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Complement-Mediated Acinar Cell Necroses in Pancreatitis Induced by Basement Membrane Antibodies

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Summary. To study the role of the serum complement system in the early necrosis of acinar cells an acute pancreatitis was produced by injection of basement membrane antibodies into the pancreatic duct of mice and rats. In all animals deposition of complement (C3) and antibasement membrane IgG could be observed in an identical position within areas of acinar cell necrosis. The extent of parenchymal damage and the intensity of complement deposits corresponded to the injected dose of antibodies. The importance of cytolytically active complement components (C5–9) was demonstrated in congenitally C5-defective old line mice which did not show typical centrolobular necroses 1 h after intraductal injection of antibodies. However, the normocomplementemic mice developed extensive necroses of acinar cells. These results support the hypothesis of a complement-induced acinar cell necrosis in acute pancreatitis.

Zusammenfassung. Die Rolle des Serumkomplementsystems für die Entwicklung der initialen Acinuszellnekrose bei akuten Pankreatitiden wurde untersucht. Nach intraduktaler Injektion von Basalmembran-Antikörpern entwickelten sich im Verlauf von 10—30 min bei Ratten und Mäusen Azinuszellnekrosen in unmittelbarer Nachbarschaft der an den Basalmembranen darstellbaren Antikörper- und Komplementablagerungen. Das Ausmaß des Parenchymschadens und der Komplementablagerungen stand in direkter Korrelation zu der injizierten Antikörpermenge. Die Bedeutung des zytolytisch aktiven Komplements für die Entwicklung der Nekrosen wurde durch Untersuchungen an Komplement-defekten Mäusen herausgestellt. Während sich bei den genetisch C5-defekten DBA2J- und B10D2 old line-Mäusen keine Azinuszellnekrosen entwickelten, zeigten die mormokomplementämischen C57 Black- und B10D2 new line-Mäuse ausgedehnte Nekrosefelder nach intraduktaler Antikörperapplikation. Die Ergebnisse lassen vermuten, daß zytolytisch aktives Komplement für den initialen Membranschaden der Azinuszelle bei der akuten autodigestiven Pankreatitis verantwortlich ist.

The formal pathogenesis of acute necrotizing pancreatitis begins with an irreversible lesion to the surface or intracellular membranes of the pancreatic acinar cell. It enables the acinar cell to be digested by its own enzymes (tryptic necrosis, Becker, 1957, 1973). Since pancreatic enzymes cannot destroy viable cells, other factors may be responsible for this initial membrane lesion. The only humoral system known up to now which attacks viable cells and induces cytolysis is the complement system. This system is activated by antigen-antibody reactions and also independently of immunologic processes. It can generate cytolytically active complement factors which induce lysis of sensitized and of nonsensitized cells. The possible participation of the complement system in acute pancreatitis which has not been induced by immunological processes has been mentioned in previous papers (Seelig and Seelig, 1975a, b; Seelig et al., 1975). There are,

however, no pertinent studies concerning the significance of the complement system in immunopathogenic pancreatitis. In the present paper the significance of the cytolytically active complement factors in the initial membrane lesion of the acinar cell is investigated by means of a pancreatitis induced by basement membrane antibodies (BM antibodies) in rats and normocomplementemic as well as in complement-defective mice.

Methods

Preparative Methods

a) Antibodies from rabbits against basement membrane antigens of porcine kidney glomeruli (BM antibodies) were used throughout all the animal experiments. Porcine glomeruli were isolated following the method of Spiro (1967) and digested with collagenase as previously described (Seelig et al., 1975). After centrifugation (40,000 g; 2 h) the porcine glomerular basement membrane (P-BM) antigens were isolated from the digested supernatant by immunoadsorption. The immunoadsorbent consisted of 19.6 mg antibody Ig against rat BM (R-BM) coupled to controlled pore glass (CPG 10, 80–120 mesh, Serva) following the method of Jungfer (1975). These antibodies against R-BM were previously made specific for BM by intravital absorption followed by elution of the intravital absorbed antibodies from rat glomerular BM as described previously (Seelig et al., 1975). To remove possible contaminations by porcine serum proteins the collagenase digested P-BM was subjected to an additional absorption with antiporcine serum IgG coupled to CPG (IgG fraction isolated from polyvalent antiporcine serum (Behringwerke) and coupled to CPG). Ten rabbits were immunized with the absorbed P-BM. In each rabbit 100 µg antigen in 50 µl 0.9% NaCl emulsified in 50 µl complete Freund adjuvant was injected into a popliteal lymph node. At weekly intervals 1 mg porcine BM antigen in 1 ml 0.9% NaCl was injected subcutanously at several sites. Animals were exsanguinated 7 days after the 8th injection. The IgG fraction of the sera was isolated (40% $(NH_4)_2SO_4$; chromatography on DEAE-cellulose), and absorbed with insolubilized serum-proteins (for method see Avrameas and Ternynck, 1969), blood cells, and acetone-dried liver powder of rats and mice (for method see Nakamura, 1974).

