INTERNATIONAL SYMPOSIUM ON FRONTIERS OF COMPLEMENT RESEARCH

September 1–3, 1983

PROGRAM

ABSTRACTS

Shima Kanko Hotel
in
Ise-Shima National Park, Japan

INTERNATIONAL SYMPOSIUM
ON
FRONTIERS IN COMPLEMENT RESEARCH

Organizing Committee:

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September 1 - 3, 1983

Shima Kanko Hotel

Kashikojima, Mie

Japan

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OUTLINE PROGRAM

Thursday, September 1, 1983

10:00 - 17:00	Registration desk open
13:00 - 13:15	Opening remarks (K. Nishioka)
13:15 - 15:05	Complement receptor Chairpersons: P.J. Lachmann, K. Iida
15:05 - 15:30	Coffee break
15:30 - 17:30	Membrane attack mechanisms of complement Chairpersons: M.M. Mayer, K. Nishioka
18:00 -	Welcome party

Friday, September 2, 1983

8:30 - 10:10	Structure and biochemistry of complement components Chairpersons: I. Gigli, K. Nagaki
10:10 - 10:40	Coffee break
10:40 - 11:30	Structure and biochemistry of complement components Chairpersons: V. Nussenzweig, T. Tachibana
11:30 - 12:40	Genetics and evolution of the complement system Chairpersons: V. Nussenzweig, T. Tachibana
12:40 -	Lunch
Afternoon	Free Boat cruise around the Ago Bay

Saturday, September 3, 1983

8:30 - 10:20	Activation and biosynthesis of complement Chairpersons: M.M. Frank, T. Okuda
10:20 - 10:40	Coffee break
10:40 - 12:40	Complement in disease process Chairpersons: K.O. Rother, M. Kondo
12:40 - 13:00	Closing remarks (P.J. Lachmann)

Thursday, September 1, 1983

13:00 - 13:15	**Opening remarks**	**K. Nishioka**

Session I. Complement receptor
Chairpersons: P.J. Lachmann and K. Iida

13:15 - 13:45	Possible role of complement receptors in the processing and traffic of C3 fragments bound to immune complexes **V. Nussenzweig,** K. Iida, L. Nadler, E. Medof; New York, USA.
13:45 - 14:15	The activity and binding properties of C3 receptors under a number of physiologic conditions. **M.M. Frank,** S. Inada, E. Brown, T.A. Gaither, C. Hammer, C. Pommier; Bethesda, USA.
14:15 - 14:35	Low C3b receptor reactivity on erythrocytes from patients with systemic lupus erythematosus. **Y. Miyakawa;** Tokyo, Japan.
14:35 - 15:05	C3 fragments and receptors. **P.J. Lachmann,** R.A. Harrison, A.E. Davis, G.D. Ross; Cambridge, UK.
15:05 - 15:30	Coffee break

Session II. Membrane attack mechanisms of complement
Chairpersons: M.M. Mayer and K. Nishioka

15:30 - 15:50	Attack site of complement on gram-negative bacteria. H. Kozono, K. Hong, J. Takeda, Y. Takata, T. Kinoshita, **K. Inoue;** Osaka, Japan.
15:50 - 16:20	Molecular composition of complement lesions and their mode of assembly. **E.R. Podack;** La Jolla, USA.
16:20 - 16:50	Activation of the attack phase of serum complement by physico-chemical means. A. Dessauer, U. Rother, **K. Rother;** Heidelberg, FRG.
16:50 - 17:30	Recent studies of membrane attack by complement. **M.M. Mayer;** Baltimore, USA.
18:00 -	Welcome party

Friday, September 2, 1983

Session III. Structure and biochemistry of complement components
Chairpersons: I. Gigli and K. Nagaki

8:30 - 9:00 Structural and functional studies on: (i) normal C1q; (ii) a defective form of C1q; (iii) C4-binding protein (C4bp).
K.B.M. Reid; Oxford, UK.

9:00 - 9:30 The β-Cys-γ-Glu thiolester in the third and fourth complement proteins.
B.F. Tack, F.F. Davidson, R.A. Harrison, M.K. Hostetter, J. Janatova, M.L. Thomas; Boston, USA.

9:30 - 9:50 Comparative studies on asparagine-linked sugar chains of subcomponents C1q of the first component of human, bovine, mouse and guinea pig complement: Are there any positive correlations between their structure and biological activities of C1q?
K. Yonemasu, T. Sasaki, Y. Dohi, H. Yoshima; Nara, Japan.

9:50 - 10:10 The functional sites of the second component (C2) of human complement.
S. Nagasawa, N. Yamashita; Sapporo, Japan.

10:10 - 10:40 Coffee break

Chairpersons: V. Nussenzweig and T. Tachibana

10:40 - 11:00 Interaction of C4-binding protein with cell-bound C4b: a quantitative analysis of binding and the role of C4-binding protein in proteolysis of cell-bound C4b.
T. Fujita, N. Tamura; Tsukuba, Japan.

11:00 - 11:30 Monoclonal antibodies as probes of complement proteins.
S. Ruddy; Richmond, USA.

Session IV. Genetics and evolution of the complement system
Chairpersons: V. Nussenzweig and T. Tachibana

11:30 - 12:00 Molecular genetics of complement.
H.R. Colten, G. Goldberger, R. Sackstein, H.S. Auerbach, F.S. Cole, D. Woods; Boston, USA.

12:00 - 12:20 The Incidence of C9 deficiency in Japan.
S. Inai, Y. Akagaki; Osaka, Japan.

12:20 - 12:40 Complement evolution in primitive vertebrates.
M. Takahashi, Kanazawa, Japan.

12:40 Lunch

Afternoon Free
Boat cruise around the Ago Bay

Saturday, September 3, 1983

Session V. Activation and biosynthesis of complement
Chairpersons: M.M. Frank and T. Okuda

8:30 - 8:50	Glycophorin inhibits activation of the alternative complement pathway of serum homologous to the glycophorin. **H. Okada**, H. Tanaka, N. Okada; Fukuoka, Japan.
8:50 - 9:20	Complement-dependent lysis of influenza virus-treated heterologous and autologous cells in serum. **M.D. Kazatchkine**, C.R. Lambre; Paris, France.
9:20 - 9:40	Endogenous inhibitor of factor B. **A. Miyama**, T. Moriyama, Y. Kawamoto; Nagoya, Japan.
9:40 - 10:00	Study on C3-like factor in the serum of a C3 deficient subject. **H. Kitamura**, H. Nishimukai, Y. Sano, K. Nagaki; Osaka, Japan.
10:00 - 10:20	A sexual dimorphism of mouse C5. **N. Tamura**, A. Baba, T. Fujita; Tsukuba, Japan.
10:20 - 10:40	Coffee break

Session VI. Complement in disease process
Chairpersons: K.O. Rother and M. Kondo

10:40 - 11:10	The heterogeneity of C1 inhibitor defects. **F.S. Rosen**; Boston, USA.
11:10 - 11:40	The role of complement in phototoxicity. **I. Gigli**, H.W. Lim; San Diego, USA.
11:40 - 12:10	Participation of complement in demyelination. **M.L. Shin**; Baltimore, USA.
12:10 - 12:40	Immune complexes, activation of complement system and cancer. **N.K. Day**; Oklahoma city, USA.
12:40 - 13:00	**Closing remarks** **P.J. Lachmann**

ABSTRACTS

Contents

Possible role of complement receptors in the processing and traffic of C3 fragments bound to immune complexes

V. Nussenzweig, K. Iida, L. Nadler, and E. Medof

New York University Medical Center and Harvard University

Membrane receptors for C3b (CR1) purified from human erythrocytes are powerful inhibitors of the complement cascade, encompassing the regulatory functions of the serum proteins β1H (H) and C4-binding proteins (C4bp). CR1 in its native configuration on the lymphocyte membrane has similar properties. These findings imply that CR1 can modulate the generation and uptake of the terminal complement components in the vicinity of cells, and may protect them from damage. Previous studies in our laboratory show also that CR1 has a unique role in the degradation by I of substrate-bound C3b into C3c + C3d, and support the idea that under physiologic conditions immune complexes bearing C3b are processed and transported on the surface of cells bearing CR1.

A number of investigators have shown that there is a defect in the expression of CR1 on erythrocytes of patients with systemic lupus erythematosus, and in certain other diseases. In view of the findings outlined above, the CR1 defect may be causally related to the presence of immune complex deposits in the tissues of the SLE patients.

