbumin retinol binding protein, C3, C4, C5, Clq, IgM, IgD, plasminogen, 9.5Sα_1 and Gc-globulin. These fractions showed a high level when the human reached adulthood and activity of the person was high, indicating that the functions of these proteins were of an advanced nature in comparison with the former type. Increase type is concerned with IgG, IgA, β-lipoprotein, haptoglobin, inter-α-trypsin inhibitor, Znα_2-glycoprotein and β_2I-glycoprotein. The level of these proteins increased gradually according to the age. IgG and IgA increased as results of antigenic stimulation with cooperation of T, B lymphocyte. Beta-lipoprotein increase depends upon food which an individual takes. The ontogenically late character of this type suggests that the functions are more advanced ones which appeared in phylogenically late like mammals. U-type included α_1-acid-glycoprotein, α_1-antitrypsin, α_2-macroglobulin, C9, α_1-antichymotrypsin and C1-inhibitor. Their level was high in young persons under 10 years and in old persons over 60 years of age. These belong to acute phase proteins which increase rapidly when a person suffers from infection or stress. Standard deviation of these proteins was usually large at all ages. The elevated mean value in young and old age suggest that persons in such age groups easily suffer from abnormal stress, even if they look healthy. M-type included ceruloplasmin, hemopexin, factor B, anti-thrombin III, α_1B-glycoprotein and β_2III-glycoprotein. M-type, a subtype of U-type, showed a decrease in old age in contrast to U-type which did not. An increase of this fraction in the acute phase was less significant than for those of U-type. A new classification of serum protein fractions will be convenient for the understanding of the meaning of pathological changes.

1) Yoikuin Hospital, Tokyo.
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(25) Sex dependent level of 35 serum protein fractions in normal individuals and their changes by estrogen or androgen administration in humans and in mice.

S. Migita, K. Motonishi, S. Natsuume-Sakai, S. Ishida, S. Hashimoto, S. Konda[1], H. Yamada[2], and H. Hisazumi[3]

The level of plasma proteins for which the adult female was higher than the adult male were α_2-macroglobulin, IgM, α_1-antitrypsin and α_1B-glycoprotein. Those for which the male is higher than the female were Znα_2, 9.5S α_1-glycoprotein, C3, C4, prealbumin, retinol binding protein Gc -globulin, β_2-glycoprotein I, C1-inhibitor and α_1 -antichymotrypsin. In cases of inbred mice, levels of C3 and C4 were higher in the male than in the female for age 3 to 8 months, though the differences became obscured by the condition of keeping, such as infection. Other fractions were not measured in mice. The question was whether sex dependent differences of serum protein fraction were primarily due to sex hormones produced or not. The effect of estrogen and androgen on the level of 35 fractions of serum protein was examined in cases of patients who took these hormone for therapy. Changes in serum protein before, during and after the hormone therapy were confirmed. Six patients of prostatic cancer were treated with estrogen. The α_1-antitrypsin and ceruloplasmin increased significantly. No decrease of fractions in the serum was observed. Seven cases of pregnancy where serum protein were serially examined, also showed elevated levels of α_1-antitrypsin, ceruloplasmin, Gc-globulin, α_1B -glycoprotein and transferrin. Sera from six patients of aplastic anemia treated with oxymetholone revealed significant temporal increases in haptoglobin, C1-inhibitor, C4, Znα_2 -glycoprotein and plasminogen during male hormone therapy. No difference was noticed between male and female patients treated with male hormone. Some of the increased components coincided with fractions that were observed to be high in normal male or female, respectively. However, ceruloplasmin in female hormone and haptoglobin in male hormale were distinct that each fraction increased rapidly and significantly after the treatment, though no difference was observed in normal sera. The synthesis of the fractions may be hormone dependent, not upon low concentration like normal, but upon high concentration therapy used. Effect of hormone administration was also examined in mice. When BALB/c mice were injected either with testosterone (TS), DHA acetate (precursor of TS) or estradiol, an increase in C4 was only observed in the TS group. The level of C4 also increased with subcutaneous injection by turpentine oil in the mouse. The added effect of C4 increasing was not observed, when TS and turpentine oil were injected into the same mouse.

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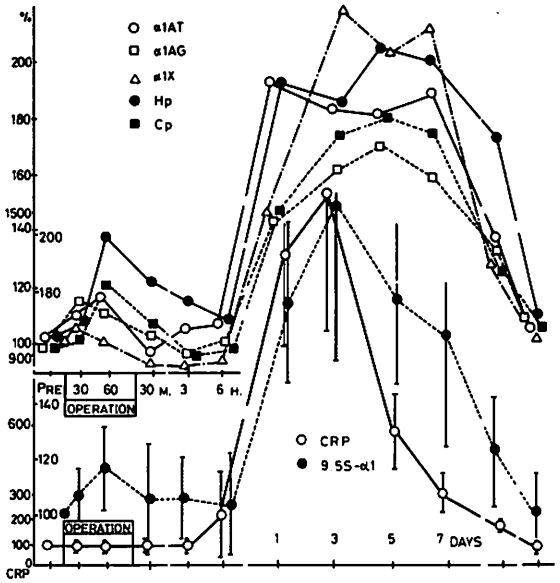
3) Dept. Urology, Kanazawa Univ. School of Medicine.

(26) Changes in thirty nine serum protein components by surgical stress.

S. Hashimoto and S. Migita

Sera from six patients aged 19 to 74 yr, undergoing operations such as hysterectomy or adnexectomy, were sampled in series until the 30th postoperative day, in order to confirm a relationship among acute phase proteins increase after surgical stress. The concentrations of 39 serum protein components were measured by the micro single radial immunodiffusion method with normal pooled lyophilized serum (QS Sera) as 100% control.