Antibodies specific for P-BM were obtained from the absorbed material by immunoadsorption of the IgG fraction with CPG to which 50 mg P-BM antigen was cross-linked. The BM antibodies showed no cross reaction with rat serum as tested by agar-gel double diffusion.

b) Rabbit IgG was isolated from pooled normal rabbit serum (40% $(NH_4)_2SO_4$); chromatography on DEAE-cellulose). Absorption with serum proteins, blood cells, and liver powder was performed as mentioned above.

Animal Studies

The experimental animals used were Sprague-Dawley rats (SD-rats) 180 g BW (Versuchstieranstalt Tuttlingen, Fa. Gassner), rats with congenital hypertension (SH rats), 100–180 g BW (Fa. Knoll, Ludwigshafen), DBA2J-mice (C5-defective) and C57BL6J mice (normocomplementemic) 20 g BW (Gl. Bomholtgard, Ry, Denmark), B10D2 old line mice (C5-defective), and B10D2 new line mice (normocomplementemic) 20 g BW (Jackson Laboratory, Bar Harbor, U.S.A.). The animal groups, numbers, and the experimental procedure are presented in Table 1.

In rats and mice the pancreatic duct was cannulated through the muscle layer of the duodenal wall. Following occlusion of the bile duct at the liver hilus the animals were injected with 1.0 ml (rat) or 0.2 ml (mouse) for 5 s under light pressure. Twenty seconds later the duct system was opened.

Blood was obtained for complement (Rapp and Borsos, 1970) and amylase (Ceska, 1970) determinations prior to intraductal injection by venesection in the flank. Before sacrifice, blood was obtained by cardiac puncture.

Table 1. Animal groups, number of experiments performed, and experimental procedure

Species	Intraductal injection	Survival time, number of animals					
		10 min	30 min	60 min	180 min	360 min	48 h
SD rats	0.13 mg anti-BM-IgG	2	2	10	2	2	10
SD rats	4.0 mg anti-BM-IgG	2	2	10	2	2	10
SH rats	0.13 mg anti-BM-IgG	2	2	2	2	2	2
SD rats	5.0 mg rabbit IgG	2	2	2	2	2	2
SD rats	0.9% NaCl	2	2	2	2	2	2
C57BL mice	0.025 mg anti-BM-IgG		5	5	5		
DBA2J mice	0.025 mg anti-BM-IgG		5	5	5		
B10D2 old line mice	0.025 mg anti-BM-IgG			5			
B10D2 new line mice	0.025 mg anti-BM-IgG			5			

Fresh pancreas from rats and mice was fixed in 10% neutral formalin. Paraffin sections were stained with H & E. Immunohistologic examination of pancreas was performed as described previously (Seelig et al., 1975).

Results

A. In vitro and in vivo Reactions of BM Antibodies with Rat and Mouse Pancreas

Rabbit antibodies against porcine glomerular basement membrane were used in all experiments. Following incubation of cryostat sections of rat and mouse pancreas the antibodies could be detected exclusively along the basement membranes of the pancreatic vessels (Fig. 1a). Pure rabbit IgG, which was absorbed in the same way as the antibodies, showed no reaction with the pancreatic tissues of rat and mouse.

B. Histologic and Immunohistologic Findings after Intraductal Injection of BM Antibodies in Rats

The progress of pancreatic lesions was studied from 10 min to 48 h after intraductal injection of 0.13 and 4.0 mg respectively of BM antibodies in normal (SD) and hypertensive (SH) rats (Fig. 2).