The C3 fragment which remains associated with immune complexes following their interaction with CR1 + I is called C3d,g. This C3 fragment is recognized by CR2, a membrane receptor expressed on certain stages of differentiation of B lymphocytes. CR2 has recently been identified by means of a monoclonal antibody as a glycoprotein of M_r 140,000. The function of CR2 is unknown. Perhaps of relevance are the observations that the retention of antigen in lymphoid follicles and the development of B memory cells are C3-dependent processes. We speculate that CR2 may be involved in the traffic of immune complexes (and therefore of antigens) into the areas of lymphoid organs where the immune response takes place.

The activity and binding properties of C3 receptors under a number of physiologic conditions. M.M. Frank, S. Inada, E. Brown, T.A. Gaither, C. Hammer, and C. Pommier. NIH, Bethesda, MD. USA.

Raji cells and several other Burkitt's lines were assayed for C3 receptor activity and conditions for optimal binding were examined. Raji cells rosetted with EC3bi, EC3d, and EC3b. Fluid phase C3b blocked Raji C3b rosettes without influencing EC3bi binding. Comparing the effect of ionic strength on Raji cell and PMN binding it was noted that binding of EC3bi to Raji cells was relatively insensitive to ionic strength; binding of EC3b was highly ionic strength dependent. In contrast EC3b binding to PMN and monocytes was relatively ionic strength independent, binding of EC3bi was ionic strength dependent. At physiologic ionic strength the C3bi receptor on PMN may be at a relative disadvantage while the PMN and monocyte C3b receptor may be more effective on a per molecule basis. EC4b bind well to the C3b receptor of cord blood lymphocytes and EB virus infected cell lines but do not bind to Raji or other Burkitt's cell lines, even at C4b densities of 50,000 molecules/E. Thus the Raji Cell C3b receptor differs markedly from that on a number of non-Burkitt's derived lymphoid lines and from the receptor on PMN and monocytes.

Circulating human monocytes express weak EC3d binding activity. On attachment to glass or plastic surfaces in the presence of Fetal Calf Serum these cells increase expression of this receptor markedly. Activity of the C3b receptor on monocytes may be markedly influenced by other events that take place at the cell surface. Fluid phase, non-cleaved, fibronectin (FN) in the reaction mixtures can bind to FN receptors on the cell surface thereby altering the activity of receptors for EC3b and EIgG. In the presence of FN the binding of IgG and C3b coated erythrocytes to the monocyte surface increases. Moreover, in the presence of FN the monocyte C3b receptor not only mediates binding of C3b coated E but phagocytosis is initiated as well. Thus fibronectin may act as a opsonin, promoting phagocytosis without binding to the material to be phagocytosed.

LOW C3b RECEPTOR REACTIVITY ON ERYTHROCYTES FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Yuzo Miyakawa

The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan

In 1953 Nelson observed that antigen-antibody complexes, when incubated in the presence of complement, adhered to primate erythrocytes. This phenomenon, designated as immune adherence, represents the binding of C3b sites generated on immune complexes to the receptor for C3b present on erythrocytes. While working on immunological assays employing immune adherence, we noticed that the reactivity for immune adherence of erythrocytes varied from person to person by a wide margin, but stayed unchanged in each individual for many years. These findings led us to look into whether or not a low reactivity of C3b receptor would be reflected on the manifestation of immune-complex-mediated diseases by an impaired disposal of C3b-bearing immune complexes in vivo.

When C3b receptor reactivity on erythrocytes was tested by immune adherence, approximately two thirds of patients with SLE were found to be defective. The defective C3b receptor reactivity persisted irrespective of the disease activity or steroid therapy, and clustered within the family members of patients with the defect (Lancet ii:493, 1981). These lines of evidence indicated that the defect is genetically determined. Iida et al. reproduced the lowered reactivity of C3b receptor by demonstrating its decreased numbers in SLE patients (J. Exp. Med. 155:1427, 1982). Wilson et al. confirmed the genetic control of C3b receptor reactivity, and extended it further to an autosomal codominant transmission (N. Engl. J. Med. 307:981, 1982).

Utilizing monoclonal antibody directed against C3b receptor, we also have found that the number of C3b receptors on erythrocytes may be classified in accordance with the HH, HL and LL phenotypes proposed by Wilson et al. LL accounted for approximately half of SLE patients while HH is extremely rare. These genetic backgrounds may be applied for the diagnosis of SLE. We phenotyped C3b receptor on erythrocytes from patients who were suspected of SLE, but still fell short of diagnostic criteria. The patients typed LL received renal biopsies, and all of them revealed immunopathological changes compatible with lupus nephritis. Sometimes, severe diffuse proliferative glomerulonephritis in the absence of pathological proteinuria was uncovered to call for an early institution of steroid therapy.

It may be naturally assumed that the erythrocyte C3b receptor helps clear immune complexes from circulation in concert with reticuloendothelial systems such as liver. Future studies will be directed toward finding any influence of the three phenotypes on the levels of immune complexes as well as on the clinicopathological pictures as their sequelae. Meanwhile, the role of C3b receptor reactivity in non-specific defense mechanism in the other immunological diseases will have to be evaluated.

C3 Fragments and Receptors

P.J. Lachmann, R.A. Harrison, A.E. Davis, G.D. Ross

Studies of C3 cleavage under physiological conditions have shown that, subsequent to the initial cleavage of C3 to C3a and C3b there occur a series of further cleavages mediated by Factor I. Initially, using Factor H as cofactor, the products produced are iC3b and C3f. Subsequent cleavage of iC3b requires CR1 as cofactor and gives rise to C3c and C3d,g (alpha 2D globulin). These are the end product of C3 catabolism, both in the fluid phase and surface bound, within the circulation. The cleavage of C3d,g to C3d and C3g is brought about by a wide variety of enzymes with tryptic activity. Other fragments are apparently also formed under non-physiological conditions. These include both the C3e of Ghebrihiwet and Muller-Eberhard and the C3d-K recently described by Meuth et al. C3d-K is closely similar to C3d,g but contains nine extra amino acids at the N terminal end. Since C3d,k and C3d,g appear to have different biological activities this region of differences is of considerable interest and some biological properties of the extra non-peptide will be described.

There are now three well-characterised cell membrane receptors for C3 fragments, CR1, CR2 and CR3. It has become clear that it is hazardous to describe them only in terms of their ligand specificity since this overlaps widely. CR1 reacts not only with C3b but also with iC3b. CR2 reacts not only with C3d but (better) with C3d,g and also weakly with iC3b. It is only CR3 that is entirely specific for iC3b among complement fragments but this receptor will also bind unopsonised zymosan. CR3 shows properties that are highly analogous with those of bovine conglutinin and appears to be of considerable biological importance as shown by the clinical effects of its deficiency in man.

TLDPERLGR.

CR
Mac
Mol
OKM1

ATTACK SITE OF COMPLEMENT ON GRAM-NEGATIVE BACTERIA.

Haruo Kozono, Kyongsu Hong, Junji Takeda, Yuko Takata, Taroh Kinoshita, and Kozo Inoue*.

Department of Bacteriology, Osaka Univeristy Medical School, Osaka, Japan.

Hypertonic medium containing 0.5 M sucrose gives a little inhibitory effect on hemolytic activity of complement. However, it inhibits very much the bactericidal activity of complement on antibody-sensitized Escherichia coli in the absence of lysozyme.

Although it is well known that the hypertonic medium causes the plasmolysis of the bacteria, the separation of the inner membrane from the outer membrane does not seem to protect the bacteria from killing action of complement, becuase the release of alkaline phosphatase (MW 80,000 - 90,000), a periplasmic enzyme, from the complement-treated bacteria is similarly inhibited by hypertonic medium. The increase of sucrose concentration in the reaction medium causes decrease of both the marker release and the bacterial death (loss of colony-forming ability) in parallel by complement in the absence of lysozyme.

Kinetic experiments show that the bacterial death by lysozyme-free complement system in isotonic medium precedes the release of alkaline phosphatase. The release of intracellular enzymes like as nucleoside phosphoacyl hydrase (MW 23,000) and β-galactosidase (MW 540,000) occurs later probably following to mechanical destruction of the inner membrane after the disturbance of outer membrane.

When complement gives the cidal lesions on the bacterial surface structure in isotonic medium, C9 molecules are deposited exclusively on the outer membrane but not on the inner membrane.

Human C9-deficient (C9D) serum can kill the sensitized bacteria but does not cause the release of alkaline phosphatase even when larger amount of C9D serum is used. The addition of purified C9 to C9D serum results in the release of alkaline phosphatase and the marked increase of the death of bacteria. C9D serum can also kill the phospholipase A-deficient mutant of E. coli. Therefore, bacterial phospholipase A does not participate in the bactericidal action of complement even when C9 is completely lacking.