From this study, the following results were obtained: (1) 9.5S-α_1-glycoprotein had characteristics of acute phase protein. (2) Acute phase proteins, such as α_1-antitrypsin, α_1-acid-glycoprotein, α_1-anti-chymotrypsin, haptoglobin, and ceruloplasmin, increased slightly during operation, and decreased slightly after operation, and then greatly increased. Initial increase in these proteins would suggest early release from the reserved pool and its consumption, followed by great increase of synthesis (Fig. 1). (3) In the complement system, components of an alternative pathway increased earlier than those of the classical pathway. (4) It was observed that the older the patients the later the increase of acute-phase proteins. (5) The changing pattern of α_1-antitrypsin was the reverse of elastase activity in the serum. (6) The cortisol level rapidly increased during operation, followed by neutrophile increase to each their peaks 3 hours after operation. On the 1st postoperative day, C-reactive protein steeply increased to 1520% of preoperative level (Fig. 1). (7) Only prealbumin and retinol-binding protein decreased concomitantly after operation.



Figeure 1. Changes of acute-phase proteins.

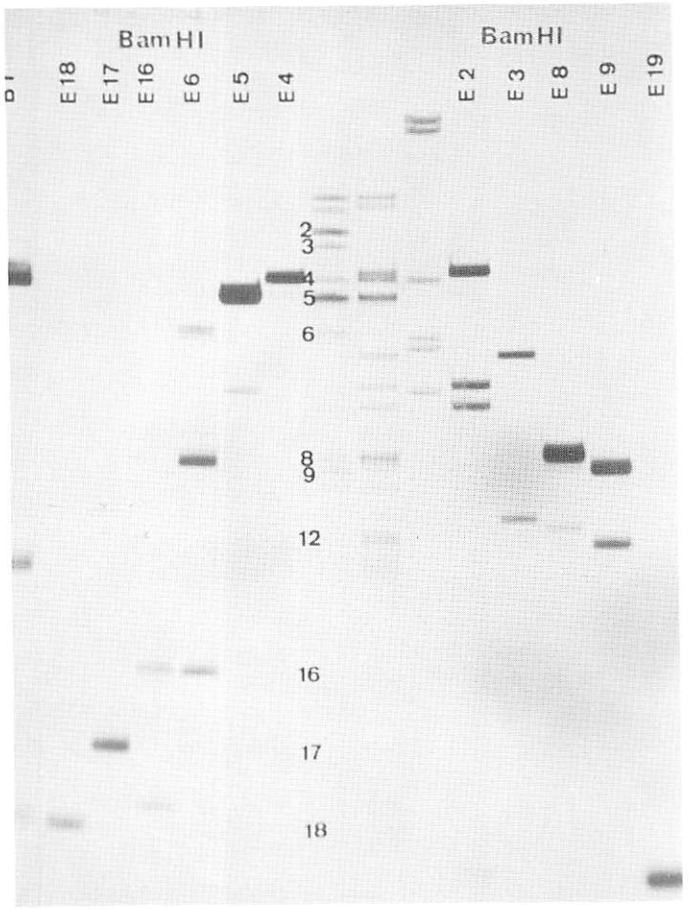
(27) Interaction of the cytolytic complex of complement (C5b-9) with the biological membrane: Influence of the proteolytic cleavage of C5b.

K. Yamamoto

The cytolytic function of complement is set in motion by the proteolytic cleavage of C5 to its major fragment, C5b. The product of the subsequent fusion reactions between C5b, C6, C7, C8 and C9 interacts with and disrupts the membrane resulting in death of the cell. Mechanisms exist in whole human plasma to regulate or restrict this potentially harmful function of complement. One of mechanisms is mediated by plasma lipoproteins and the S-protein which inhibit the binding of the complement complex to the membrane by interacting with the membrane -binding sites in the complex.

Another and quite different form of control mechanism was found which involved the limited cleavage of the α' chain of C5b by the proteolytic serum enzymes. The product of this cleavage, designated C5b′, consisted of three polypeptide chains, i.e., two fragments of the α' chain designated α'_1 and α'_2, and the β chains, and retained the ability to form a complex with C6 as revealed by gel filtration analysis. However, this cleavage uniquely affected the function of C5b. The complex formed from C5b′ and C6 (designated C5b′-6) did not form the macromolecular aggregates upon reaction with C7, and readily dissociated into C5b′ and C6 during ultracentrifugation in sucrose solution. The C5b′-6 complex had a binding efficiency to the membrane of sheep erythrocytes comparable to that of the C5b-6 complex as measured by the binding of ^{125}I-C5b′ -6 to the membrane in the presence of C7, but the hemolytic potential of the bound C5b′-6 complex seemed to be reduced. The cytolytic complex of complement was successfully solubilized by sodium deoxycholate from the complement-lysed membrane of sheep erythrocytes. The solubilized complex showed a polydisperse distribution and was partitioned with the apparent molecular weight of 2.2 million daltons on gel filtration, in contrast to the complex formed in the fluid phase which had the apparent molecular weight of about 1 million. The complex extracted from the complement-lysed membrane apparently have aggregated or carried the substantial amounts of the membrane-derived lipid. Analysis by SDS -polyacrylamide gel electrophoresis identified C5b as the form of the C5 -derived fragment incorporated into the cytolytic complex of complement. C5b′ was not found in the complex, suggesting that C5b′ was incapable of forming the cytolytic complex of complement.

Biophysics



Fluorography of ^{3}H-labeled DNA fragments produced by various restriction endonucleases. (see Abstract 29, and 30).