In normal rats receiving 0.13 mg of BM antibodies, scattered foci of necrotic acinar cells could be seen in centrolobular areas just after 10 min. Some of the necrotic acinar cells appeared greatly enlarged. After 30 min there was only a minor accumulation of leukocytes within the venules and capillaries while necroses had progressed. Within 60 min there was a further extension of the necrotic areas. A pronounced leukodiapedesis through the walls of venules and capillaries had started, which developed to a diffuse interstitial leukocytosis after 3 h. The necrotic acini were enveloped by polymorphonuclear leukocytes, whereas intact parenchymal cells remained unaffected. The infiltrative inflammatory process had further progressed after 6 h. At this time hemorrhages from ruptured vessels

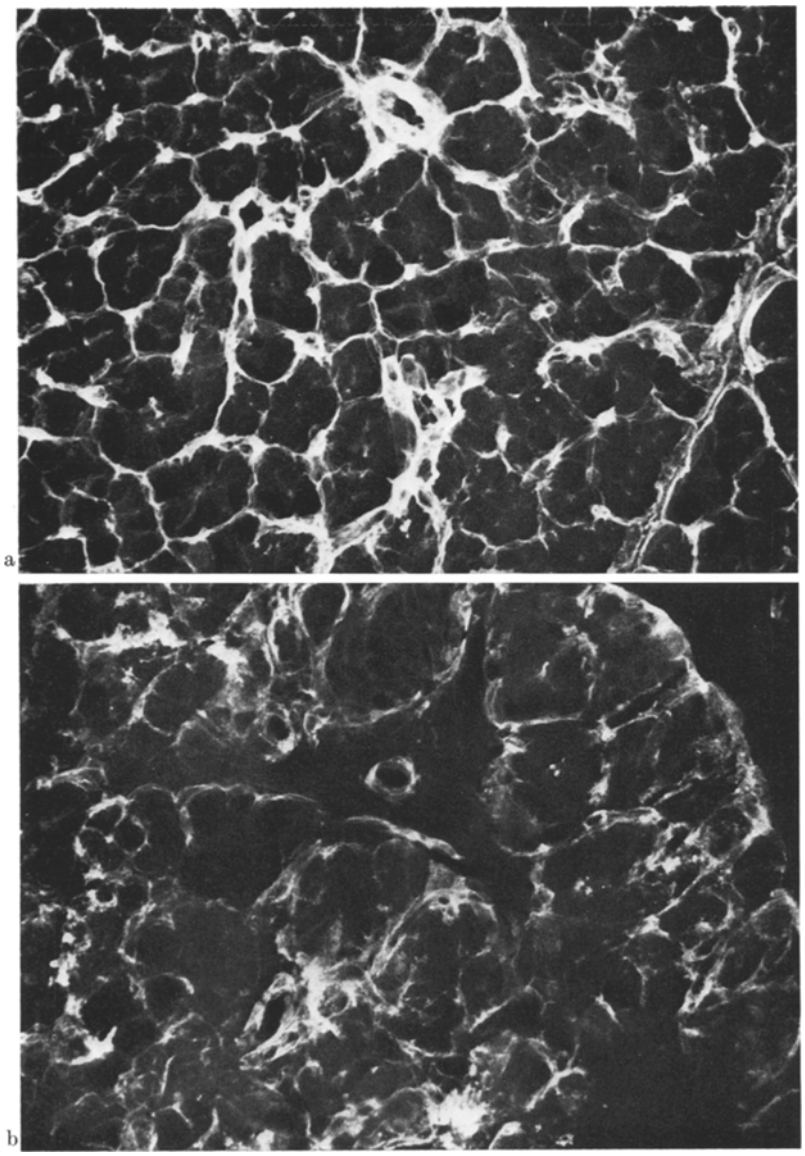

Fig. 1. (a) Rat pancreas, cryostat section. Incubated with 2.0 mg/ml antiporcine BM-IgG from rabbit, FITC labeled. Deposits of basement membrane antibodies along pancreatic vessels. ×125. (b) Rat pancreas 10 min after intraductal injection of 0.13 mg antiporcine BM-IgG. Deposition of antibodies at basement membrane of capillaries in focally varying degree. Deposits of complement (C3) show identical patterns. Cryostat section, antirabbit IgG, FITC labeled, ×125

infiltrated with leukocytes were also to be seen. After 48 h there was a pronounced mesenchymal reaction with abundant fibroblasts, newly formed connective tissue fibers, macrophages, and eosinophil leukocytes in the former necrotic regions.