Molecular composition of complement lesions and their mode of assembly.
E.R. Podack, Research Institute of Scripps Clinic, La Jolla, California.

Membrane attack by complement involves the transition of hydrophilic precursor proteins to amphiphilic complexes interacting with the hydrocarbon core of the membrane. Membrane insertion follows binding of C7 to C5b-6 and conversion of the two hydrophilic proteins to amphiphilic C5b-7. Labeling with membrane restricted photoaffinity probes and localization of biotinated C7 in C5b-7 with colloidal gold-avidin suggests that the C7 subunit contributes to the membrane binding domain of the complex. Furthermore, isolated C7, but not C5 and C6, forms amphiphilic C7 dimers upon incubation with sodium deoxycholate above its critical micellar concentration at 37°. C7 dimers reconstitute with single bilayer vesicles upon detergent removal. They contain more β-structure and show an increased length when compared to monomeric C7 in the electron microscope. Expression of hydrophobic domains may be caused by a conformational change involving restricted unfolding of C7 upon interaction with itself. A similar or identical conformational change of C7 occurs upon reaction with C5b-6 in the absence of detergents. It is suggested that protein-protein interactions provide the energy for the partial unfolding of C7 and expression of hydrophobic domains.

Membrane lysis ensues upon binding and rapid circular polymerization of 12-16 C9 molecules by C5b-8. The resulting complex (MAC) is composed of the 100 Å wide, 160 Å long poly C9 tubule in association with the 320 Å long C5b-8 rod. C9 polymerization is blocked in the presence of complex bound S-protein. During prolonged incubation at 37°, isolated C9 undergoes slow spontaneous polymerization to a tubular complex. The tubule is rimmed on one end by a torus of 200 Å outer diameter and on the opposite end by a 40 Å long membrane binding domain that affords poly C9's membrane insertion and is accompanied by release of encapsulated markers. Subunits in poly C9 have twice the length of monomeric C9, they express neoantigens, contain increased β-structure and are amphiphilic. C9 subunit interactions in circular poly C9 are resistant to dissociation by SDS but may be broken by 8 M guanidine thiocyanate. It is suggested that the expression of hydrophobic domains of C9 is the result of a conformational rearrangement involving restricted unfolding of C9 molecules upon C9-C9 interaction. The hydrophilic-amphiphilic transition of terminal complement proteins thus appears to be the result of conformational changes mediated by high affinity protein-protein interactions in the form of homopolymers (poly C9) or heteropolymers (C5b-7).

Activation of the attack phase of serum complement by physico-chemical means

A. Dessauer, U. Rother and K. Rother

Institute of Immunology, The University of Heidelberg
West - Germany.

Starting with an investigation of the acid lysis test used for the diagnosis of PNH, our Heidelberg group has previously reported on the activation of the attack phase by brief exposure of human serum or of C5 plus C6 to pH 6.4. The observations were not reconcilable with the interpretations based on the activation by the convertases in that

(1) PNH cells were not needed for activation

(2) The lytic activity was stable in serum

(3) Activation could be achieved within seconds at $0^{\circ}C$ and in the absence of bivalent cations.

(4) Components of the convertases were not needed

(5) C5a was not found after activation (RIA or function).

Similar activations of C5 were induced by physico-chemical means as used for storage or in some purification procedures such as PEG precipitation, low or high ionic strength or freezing and thawing. The activations were dependent on the presence of at least traces of C6. Freezing and thawing of C5 plus C6 was as efficient as the convertases and was used for further analysis. When C5 and C6 were incubated, a time and temperature dependent formation of an inactive C56 complex was observed. The complex was converted into the active state by freezing. The two forms of the C56 complexes were separated by FPLC anion exchange chromatography. The activated complex was more stable and more hydrophobic and had a higher negativ charge.

The results further support our view (U.Rother 1977; U.Rother et al.1978) that a configurational change of the tertiary structure of C5 is the critical event in the activation process. It can be achieved by different means, the activity of the convertases among them. The liberation of C5a by the convertases, then, would appear of concomitant nature with respect to the lytic process.

RECENT STUDIES OF MEMBRANE ATTACK BY COMPLEMENT

Manfred M. Mayer

[1] The characteristics of the C5b-8 and C5b-9 transmembrane channels (Ramm): Small unstable channels are formed by C5b-8. Incorporation of one molecule of C9 yields a rigid channel (molecular composition $C5b_1C6_1C7_1C8_1C9_1$). Both channel types have functional diameters of ca. 1 - 3 nm, but marker flux through the latter type is much faster, indicating a larger diameter and/or longer open time. Uptake of a second molecule of C9 yields a larger channel (>3 nm; molecular composition $C5b_1C6_1C7_1C8_1C9_2$). There is no evidence, pro or con, with respect to further channel enlargement by uptake of additional molecules of C9. Our results indicate that the transmembrane part of the channel structure is a hetero-polymer comprising C9 and C5b-8 subunits, rather than a homo-polymer of C9 molecules.

[2] Penetration of the C9 subunit in C5b-9 across the membrane into the cytoplasmic space (Whitlow): C9 penetration into the cytoplasmic space was assessed with resealed erythrocyte ghosts containing trapped transglutaminase, a cross-linking enzyme, and carrying C1-8 on their surface. Complement channels were made with radioiodinated C9 and the transglutaminase activated by addition of Ca^{++} through the channels. Cross-linking of C9 was assayed by measuring molecular weight on SDS gels under reducing conditions. These analyses showed that C9 was cross-linked from the inside which indicates that C9 within the C5b-9 complex is a transmembrane protein penetrating into the cytoplasmic space.

[3] The basis of the multi-channel requirement for killing a nucleated mammalian cell (Ramm): Koski et al. showed recently that the cytolytic dose-response curves of two nucleated cell lines, Molt 4 and U937, with respect to C6, are sigmoidal, in sharp contrast to the monotonic lytic curves of erythrocytes treated similarily. The release of ^{86}Rb, an indicator of channel formation, from U937 cells displayed a monotonic dose-response. Therefore, several channels are required to lyse a nucleated cell. Ramm et al. have shown that channels are eliminated very rapidly by U937 cells. Therefore, lysis is dependent on formation of several channels within a very short time span. We have now found that ouabain, at a concentration sufficient for 95% inhibition of the Na^+/K^+ membrane pumps, does not affect the sigmoidal dose-response of U937 cells, indicating that these pumps are not responsible for the multi-hit characteristics. By contrast, incubation of the cells with puromycin reduced the rate of channel elimination and changed the cytolysis curve from a sigmoidal to a monotonic shape. Hence, the multi-channel requirement, in the absence of puromycin, is due to rapid elimination of channels.

[4] Consequences of cell membrane attack by complement: Release of arachidonate and formation of inflammatory derivatives (Imagawa): When antibody-sensitized Ehrlich ascites cells are attacked by rabbit or guinea pig serum complement, free ^3H-arachidonic acid is released from the cells into the fluid phase (a maximum of about 20% of the available arachidonic acid can be released). The dose-response curve of release is approximately in the same range as the cytolytic response. C6-deficient or C8-deficient complement sera do not cause cytolysis or phospholipase activation, but both activities are restored by reconstitution with purified C6 or C8, respectively, which implicates these terminal complement proteins. With Ehrlich cells, formation of oxygenated inflammatory derivatives of arachidonic acid, such as prostaglandins, thromboxanes and HETES was nil. By contrast, complement attack on antibody-sensitized mouse peritoneal macrophages induced formation of significant quantities of these oxygenated derivatives, together with free arachidonic acid.

Structural and Functional Studies on : (i) Normal C1q; (ii) A Defective form of C1q; (iii) C4-Binding Protein (C4BP)

K.B.M. REID

MRC Immunochemistry Unit, Department of Biochemistry, Oxford, UK

(i) Structural studies have allowed a molecular model to be proposed for C1q which helps to explain how it interacts with: activators; the $C1r_2C1s_2$ complex; other molecules, such as fibronectin, and receptors on cells. For example, binding assays using intact C1q, collagenase-digested C1q, pepsin-digested C1q and ^{125}I-labelled fibronectin confirmed that both the collagenase-digested and pepsin-digested C1q preparations bind fibronectin. This indicates the presence of two sites on C1q for fibronectin binding or that a short collagenous section, located just prior to the 'head' region and present in both types of digest tested, represents the fibronectin binding site.

(ii) C1q has a mol. wt. of 460 000 in normal human serum but a C1q-like molecule of 150 000 mol. wt. is found in the sera of patients homozygous for a genetically linked deficiency of C1q activity. This defective form of C1q may be the consequence of an abnormal C1q gene which produces an antigenically deficient, non-functional molecule. The defective molecule contains subunits of a similar size to those of the normal C1q in SDS-PAGE without reduction of disulphide bonds but shows an unusual pattern when examined after reduction of disulphide bonds. The defective molecule contains collagenous and non-collagenous domains of a similar size to those found in the normal molecule. These observations allow a structural model to be proposed for the defective molecule.

(iii) C4BP of approx. 540 000 mol. wt. is composed of 6-8, probably identical, chains of 70 000 mol. wt. Over 60% of the amino acid sequence of the 70 000 mol. wt. chain has been determined by examination of fragments generated by CNBr digestion, hydroxylamine treatment, trypsin digestion and limited proteolysis by chymotrypsin. The sequence data and the fragments generated by limited proteolysis allow a structural model to be proposed which is consistent with electron microscopy studies, performed in other laboratories, which have shown that C4BP has an unusual, spider-like, structure consisting of 7 highly flexible strands joined in a compact central body. The relationship between the structure and function of C4BP in the cleavage of C4b by factor I has been studied by using C4BP which has been subjected to limited proteolysis by trypsin or chymotrypsin.

THE β-CYS-γ-GLU THIOLESTER IN THE THIRD AND FOURTH COMPLEMENT PROTEINS. B.F. Tack (1), F.F. Davidson (1), R.A. Harrison (1), M.K. Hostetter (1), J. Janatova (2) and M.L. Thomas (1). Department of Pediatrics, Harvard Medical School, Boston, Mass. (1), and Department of Pathology, University of Utah, Salt Lake City, Utah (2).

The third (C3) and fourth (C4) components of human complement react stoichiometrically (1:1 molar ratio) and covalently with [^{14}C]methylamine. The reaction with methylamine is further characterized by loss of hemolytic function and the appearance of a thiol group. The methylamine-reactive site in each protein is associated with the pentapeptide sequence Gly-Cys-Gly-Glu-Glu, where the sidechains of the cysteinyl and second glutamyl residues in the native proteins bridge to form a thiolester loop. The assignment of the second glutamyl residue as acyl group donor to the proposed thiolester is largely based on the recovery of γ-glutamyl[^{14}C]methylamide at this position. Further chemical evidence in support of the thiolester assignment in human C3 has been recently obtained using [^{3}H]$NaBH_4$. On trypsin activation of C3 in the presence of [^{3}H]$NaBH_4$, [^{3}H] was observed to incorporate specifically into the α'-chain of C3b. The [^{3}H]-label was found to reside in a limit tryptic peptide containing the above pentapeptide sequence. On Edman degradation of this peptide, the [^{3}H]-label was associated with the position of the second glutamyl residue earlier implicated in formation of the thiolester. As would be predicted from reduction of a β-Cys-γ-Glu thiolester with [^{3}H]$NaBH_4$, α-amino-δ-[^{3}H] hydroxyvaleric acid was identified at this position by HPLC analysis. An identical site is also found in the major blood protease inhibitor α_2-macroglobulin. The thiolester present in each protein can participate in a transesterification reaction with appropriate acceptor molecules. For C3, the glutamyl component has been shown to acylate glycerol and L-threonine on proteolytic activation with the resultant formation of an oxy-ester and amide bond, respectively.

COMPARATIVE STUDIES ON ASPARAGINE-LINKED SUGAR CHAINS OF SUBCOMPONENTS C1q OF THE FIRST COMPONENT OF HUMAN, BOVINE, MOUSE AND GUINEA PIG COMPLEMENT: ARE THERE ANY POSITIVE CORRELATIONS BETWEEN THEIR STRUCTURE AND BIOLOGICAL ACTIVITIES OF C1q?

Kunio Yonemasu[1], *Takako Sasaki*[1], *Yoshiko Dohi*[1] *and Hideo Yoshima*[2]; [1]Nara Medical College, Nara 634 and [2]Osaka Prefectural Hospital, Osaka 546, Japan.

The *C*-terminal globular regions of C1q(GF) are considered to contain the binding sites to antibody molecules(Hughes-Jones & Gardner, Mol. Immunol. 16,697,1979; Pâques et al., Hoppe-Seyler's Z. Physiol. Chem. 360,177,1979) and one biantennary complex type asparagine-linked sugar chain(J. Biol. Chem. 253,7404,1978) presumably at position 124 of each A chain(Reid et al., Biochem. J. 203,559,1982). This investigation was undertaken to characterize GF over mammalian species and to elucidate the possible role of asparagine-linked sugar chains in the biological activities of C1q.

Human, bovine, mouse and guinea pig C1q were highly purified by a combination of precipitation with EGTA, ion-exchange chromatography and/or gel filtration. The GF of these C1q was prepared by collagenase digestion. The overall amino acid composition of each of these C1q was very similar to each other. Great similarities of amino acid compositions were found between highly purified human and bovine GF, among three non-covalently linked chains of each GF as well as between the corresponding chains of human and bovine GF. These results suggested that the GF on the C1q molecule remained highly conserved in its evolution.

Each of these C1q contained one asparagine-linked sugar chain on each GF (six chains/molecule). After *N*-acetylation and successive NaB^3H_4-reduction of asparagine-linked sugar chains liberated by hydrazinolysis of these C1q, their structure was analysed by sequential exoglycosidase digestion in combination with methylation analysis or with sugar composition analysis. These sugar chains were composed of the biantennary complex type sugar chains with the following outer chains in various combinations: (±NeuNAcα2→6)Galβ1→4GlcNAcβ1→2 for human, NeuNGcα2→6Galβ1→4GlcNAcβ1→2 and Galβ1→3Galβ1→4GlcNAcβ1→2 for bovine, and (±NeuNAcα→)Galβ1→GlcNAcβ1→ and Galβ1→Galβ1→GlcNAcβ1→ for both rodents C1q, respectively. These outer chain moieties were found to be linked to a common core structure of Manα1→(Manα1→)Manβ1→GlcNAcβ1→(Fucα1→)GlcNAc. Thus, asparagine-linked sugar chains were similar to each other and these seemed not to be specific to each of these species.

On the other hand, only human and mouse C1q had the positive agglutinating activity of human IgG-coated latex particles(IgG-latex), and neither bovine nor guinea pig C1q did. Since it seems to be unreasonable that differences of their agglutinating activity is attributable to those of these sugar chain structures themselves located in GF, we have further tried to elucidate more directly whether these asparagine-linked sugar chains have any influence on biological activities of C1q by using human C1q before and after sialidase digestion; incidentally sialic acid is supposed to be the most important sugar to keep the conformation of peptide chain(s) as well as that of sugar chains themselves natural by its negative charge(Sharon & Lis, Chem. Eng. News, 21,1981). Both the haemolytic activity of human C1q and its binding activity to immune complex made of human or rabbit IgG were unimpaired even after the complete removal of sialic acid from it.

From these results(great similarities of amino acid compositions of GF, the lack of any positive correlations between these sugar chain structure and agglutinating activity of IgG-latex, and no influence of the removal of sialic acid from these sugar chains on biological activities of C1q), it might be concluded that these sugar chains of C1q have no direct influence on biological activities of C1q.

The Functional Sites of The Second Component (C2) of Human Complement

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Human C2 is supposed to have at least two thiol residues, which upon treatment with iodine, formed a disulfide linkage and stabilzed C3 convertase, C4b,2a. Then we have investigated the number and location of the thiol residue in C2.

Titration of the thiol residue in C2 using 5,5'-dithio-(2-nitrobenzoic acid), N-(dimethylamino-4-methylcoumarinyl)-maleimide (DACM), and 2,2'-dithiopyridine in the presence of SDS showed that human C2 contained a single thiol residue. The thiol residue in C2 did not react with a fluorescent thiol reagent, DACM, in the absence of SDS but reacted with a more hydrophobic fluorescent thiol reagent, pyrenyl maleimide (PMI) in the absence of SDS. These results suggest that the thiol residue is located not at the surface of C2 but in a hydrophobic inner portion of C2 molecule. Labeling of the thiol residue with PMI did not affect the C3 convertase activity of C2.

Iodine treated C2 failed to react with DACM even in the presence of SDS, suggesting the modification of the thiol residue upon iodine treatment of C2.

SDS-PAGE of C2a and C2b after treatment with DACM revealed that the thiol residue was located in the C2a domain. Interestingly enough, the thiol residue in C2a was no longer labeled with PMI in the absence of SDS, suggesting that the thiol residue becomes much more buried in an inner portion of C2a.