Animals receiving 4.0 mg of BM antibodies in general developed a similar morphologic picture; the acinar cell necroses, however, were more extended and pronounced leukocytic infiltration and vessel ruptures with hemorrhages developed just within 60 min.

In hypertensive rats (SH) with advanced arteriosclerosis of the pancreatic vessels (blood pressure 180–200 mmHg) 0.13 mg of BM antibodies were sufficient to produce lesions corresponding to 4.0 mg of antibodies in normotensive SD rats. In addition these hypertensive animals developed more severe hemorrhages compared to normal animals.

The immunohistologically demonstrable immunoglobulin deposits showed roughly the same pattern in all animal groups up to 6 h following intraductal injection (Fig. 1 b). Some of the immunoglobulin deposits on the capillary basal membrane were focal while others extended over whole lobes. Complement deposits (C3) were seen in all animals in a pattern corresponding to the immunoglobulin deposits. Necrosis of acinar cells only occurred at sites of immunoglobulin and complement deposits.

SD rats receiving an intraductal injection of 5 mg pure rabbit IgG in 1.0 ml NaCl or 1.0 ml 0.9% NaCl served as controls. In these animals acinar cell necrosis or leukocytic infiltration could not be detected. The initial edema and hyperemia had usually disappeared after 6 h and the lobules appeared relatively compact. The deposition of rabbit IgG resembled the BM-antibody pattern, however, the deposits appeared more granular in form. In the course of 6 h, the rabbit immunoglobulins were largely resorbed and could only be demonstrated occasionally on the periphery of the lobules. Complement deposits could not be detected immunohistologically in these animals.

C. Histologic Findings after Intraductal Injection of BM Antibodies in Normocomplementemic and C5-Defective Mice

To evaluate the role of the complement system in the inflammatory process evoked by BM antibodies, an acute pancreatitis was induced in normocomplementemic mice and compared with mice genetically lacking the 5th component of complement essential for building the cytolytically active complex C5–C9. Normocomplementemic C57 BL 6J mice were intraductally injected with 0.025 mg BM antibodies. An interstitial edema and small centrolobular single cell necroses near hyperemic veins were found 10 min after intraductal injection. After 30 min centrolobular necrotic areas developed. These progressed to subtotal lobular necroses after 1 h. The periacinar edema increased and leukocytosis was observed in the vessel lumina. A pronounced intravascular and interstitial leukocytic infiltration occurred within 3 h. In contrast to these normocomplementemic animals C5-defective DBA2J mice receiving the same doses of BM antibodies showed no typical centrolobular necroses of acinar cells 30 min after intraductal injection. A moderate intravascular leukocytosis was found 1 h after the injection.

To exclude that this observation was rather the result of genetically different strains of experimental animals than the effect of a complement defect we repeated these experiments with B10D2 old and new line mice. These two strains are genetically identical and differ only in a C5 defect in the old line mice. Extensive necrosis of acinar cells radiating from the lobule center developed within 1 h