Although C2a has been shown to be the catalytic subunit of the assembled C3 convertase, little is known on the role of C2b upon the assembly of C3 convertase. Antiserum against C2a effectively inhibited the C3 convertase activity, while antiserum against C2b failed to inhibit the C3 convertase activity. However, addition of increasing amounts of C2b to C2 resulted in the gradual inhibition of the activation of C3 convertase. These results suggest that although C2b domain is not necessary for the C3 convertase activity, binding of C2 via C2b domain to C4b is a prerequisite for the assembly of C3 convertase, C4b,2a.

Interaction of C4-binding protein with cell-bound C4b: a quantitative analysis of binding and the role of C4-binding protein in proteolysis of cell-bound C4b.

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It has been reported that a serum protein C4-binding protein (C4-bp), which has several binding sites for fluid-phase C4b, functions as an essential cofactor for C3b/C4b inactivator (I) in the proteolysis of fluid-phase C4b. It has also shown that C4-bp accelerates the decay of C4b2a by dissociating C2a from cell-bound C4b, suggesting an interaction between C4-bp and cell-bound C4b. However, the direct binding of C4-bp to cell-bound C4b has not actually demonstrated.

In this study, we provided evidence that C4-bp binds specifically to cell-bound C4b. Quantitative analysis of C4-bp binding shows that the number of C4-bp molecules bound per cell is proportional to the number of C4b molecules on the cell surface. However, only small amounts of C4-bp bound to EAC4 bearing less than 3,000 C4b-molecules/cell on thier surface, suggesting that a relatively high C4b-density per cell is required for the binding. Scatchard analysis of binding of C4-bp indicated an equilibrium constant of 4.6 X 10^5 L/M and a maximum of 0.43 C4-bp molecules bound per C4b molecule, equivalent to an average of one molecule of C4-bp per two or more molecules of C4b.

Fluid-phase C4b inhibited the binding of C4-bp to cell-bound C4b in a dose-dependent manner, whereas native C4 had little effect. C2 inhibited this binding and also released C4-bp from EAC4,C4-bp cell. However, C2 was 27 times less effective than unlabeled C4-bp on a molar basis and a considerable amount of C4-bp remained bound to C4b on the cell even in the presence of a large excess of C2.

We also examined the cofactor activity of C4-bp in the cleavage of cell-bound C4b by I. Cleavage of the α' chain of C4b on the cell surface by I alone was incomplete and an intermediate product was observed. When C4-bp bound to C4b on the surface, the α' chain of the C4b cleaved into three fragments, $\alpha 2$, $\alpha 3$, and $\alpha 4$. The $\alpha 3$, $\alpha 4$, β, and γ peptides (C4c) were released into the fluid phase, and the $\alpha' 2$ fragment (C4d) remained linked covalently to the cell membrane via an ester bond. In some situations, therefore, C4-bp enhances the proteolytic activity of I on the cell-bound C4b. As mentioned above, binding of C4-bp to cell-bound C4b depends on the density of C4b on the cell surface. Thus, the effect of C4-bp is greatly influenced by the microenvironment in which the reaction takes place.

MONOCLONAL ANTIBODIES AS PROBES OF COMPLEMENT PROTEINS.

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Because they recognize individual epitopes, monoclonal antibodies to complement proteins provide useful reagents for exploring the activities associated with such epitopes. To take advantage of this probing function, we have prepared rat monoclonal antibodies to constituents of the alternative pathway convertase, produced by fusing spleens from rats immunized either with purified human factor B, C3, or the active enzyme C3bBb to the rat myeloma lines Y3.Ag.1.2.3 or 210.RCY0.

Of three rat monoclonals that react with human factor B, two which are specific for Bb inhibit the hemolytic activity of B and one which reacts with Ba does not. The inhibition of C3bBb activity is effectively instantaneous and occurs with preformed convertase, consistent with binding of the monoclonal antibodies in or near the active site of the Bb fragment. Blocking of monocyte spreading induced by Bb, a function previously also shown to require an active enzymatic site in Bb, supports this conclusion.

Of two rat monoclonals reactive with C3d (trypsin), one preferentially blocks formation of active C3bBb. Treatment of cells bearing this convertase with the monoclonal appears to shorten their half-life. The inhibition of hemolytic activity is correlated with inhibition of uptake of ^{125}I-B. The same monoclonal inhibits the uptake of ^{125}I-H onto EAC43b. This antibody specifically precipitates the alpha chain of C3. It appears to recognize an epitope on the alpha chain of C3 which is involved in binding of both B and H.

MOLECULAR GENETICS OF COMPLEMENT: HR Colten, G Goldberger, R Sackstein, HS Auerbach, FS Cole, D Woods.

Recently, complementary DNA (cDNA) clones corresponding to sequences coding for complement proteins factor B, C4 and C3 have been isolated. With these clones it has been possible to determine the structure of the factor B gene, identify and partially characterize precursor and mature mRNA specific for factor B, determine the entire sequence (nucleotide and amino acid) and to generate a three dimensional model of the factor B protein. A comparison of sequence analysis of mouse and human factor B cDNA revealed 80% nucleotide sequence homology. The cDNA probe has also been useful in the elucidation of molecular mechanisms accounting for changes in factor B synthesis that accompany maturation of monocytes and macrophages in vitro.

With the C4 cDNA it has been possible to undertake structural studies of C4, to determine genomic variants of C4, to determine that genetic deficiency of C4 in guinea pigs is probably a result of a defect in post-transcriptional processing of C4 RNA and to define the mechanism of feedback inhibition of C4 biosynthesis.

With C3 genomic and cDNA clones, Fey and his colleagues have localized the human C3 gene to chromosome 19 and elucidated C3 gene structure and a probable signal peptide of pre pro-C3.

The Incidence of C9 Deficiency in Japan

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Since the first case of C9 deficiency (C9D) was reported by Inai et al. in 1978, more than 10 cases with C9D have been reported in Japan. Kisu et al. reported an interesting C9D with gastric cancer who developed an antibody against C9 after she was given 6 units of blood and 7 units of fresh plasma during the operation.

We intend to examine the incidence of C9D in Japan. Two groups were selected for the mass examination of C9D, one being a blood donor group and the other a random group of patients. Sera obtained from the donors who visited the Red Cross Blood Center of Osaka was used for the screening test. The sera taken from the random group of patients had been sent from hospitals all over Japan to the Special Reference Laboratory for laboratory examination and was used by us for the screening test. For this test, hemolytic activity of complement in the serum specimens was assayed by micro-method using sucrose gelatin veronal buffer (SGVB). SGVB was used because, although the CH50 level of C9D had been known to be approximately 15 units when assayed in gelatin veronal buffer (GVB), it has been reported by Kitamura et al. that CH50 of C9D decreased to about 5 units when assayed in SGVB. Sera which showed no hemolysis in this test were used for further examination of the complement system, including hemolytic assay and immunochemical analysis of C9. When C9 protein was undetected in the test serum and the hemolytic activity of C9 was less than 0.5% of normal human serum, the subject was classified as C9D. The results of the mass examination of C9D in the two groups were as follows.

1. The Blood Donor Group: Of 52,175 blood donors, 55 were C9D. Therefore, the incidence of C9D in the Osaka area was considered to be 0.1%. This value is almost equivalent to that reported by the Red Cross Blood Center of Saitama, but it was 1.5 times higher than that reported by the Red Cross Blood Center of Fukuoka. The average CH50 unit of C9D found in this group was 12.1±3.8. In this group, all the donors with C9D were healthy and their laboratory examination results were in the normal range. In addition, five donors were found to be deficient in C7 and two in C8.

2. The Random Group of Patients: Of 39,025 patients, 37 patients were classified as C9D. Therefore, the incidence of C9D in this group was 0.09%. In order to clarify the difference in the incidence of C9D throughout Japan, Japan was divided into eight areas and the incidence of C9D in each area was calculated. No C9D has been found in two areas of Japan, namely Kyushu and Shikoku. Therefore, the incidence of C9D in these two areas is considered to be definitely lower than that of any other area of Japan. The incidence of C9D in the other areas of Japan is approximately 0.1%. This incidence is equivalent to that of Osaka or Saitama. Most of the C9D patients in this group had CH50 levels in the normal range, which is between 30 and 50 units. The CH50 level of a patient with C9D was 63.8. From these results it was found that the level of CH50 of people with C9D in the random group of patients was quite different from those of the blood donor group.

COMPLEMENT EVOLUTION IN PRIMITIVE VERTEBRATES

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(I) Recent studies in my laboratory have identified complement system in two primitive vertebrates,lamprey and rainbow trout. Several general conclusions concerning complement evolution have been derived from these studies. (1)Lamprey,one of the most primitive extant vertebrates,has an apparently very simplified complement system consisting of a primordial C3 and a few additional components. The primary function of lamprey C3 is not as the mediator of target cell lysis,but as the essential phagocytosis-promoting factor. (2)Teleostean rainbow trout has highly developed complement system complete with the classical and the alternative pathways. Rainbow trout complement mediates both target cell lysis and phagocytosis. (3)Component C3 and C5 had been differentiated already at the phylogenetical level of rainbow trout,suggesting that gene duplication leading to divergence of these two proteins had preceded the divergence of the common ancester of teleost and mammals. (4)The unique thiol ester site that functions as the metastable binding site for mammalian C3 and C4 have been demonstrated in α chains of all of C3-like proteins of primitive vertebrates studied. (5)Phagocytosis-promoting activity of promitive vertebrates appears to be mediated by the interaction between cell-bound C3 and receptors at the phagocyte membranes,in a similar manner as mammalian complement.

(II) Four complement proteins have been isolated from lamprey and rainbow trout. Their physicochemical characters are as follows. (1)Primordial complement protein of lamprey(denoted lamprey C3) shows the m.w. of 190K,consisting of three polypeptide chains(84K-α,74 K-β,and 32 K-γ) linked by disulfide bonds. This protein has to be activated by B-like factor to be bound to receptive surfaces like zymosan. Activation of lamprey C3 is associated with split of a 13 K-fragment from α chain and cocommitant appearance of one sulfhydryl group in α chain. Lamprey C3 retains the unique thiol ester site that serves,upon activation,as the metastable binding site. Lamprey C3 bound to particles strongly enhances phagocytosis,but it is not part of lytic activity present in lamprey serum.
(2)Rainbow trout C3 consists of two isotypes that share a partly identical and partly distinctive antigenecity(C3-1 and C3-2). Isotype specific antisera each specific for C3-1 and C3-2 can be prepared. Tryptic mapping revealed $\sim$85% identical and $\sim$15% distinctive sponts for these two isotypes. NH_2-terminal sequence of C3-1 and C3-2 showed a significant homology to those of mammalian C3,C4 and C5 as well as to cobra venom factor. Rainbow trout C5 shows the m.w. of 190 K,consisting of disulfide linked two polypeptide chains(133 K-α and 86 K-β). This protein is involved in the assembly of membrane attack complex during complement activation in a similar manner as mammalian complement.

The present work was performed by a close collaboration between my laboratory(Drs.S.Sakai,M.Nonaka,T.Kaidoh,M.Nonaka) and Dr.T.Fujii of Hokkaido University, Drs.M.Nozaki and M.Iwaki of Shiga Medical College and Dr.K.Tanabe of Aichi Cancer Center Research Institute.

GLYCOPHORIN INHIBITS ACTIVATION OF THE ALTERNATIVE COMPLEMENT PATHWAY OF SERUM HOMOLOGOUS TO THE GLYCOPHORIN

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The alternative complement pathway (ACP) was first claimed by Pillemer et al (1954), under the name of properdin system, to play a role as a natural defense mechanism in body fluids. Recently, bactericidal activity was demonstrated in a reconstituted ACP consisting of highly purified components, indicating that no immunoglobulin is required for the reaction (Schreiber et al, 1979). Then the question to be answered is how the ACP, in the absence of antibody, recognizes the non-self substances with which it should react. If complement could recognizes self-cell surface as the place where the ACP reaction must be prevented, complement may be able to accomplish the recognition of non-self constituents without diversity of recognition sites for a veriety of foreign substances (Okada et al. 1983). Since the ACP is under a continuous pressure of activation (Pangburn et al, 1981), invading microorganisms might offer a place for the complement activation because there might be no species-specific inhibitory molecules on the invaders. As an approach to the identification of the putative membrane inhibitor which restrict ACP activation of homologous serum, we tried to obtain monoclonal antibody to the putative membrane molecules. Hybridoma cells producing antibody to erythrocytes (E) of guinea pig (GPE) were prepared by fusing mouse myeloma cells and immune spleem cells from mice. Most of antibodies produced by those hybrid cells could not sensitize GPE for hemolysis by homologous C4 deficient serum (C4D-GPS), although they could cause agglutination of GPE. Out of over 200 antibody forming wells, a clone of hybridoma has been isolated which produces monoclonal antibody (MCA) capable of sensitizing GPE for hemolysis by C4D-GPS. The MCA was expected to have sensitized GPE by reacting on the putative inhibitor of GPE membrane. Since the MCA was found to react with glycophorin of GPE membrane, glycophorin was speculated to be a membrane inhibitor which restrict reaction of ACP of homologous serum.

To verify this speculation, glycophorins extracted from human E (glycophorin-Hu) and GPE (glycophorin-GP) were adsorbed to rabbit-E. The adsorption of glycophorin-Hu and glycophorin-GP to rabbit-E made the E resistant to hemolysis by human serum (HuS) and guinea pig serum (GPS), respectively, via the ACP. On the other hand, glycophorin on rabbit-E did not inhibit the hemolysis by serum heterologous to the glycophorin adsorbed. To obtain indisputable results, glycophorin-GP was incorporated in the liposome membrane which consisted of cholesterol, dimyristoylphosphatidylcholine and trinitrophenyaminocaproyldipalmitoyl phosphatidylethanolamine (TNP-Cap-DPPE). The presence of glycophorin-GP on the liposome membrane effectively inhibited the ACP activation of GPS but not of HuS. This species specific inhibition by glycophorin of ACP activation should play a role in restricting ACP activation on self cell membranes. By recognizing the self-cell surface as the place where the complement reaction must be prevented, ACP will accomplish the recognition of non-self constituents without diversity of recognition sites for a variety of foreign substances.

We thank Dr. T. Yasuda for advices for liposome experiments and Dr. A. Hamada for kind gift of some glycophorin preparations. This work was supported by Grants-in-Aid from the Ministry of Education, Culture and Science, from the Ministry of Health and Welfare, and from the Adult Disease Clinic Memorial Foundation.

what stage?

Complement-dependent lysis of Influenza virus-treated heterologous and autologous cells in serum.

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Cell surface sialic acids play an important role in regulation of complement activation. Influenza viruses may attach to peripheral blood cells by binding to membrane receptors bearing terminal sialic acid residues. At 37 °C, viruses spontaneously elute from the cells through cleavage of these receptors by a viral neuraminidase, resulting in loss of sialic acid from the virus-treated cells. Treatment of guinea pig erythrocytes (E^{gp}) with Influenza virus in vitro, resulted in lysis of the cells in human serum in the absence of antibodies. The acquired capacity of the virus-treated heterologous erythrocytes to activate the human alternative complement pathway was dependent on an acquired resistance of the bound P,C3b,Bb amplification convertase to the regulatory action of H, that was directly related to desialation of the cells induced by the virus. Virus-treated E^{gp} also lyzed in guinea pig serum; however, complement activation in homologous serum occured through the classical pathway and was mediated by natural antibodies to neoantigens expressed by the desialated cells. A third type of interaction between Influenza virus-treated cells and complement was observed using human platelets and autologous serum. Platelets, after their contact with virus, lyzed in autologous serum through activation of the classical pathway in an antibody-dependent mechanism that didi not occur in agammaglobulinemic serum. Complement activation was not dependent on natural antibodies to the desialated cells as desialation of platelets using Vibrio cholerae neuraminidase did not result in complement activation or cell lysis. Electrophoretic analysis of surface-labeled platelets demonstrated the presence of a 72,000 mol.-wt protein in the membrane of virus-treated platelets that could be identified as viral hemagglutinin in blotting experiments and to which serum cytolytic antibodies were found to be directed. Thus heterologous and autologous peripheral blood cells may activate complement through various mechanisms following biochemical alteration of their membrane after contact with myxoviruses. Binding of a viral protein to membrane proteins of human platelets that is responsible for complement-dependent lysis of the cells in autologous serum may provide a model for immune thrombocytopenias occuring during acute viral infection at the time of the specific immune response.