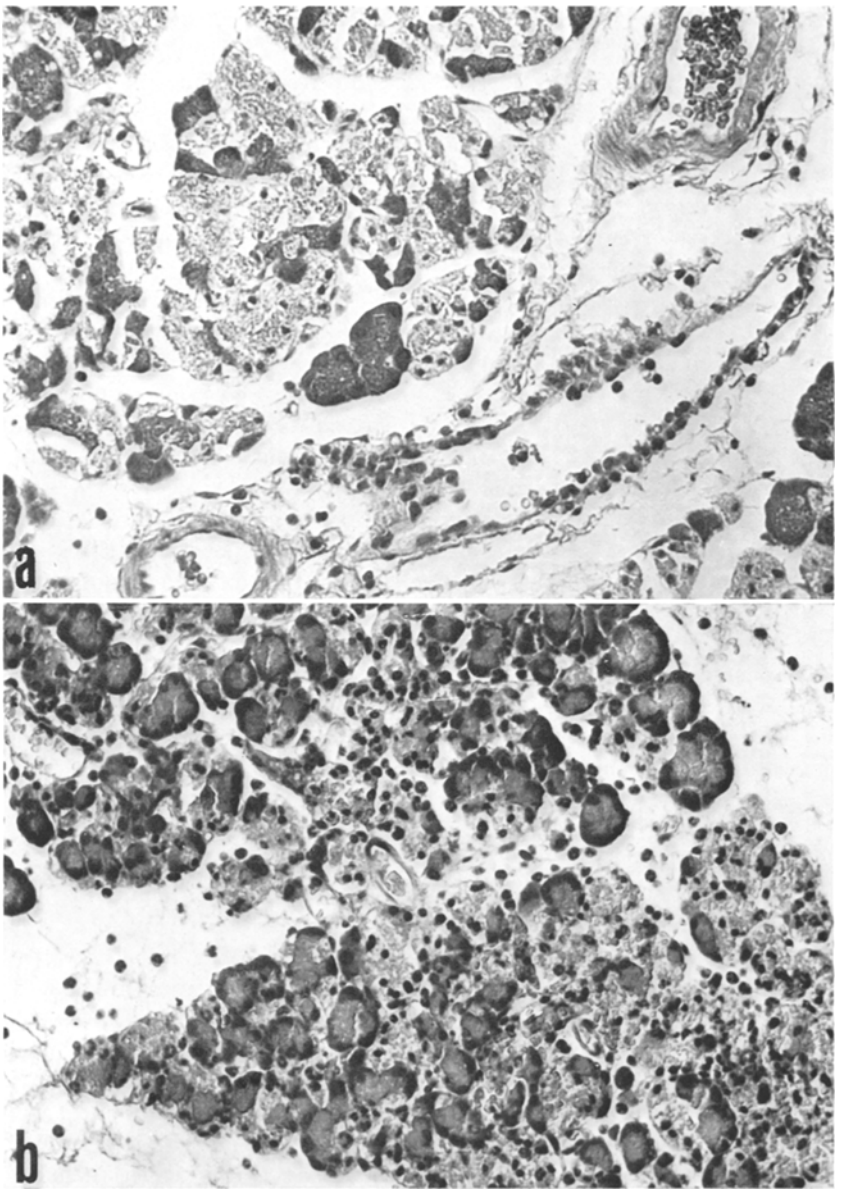

Fig. 2a—c. Rat pancreas after intraductal injection of 0.13 mg antiporcine BM-IgG. (a) 30 min after injection; pronounced acinar cell necroses and incipient leukodiapedesis. (b) 180 min after injection; infiltration of necrotic areas with polymorphonuclear leukocytes. (c) 48 h after injection; marked mesenchymal reaction: fibroblastic cells and eosinophilic leukocytes in filtrating the newly forming fibroses. Formalin, paraffin, H & E. ×125

after intraductal administration of 0.025 BM antibody in normocomplementemic new line mice. There was intra- and perivascular leukocytosis. Septal hemorrhages occurred occasionally. On the other hand, typical centrolobular necroses could not be shown in any of the C5-defective animals. In contrast the intra- and peri-

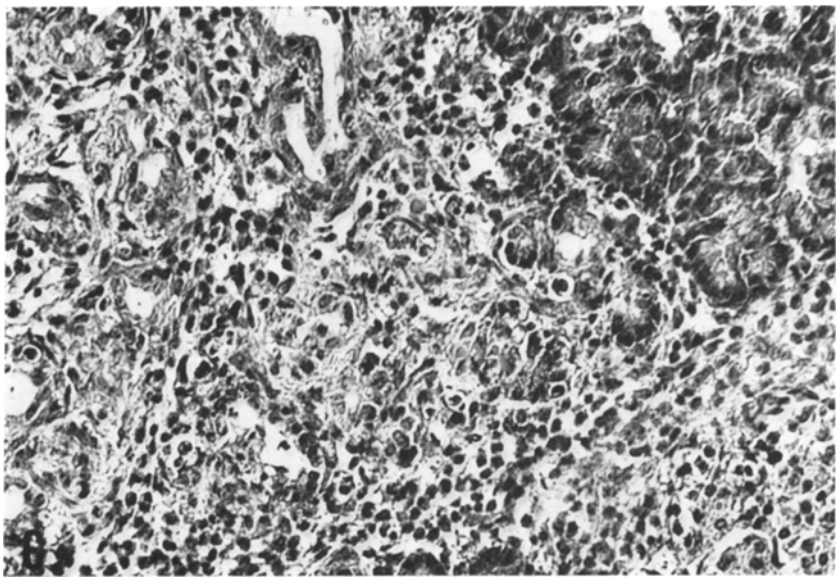

Fig. 2c

vascular leukocytosis appeared to be only slightly less pronounced compared to the genetically identical normocomplementemic animals.