1) Sialic → C3bBb

Heterogous

2) Classical

Endogenous inhibitor of factor B

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Endogenous inhibitors were isolated from lysates of mouse-established cells, M1 and L cells. Inhibitors were first discovered in precipitate formed between lysate of ^{14}C-labeled M1$^-$ cells in the presence of mouse serum as a carrier and $F(ab')_2$ of rabbit anti mouse factor B. Precipitate prepared from L cell-lysate in a large scale was solubilized and separated by preparative SDS-PAGE, and 4 distinctive proteins were separated from B protein, with Mr of 25K, 28K, 33K and 35K respectively. These proteins appeared to combine with mouse B, judging from mobility of the reactant on conventional PAGE, and inhibited its hemolytic activity. One of these inhibitors, the 25K protein, inhibited about 80% of mouse B-activity at mole ratio of more than 1:1 and raised neutralizing but not precipitating antibody against rabbit. The 25K protein inhibited also purified human factor B and CVF-Bb but not C2 in mouse serum or purified human C2. Localization of the inhibitor on cell membrane was studied by using ^{125}I surface-labeled cells. Ratio of ^{125}I-incorporation to anti 25K-immune complex against ^{125}I-counts of TCA-insoluble precipitate was 0.54% in M1$^-$ cells and 0.42% in L cells. Autoradiography of SDS-PAGE also exhibited a band at 25K position as to anti 25K-immune complex obtained from the lysates of ^{125}I surface-labeled M1$^-$ and L cells and mouse spleen adherent cells in early culture, but not of peritoneal resident macrophages. It is uncertain whether difference in distribution of the 25K protein among various cells is either quantitative or qualitative.

We designed to isolate this inhibitor by simple procedure. Because the 25K protein could react with purified human factor B, human B-Sepharose 4B affinity gel was prepared for isolating inhibitor(s) from L cell-lysate. Lysate of ^{14}C-labeled L cells in 0.1 M borate buffer (pH 8.0)was applied on affinity column and elution was performed by 0.1 M Tris-glycine buffer(pH 8.7)containing 0.2% SDS. The protein recovered in a void volume of the gel is inhibitory against mouse B-activity and reactive with anti 25K antibody, indicating that the affinity-purified inhibitor is identical to the 25K protein. Average recovery of the inhibitor was about 67% determined by hemolytic assay and 70% by immunoassay. Specific activity increased about 200-fold. High recovery of the inhibitor would mean that the inhibitor in L cell is produced in large excess than factor B and, therefore, nearly all of the inhibitor are present in a free form. The inhibitor can not prevent trypsin and papain, but slightly inhibits partially purified elastase of porcine pancreas(10-15% inhibition), suggesting that it is highly selective for factor B and its activated form.

Study on C3-like factor in the serum of a C3 deficient subject

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In a recent report (Sano et al. Arthritis Rheum. 24, 1255, 1981) we described a family in which two sisters with systemic lupus erythematosus (SLE)-like symptoms were found to have a homozygous deficiency of C3 (C3D). Despite the finding that C3 protein could not be detected in their sera by immunochemical methods, a low level of C3 hemolytic activity was found. Furthermore, it was found that the total complement activity (CH50) of these sera were 12-15 % of pooled normal human serum (NHS) and that complement mediated bactericidal activity was also present. The present study was performed to analyse the C3-like factor, responsible for this C3 activity in C3D serum.

It was found that C3 hemolytic activity in C3D serum was not depleted after incubation with anti-C3 but neutralized by incubation with anti-C5. Antisera against serum proteins other than C3 and C5 (Gc, transferrin, B, C4, C8 and C9) did not neutralize C3 activity in C3D serum. These findings suggest that the C3-like factor has C5 antigenicity. To know the electrophoretic mobility of C3-like factor, C3D serum was subjected to electrophoresis followed by overlaying a gel containing $EAC1_4^{oxy}2$ and C5-C9 to develop hemolytic bands of C3 activity. It was found that the C3-like factor in C3D serum migrated more anodally than purified C3. Its position corresponded to that of C5. This hemolytic band reflecting C3-like activity was present when the C3D serum was electrophoresed after incubation with anti-C3 but was absent in C3D serum after incubation with anti-C5. NHS produced two C3 hemolytic bands. The major band coincided with that of purified C3 and the minor band corresponded in position to the hemolytic band of the C3-like factor. Thus, C3-like factor was shown to have the antigenicity and electrophoretic mobility of C5. This factor was found in the sera of three other C3D subjects and even in NHS.

These findings suggest that, in cases of C3D, C5 compensates for the genetic lack of C3 and serves a protective function and that sensitized erythrocytes (EA) may be lysed by complement components without participation of C3.

A sexual dimorphism of mouse C5

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In the course of study on the polymorphism of mouse C5, we found an electrophoretic difference of mouse C5 between males and females. Desialated plasma from BALB/c, C3H/He, C57BL/6, CBA, DBA/1, and SJL mice were analyzed by non-equilibrium isoelectric focusing (IEF) on agarose gel, and immunofixation with anti-mouse C5. In all strains tested, male plasma had two C5 bands, basic and acidic, while female had only one band. The male basic band and the female band had the same electrophoretic mobilities. To clarify the nature of these bands, male or female plasma separately and a combination of both were analyzed by IEF, followed by crossed immunoelectrophoresis. Simultaneously, the eluates from gel slices after IEF were assayed for C5 hemolytic activity. On crossed immunoelectrophoresis male plasma produced a single precipitin line with bipeaks and C5 hemolytic activity corresponded well to the peaks, showing that the male two bands are antigenically and hemolytically indistinguishable. The mixture of male and female plasma also exhibited a single precipitin line with bipeaks, but the acidic peak decreased to the half. This result indicates that the male basic and female bands are identical. Thus, the acidic band is specific for male mice.

In view of the results above, we examine the C5 pattern in the neonatal mice. The basic C5 of both sexes was observed at the same levels, but the male acidic C5 was only slightly detected, suggesting that the acidic C5 is influenced by testosterone. Injection of testosterone resulted in an appearance of the acidic C5 in female mice and its increase in male mice. By contrast, the acidic C5 decreased with injection of estradiol. Both sex hormones had little effect on the basic C5 band. The hemolytic activity and protein concentration of C5 change with the amount of the acidic C5. Therefore, it is clear that expression of the acidic C5 is under the control of testosterone.

THE HETEROGENEITY OF C1 INHIBITOR DEFECTS

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THE ROLE OF COMPLEMENT IN PHOTOTOXICITY

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In vitro irradiation with ultraviolet light of sera of patients with erythropoietic protoporphyria (EPP) and porphyria cutanea tarda (PCT), or of normal sera to which phototoxic substances (protoporphyrin, uroporphyrin, hematoporphyrin or demethylchlortetracycline: DMCT) have been added, resulted in activation of the complement system. Complement activation in vivo was also observed following irradiation of the dorsal skin surface of the forearms of patients with PCT and EPP. The diminution of complement hemolytic activity noted was associated with changes in the electrophoretic mobility of C3 and the generation of chemotactic activity for polymorphonuclear cells. Using albino guinea pigs as an animal model, and the extravasation of ^{125}I-BSA or Evans blue injected intravenously to measure the increase in vascular permeability associated with the clinical response, the role of complement in phototoxicity was further investigated. We demonstrated that in normal animals the injection of DMCT followed by light exposure resulted in edema and erythema at the irradiated site. In contrast in guinea pigs that had been depleted of complement activity by the injection of CVF, there were no clinical findings and increased vascular permeability was abolished by 93%. These experiments support the concept that the clinical lesion seen in phototoxicity are mediated by the cleavage products of complement activation C3a and C5a.

PARTICIPATION OF COMPLEMENT IN DEMYELINATION.

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Review of the literature on demyelination reveals that no serious consideration has been given to the possibility that complement (C) may be the agent responsible for myelin destruction and its disposal. We have studied the interaction between C and myelin, and specifically the involvement of C5b-9 (MAC) as an effector. Experiments were designed to explore two issues: the role of C in immunologically-mediated demyelination, such as multiple sclerosis, and the participation of C in myelin damage and disposal in injuries of the central nervous system (CNS), such as trauma or infarct.

The Role of C in Antibody (Ab)-Mediated Demyelination: Anti-spinal cord antiserum from EAE rabbits was screened initially for its potency to demyelinate different batches of mouse cerebellar explants. Densely myelinated mouse cerebellar explant cultures (18-24 days) were then treated with IgM fraction of the Ab plus normal or heated human serum (NHS or H-NHS), C8 deficient human serum (C8D-HS) with or without human C8, or C8 alone. Demyelination was evaluated blindly by three investigators 18 hours later (endpoint of demyelination). None of the cultures treated with C8D-HS, H-NHS or C8 alone were demyelinated, while C8D-HS with C8 or NHS produced extensive demyelination. In a separate experiment, an identical result was obtained when cultures were treated first with antiserum, washed, then treated with C8D-HS with or without C8. Results of these experiments indicate the essential requirement of C5b-8 or C5b-9 in Ab-mediated *in vitro* demyelination. When cultures that had been demyelinated were further grown in the presence of ^{14}C-acetate, active uptake of ^{14}C by the explants and by newly synthesized myelin was observed indicating that cells responsible for myelin synthesis were viable in this demylinated explants and the demyelination was not caused by non-discriminative killing of neurons and oligodendrocytes by C5b-9 attack. At present, the mechanism of demyelination by C in CNS explants is unknown. We found that removal of Ab and C two hrs following incubation did not reduce the potency or kinetics of myelin destruction (which takes 8-24 hrs in our system). This suggests the possible involvement of other metabolic factors following C attack. Accordingly, the fates of myelin basic protein (MBP) and lipid of myelin and heavy membranes during demyelination were evaluated. Results revealed that no or little loss or proteolysis of MBP was triggered by C attack. In studies of lipids, ^{14}C-oleic acid was incorporated in explants during myelination. Samples taken at a different time during demyelination and the distribution of the label in each lipid fraction in isolated myelin and "heavy" membranes were studied by TLC. While no difference was seen in heavy membranes between demyelinating and control explants, a progressive decrease in ^{14}C phosphatidylethanolamine (PE) was observed with myelins from demyelinating cultures. Decrease in PE may be due to selective activation of PE-cleaving enzymes, as shown in canine demyelination by dystemper virus, due to transmethylation enhanced by C5b-9 as we have shown with Erlich ascites cells (Fed. Proc. 42:1234, 1983)

Interaction of Myelin with C in the Absence of Ab: The role of C as a non-specific effector was studied because myelin damage and its subsequent removal are natural consequences of CNS injuries such as infarction and trauma, during which, breakdown of blood-brain barrier results in exposure of myelin to serum C. Recently, we showed that myelin membranes, but not heavy membranes of CNS, activate C1 directly, and produce C3 and C5 cleavage. Further studies of C-treated myelin with density gradient ultracentrifugation, SDS-PAGE analysis, electroblotting and immunostaining have demonstrated that C activation by myelin leads to the generation of MAC in myelin membranes.

IMMUNE COMPLEXES, ACTIVATION OF COMPLEMENT SYSTEM AND CANCER

N.K. DAY

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Our laboratory has studied both inherited and acquired deficiencies of the complement (C) system since the late 1950's. Dr. Robert Good and his associates were the first to demonstrate that isolated inherited deficiencies of the earlier complement components are associated with serious mesenchymal diseases and recurrent infections. We were also the first to demonstrate that not all C components are linked to the major histocompatability complex. We previously reported that acquired C1 esterase inhibitor deficiency was observed in the serum of a patient with chronic lymphocytic leukemia. A cold-reactive IgM antilymphocyte antibody determined by C-dependent cytotoxicity and indirect immunofluorescence exhibited specificities for both autologous leukemia cells and lymphocytes from normal donors. Studies of an animal model, i.e., leukemia in the cat, resembles closely our patient with CLL, i.e., hypocomplementemia, low C1s inhibitor and high levels of circulating immune complexes. We will present our recent studies using Protein A that induces these cats to undergo remission of their leukemia and lymphoma associated with transient loss of anemia, and development of high levels of C-dependent cytotoxic antibodies with return to normal of their complement system at the time the leukemia undergoes remission. (Funded by grants from ACS #IM-298, NIH #CA-34103 and Oklahoma Medical Research Foundation.)

HETEROGENEITY OF C1 INHIBITOR

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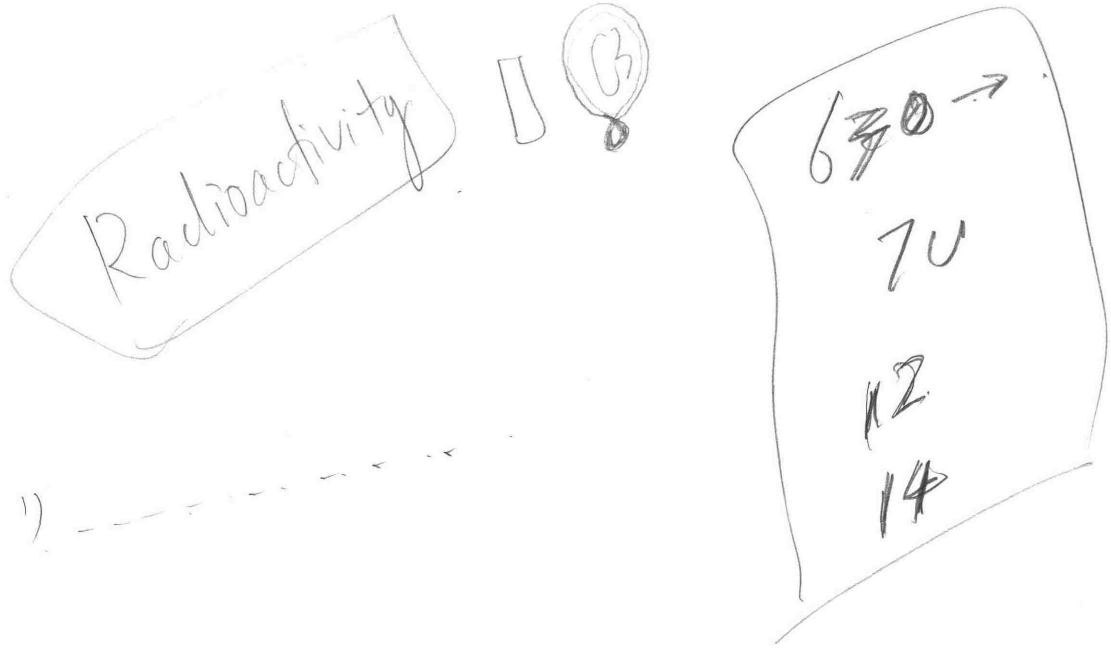
The C1 inhibitor (C1 INH) is a single chain protein of ~100,000 daltons. Upon treatment with CNBR it is cleaved into 3 major fragments of 45K, 25K, 14K and at least 4 minor fragments. One of these is N-terminal as a Met residue is present at position 30. The 45K fragment is second in order; the remaining fragments have not been ordered as yet.

Eight different dysfunctional C1 INH's have been isolated from patients with HANE. One of them (protein Ta) has a 29K instead of 25K CNBR peptide. This genetic insertion or frame shift mutation is due to elongation of the peptide chain rather than to carbohydrate additions. Another (protein We) appears to have a substituted methionine as 2 small CNBR fragments are missing and an 8K fragment, not present in normal C1 INH, appears. The remaining six dysfunctional proteins appear to have mutations in the 45K fragment but this has not been proved.

Dysfunctional proteins have been tested for their ability to inhibit C1s, C1r, plasmin, kallikrein, Factor XIa and Hageman Factor fragments. The mutants vary widely in this inhibitory activity but all fail to inhibit plasmin.

The fractional catabolic rate (FCR) of C1 INH is approximately 2% of the body pool/hr in normal people but 3.5% in patients with HANE. Proteins Ta and At have FCR of 1.25% of the body pool/hr in normals and patients with HANE. Based on calculations of FCR's and synthetic rates it appears that the less than anticipated amount of C1 INH in HANE serum results from increased FCR of a single gene dose of C1 INH synthesis.

The acquisition of C1 INH deficiency in B cell malignancy will be discussed.



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Corrections of the Abstract

Title. The Incidence of C9 Deficiency in Japan

S. Inai and Y. Akagaki

Paragraph 2. The Random Group of Patient

is changed as follows:

2. The Random Group of Patients: Of 49,577 patients, 48 patients were classified as C9D. Therefore, the incidence of C9D in this group was 0.097%. In order to clarify the difference in the incidence of C9D throughout Japan, Japan was divided into eight areas and the incidence of C9D in each area was calculated. The incidences of C9 deficiency in the eight areas were as follows. HOKKAIDO: 0.08%, TOHOKU: 0.14%, KANTO: 0.1%, HOKURIKU, CHUBU: 0.11%, KINKI: 0.06%, CHUGOKU: 0.15%, SHIKOKU: 0.12%, and KYUSHU: 0.06%. Most of the C9D patients in this group had CH50 levels in the normal range, which is between 30 and 50 units. The CH50 level of a patient with C9D was 63.8. From these results it was found that the level of CH50 of people with C9D in the random group of patients was quite different from those of the blood donor group.

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