E. Serum Complement and Amylase in Rats

One hour after intraductal injection of BM antibodies, the total hemolytic activity of the serum complement fell to an average of 64% of the preoperative values. In contrast control animals injected intraductally with rabbit-IgG or 0.9% NaCl still had 90% of their preoperative complement activity after 1 h. A rise in amylase activity did not relate to the histologically demonstrable damage of the parenchyma and occurred in control animals as well. This may be attributed to luxation of the duodenal loop and retrograde instillation of pancreatic juice into the interstitial tissue.

Discussion

Acute necrotizing pancreatitis can be induced by intraductal injection of antibodies to vascular basement membranes. The initial lesions seen by light microscopy within 10–30 min are centrolobularly localized acinar cell necroses which lie in the areas of immunohistologically observed immunoglobulin and complement depositions. The extent of the necroses correspond to the amount of injected antibodies. In every case the necrosis precedes the infiltration of polymorphonuclear leukocytes, which starts after 30–60 min and reaches a maximum within 3–6 h. Within 48 h, resolution and early fibrosis with infiltrations of mononuclear cells develop.

The cause of parenchymal damage in these forms of immunopathogenic pancreatitis has to be regarded as a consequence of the antigen-antibody reaction, which leads to an activation of the complement system with resultant release of

biologically active mediators of inflammation and formation of cytolytically active complement factors. Leukotactic factors from C3 and C5 and the powerful leukotactic factor C567 are released at the same time (Cochrane, 1968; Bokisch et al., 1969, Müller-Eberhard et al., 1972). The effect of these leukotactic factors can be discerned under the light microscope only after a latency period. Intravascular leukocytosis, leukocyte-emigration, and destruction of the vessel wall (intracellular substance) by leukocyte enzymes (Cochrane, 1968) begins after about 60 min.

The acinar cell necrosis which was earlier demonstrated by light microscopy may possibly be induced by the direct attack of cytolytically active complement factors from the components C5–C9 on the membrane of the acinar cell. This cell damage is a complement-mediated lysis of non sensitized acinar cells since the antibody reacts selectively with the basal membrane of the vessels. The complement system is activated on the basal membrane by the antigen-antibody reaction. Cytolytically active complement factors must be transferred from the basal membrane to the pancreatic acinar cell. The acinar cell necrosis in the direct vicinity of the complement activation might be explained as the result of mechanisms resembling the phenomena of "deviated lysis" (Rother et al., 1974) or "reactive lysis" (Lachman and Thompson, 1970; Goldman et al., 1972).

The role of the cytolytically active complement factors C5–C9 in induction of acinar cell necroses is emphasized by the data from C5-defective mice. The two genetically identical inbred strains of the B10D2 old line and new line mice only differ in a C5 defect in the old line mice. One hour after intraductal injection of basal membrane antibodies there were no necroses in the C5-defective old line mice while extensive acinar cell necroses appear in the C5 normal new line animals.

The initial acinar cell necrosis of this form of immunologically induced pancreatitis does not differ in its morphologic appearance from the necroses induced by nonimmunologic mechanisms. In 1964 Doerr already pointed to the identical picture of acinar cell damage induced by a variety of substances such as Na-taurocholate, olive oil, or after induction of a Popper mechanism.

Despite different etiologic factors a common formal pathogenic mechanism may lead to initial membrane lesion of the acinar cell. We believe this common mechanism to be the cytolytically active complement that can be generated by immunologic as well as by various nonimmunologic reactions. For example the nonimmunologic activation of complement in the mechanical edema of pancreas by intraductal instillation of a complement activating protein (cobra venum factor; Seelig et al., 1975) leads to an acute pancreatitis with acinar cell necroses and leukocytosis.

References

Avrameas, S., Ternynck, Th.: The cross linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. Immunochemistry **6**, 53 (1969)

Becker, V.: Sekretionsstudien am Pankreas. Stuttgart: Thieme 1957

Becker, V.: Bauchspeicheldrüse. In: Doerr, W., Seifert, G., Uehlinger, E.: Spezielle pathologische Anatomie, Bd. 6. Berlin-Heidelberg-New York: Springer 1973

Bokisch, V. A., Müller-Eberhard, H. J., Cochrane, Ch. G.: Isolation of a fragment (C3a) of the third component of human complement containing anaphylatoxin and chemotactic activity and description of an anaphylatoxin inactivator. J. exp. Med. **129**, 1109 (1969)

Ceska: In: Bergmeyer, U. (Hersg.), Methoden der enzymatischen Analyse, Bd. 1, S. 928. Weinheim: Verlag Chemie 1974

Cochrane, C.: Immunologic tissue injury mediated by neutrophilic leukocytes. Advanc. Immunol. **9**, 97 (1968)

Doerr, W.: Pathogenese der akuten und chronischen Pankreatitis. Verh. dtsch. Ges. inn. Med. **70**, 718 (1964)

Goldman, J. N., Ruddy, S., Austen, K. F.: Reaction mechanism of nascent C567. Reaction characteristics for production of EC567 and lysis by C8 and C9. J. Immunol. **109**, 353 (1972)

Jungfer, H.: Immunoadsorption with protein-glass derivatives. Ärztl. Lab. **21**, 80 (1975)

Kolb, W. P., Müller-Eberhard, H. J.: The membrane attack mechanism of complement. Isolation and subunit composition of the C5b-9 complex. J. exp. Med. **141**, 724 (1975)

Lachman, P. J., Thompson, R. A.: Reactive lysis: The complement mediated lysis of unsensitized cells. II. The characterization of activated reactor as C56 and the participation of C8 and C9. J. exp. Med. **131**, 643 (1970)

Mayer, M. M.: Mechanism of cytolysis by complement. Proc. nat. Acad. Sci. (Wash.) **69**, 2954 (1972)

Müller-Eberhard, H. J.: Patterns of complement activation. Progress in Immunology II. **1**, 173 (1974)

Müller-Eberhard, H. J., Vallota, E. H., Götze, O., Zimmerman, T. S.: Mediators to inflammatory response complement. In: Lepow, I. H. and Ward, P. A., eds., Inflammation mechanism and control, p. 83. New York, London: Academic Press 1972

Nakamura, R. H.: Immunopathology. Clinical laboratory concepts and methods. Boston: Little, Brown and Company 1974

Polley, M. J., Müller-Eberhard, H. J., Feldmann, J. D.: Production of ultrastructural membrane lesions by the fifth component of complement. J. exp. Med. **133**, 53 (1971)

Rapp, H. J., Borsos, T.: Molecular basis of complement action. New York: Appleton-Century Crofts Meredith Corporation 1970

Rother, U., Hänsch, G., Menzel, J., Rother, K.: Deviated lysis: Transfer of complement lytic activity to unsensitized cells. I Generation of a transferable activity on the surface of complement resistant bacteria. Z. Immunforsch. **148**, 172 (1974)

Seelig, H. P., Seelig, R., Drescher, M.: Der Einfluß der Autolyse auf die immunhistologische Darstellbarkeit gewebegebundener Immunglobuline in der Niere. Virchows Arch. Abt. A **365**, 151 (1975)

Seelig, H. P., Seelig, R., Roth, E., Roth, E.: Antibodies reacting with the glomerular mesangium. Isolation and Immunopathology. Virchows Arch. Abt. A **366**, 313 (1975)

Seelig, R., Ehemann, V., Tschahargane, C., Seelig, H. P.: The serum complement system—a mediator of acute pancreatitis. Virchows Arch. Abt. A **365**, 193 (1975)

Seelig, R., Seelig, H. P.: The possible role of serum complement system in the formal pathogenesis of acute pancreatitis. I Immunopathogenic pancreatitis-local Shwartzman-Sanarelli phenomenon-endotoxin induced pancreatitis. Acta hepatogastroenterol. **22**, 223 (1975)

Seelig, R., Seelig, H. P.: The possible role of serum complement system in the formal pathogenesis of acute pancreatitits. II Cobra venom factor and Na-taurocholate induced pancreatitis. Acta hepato-gastroenterol. **22**, 335 (1975)

Spiro, R. G.: Studies on the renal glomerular basement membrane. Preparation and chemical composition. J. biol. Chem. **242**, 1915 (1967)

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