Immunology

Are complement lysis and lymphocytotoxicity analogous?

from P.J. Lachmann

THE most dramatic effect of the complement system is its ability to cause lysis of red cells and of a variety of other cell types extending from viruses and bacteria to platelets and nucleated mammalian cells. The mechanism of this lytic action has proved a fascinating subject. Particularly intriguing is the possibility that a similar mechanism is involved in lymphocytotoxicity. Several recent studies throw light on the relationship between the two systems.

Very early on it was discovered that the final events in complement lysis were independent of the presence of divalent cations and that following addition of all the complement components lysis could still be prevented by lowering the temperature or by adding such agents as heparin or concentrated EDTA[1]. The existence of this so-called 'S*' stage prompted the idea that target cells participate in their own lysis in some active way, but this notion was disproved by the observation that complement lysis of liposomes can be brought about in a highly simplified system using only the terminal complement components from C5 onwards[2,3]. Using this system it also rapidly became clear that complement lysis is not accompanied by an enzymatic action on the target cell[3,4].

A new dimension was added to the study of complement lysis in 1964 when Borsos, Dourmashkin and Humphrey[5] first demonstrated under the electron microscope the 'lesions' that typically accompany complement lysis. These ring-like structures are around 10 nm in internal diameter with a wall thickness of about 1–2 nm and appear only when all the complement components have reacted. They can be used to identify complement lysis with certainty. The lesions were initially believed to represent holes through the membrane, an interpretation which was subsequently denied when it was shown that the functional complement 'hole' measured by molecular sieving experiments was substantially smaller than the 10 nm of the structural lesion. More recently, however, spectacular freeze-electron micrographs[6] have shown beyond reasonable doubt that while the bulk of the lesion is a funnel-shaped projection above the outer lamella of the membrane, a corresponding defect in the inner lamella is found wherever such a funnel occurs and that the centre of the lesions do constitute a 'hole' through the membrane.

For some time there has been general agreement that complement lysis is due to the insertion of a 'plug' into the membrane, causing it to leak. There has, however, been surprisingly heated debate about the details of the process. Mayer[7] and his group have long propounded the 'doughnut' hypothesis which states that the plug is hollow and inserts a protein-lined transmembrane channel into the membrane; on the other hand, Muller-Eberhard and the La Jolla group put forward the view that the plug is solid and causes lysis by disturbing the lipid bilayer, leakage therefore occurring around the plug rather than through it and through a lipid region rather than through a protein-lined channel — this is called the 'leaky patch' model[8,9].

Within the last two years, however, the La Jolla group has undergone a dramatic conversion. This followed their observation that C9 alone, if left for prolonged periods or heated, can undergo polymerization and form structures which closely resemble the complement lesions seen on electron microscopy[10]. They are now convinced that this tubular complex of polymerized C9 does indeed form a hollow plug which can cause the membrane to leak[11]. It had previously been thought that the electron-microscopic (EM) lesion consisted of a dimer of the C5–C9 complex with dimerization accounting for the S* step[12]. Now it has been claimed that the lesion seen in the electron microscope is wholly poly C9, the C5–C8 moiety not being visualized in the usual conditions of negative staining. The function of C5–8 is seen as that of polymerizing agent for C9[11].

It may therefore be concluded with fair confidence that the doughnut hypothesis is essentially correct and that the mechanism of complement lysis is the introduction of a protein-lined channel stably into the membrane. Estimates of the extent of C9 polymerization needed to form a complete lesion vary, the most recent estimate[13] being that between 12 and 18 C9 subunits make up the poly C9 cylinder and are associated with one or two C5–8 complexes.

Some observations still require explanation, however. One is the claim that a single molecule of C9 can give rise to a channel large enough to allow sucrose to pass and which should therefore lyse the cell[14]. Another is that complement lysis will proceed, albeit more slowly, in the absence of C9. This has long been known from experimental studies but it has been emphasized more recently by the discovery of individuals who are homozygous for C9 deficiency, yet who have significant plasma lytic activity and seem to suffer no obvious defect in their complement-mediated functions[15]. C9 deficiency occurs in about 1 in 10,000 Japanese blood donors[30], suggesting that the selective pressure against it is, at best, weak. One must therefore conclude that there is a method of producing cell lysis additional to that provided by poly C9 and it is perhaps not implausible — though there is no proof — that this may be a 'leaky patch' produced by insertion of C5–8.

The idea that a process analogous to complement lysis is involved in cell-mediated cytotoxicity has been considered for some years. Thus there is evidence that lymphocyte cytotoxicity shows a one-hit dose response, as does complement lysis[16]. Extracellular macromolecules inhibit T-cell lysis just as they do complement lysis and by molecular sieving techniques the ADCC lesion seems to be larger than the complement lesion[17]. Moreover, lymphocytotoxicity has a step equivalent to S*, where target cells that have received a lethal hit from killer lymphocytes may be prevented from lysing by chilling or heparin[18].

Early work attempting to demonstrate the involvement of the serum complement components themselves in lymphocyte killing by showing inhibition of lymphocytotoxicity by antibodies against complement gave negative results[19]. Claims to the contrary have been made more recently[20], but there is so far no convincing evidence that antibodies that react with serum complement components are capable of inhibiting lymphocyte lysis. Furthermore, antibody-dependent cytotoxic reactions have been shown to be normal in patients with deficiencies of the terminal complement components (for example, ref. 21). If, therefore, there is an analogue of the complement system in lymphocytes it presumably involves not the terminal components themselves but an analogous set of proteins possibly evolved by gene duplication from the terminal complement components[22].

In accord with this idea are EM studies of lymphocyte lysis by Dourmashkin *et al.*[23] and by the Henkarts[24] which show lesions that bear a strong resemblance to the complement lesion but are clearly not identical with it, being, for example, larger. Podack and Dennert have more recently provided further support for this view in experiments using both a cloned natural killer (NK) cell line[25] and a cloned cytotoxic T-cell line[26] as killers. They find EM lesions that are essentially similar to those described by Dourmashkin and by the Henkarts although they describe lesions of two distinct sizes with internal diameters of 16 nm and 5 nm in contrast to the 10 nm internal diameter of poly C9. They suggest that the precursor subunits of the tubular structures, for which they propose the names perforins 1 and 2, are produced from the dense granules of the NK cells and are assembled into their polymers (poly-perforins 1 and 2) at the killer cell–target cell interface and incorporated into the target cell by membrane fusion. This explanation is wholly plausible, but the processes involved need to be worked out by

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other than morphological techniques.

The evidence is therefore growing that there is indeed a plug-insertion mechanism analogous to complement lysis involved in lymphocyte killing. This is presumably distinct from the oxygen-radical killing mechanisms largely used by myeloid cells and it remains to be seen whether individual cell types always use only one or other of these methods or whether they may use both. Oxygen-radical killing is characteristically accompanied by chemiluminescence and can be inhibited by superoxide dismutase or catalase or strict anaerobiosis. There is fairly general agreement that these procedures do not inhibit T-cell killing. It has been claimed, however, that NK cells do kill by an oxygen metabolite mechanism[27], that they are of myeloid origin[28] and that their killing mechanism is distinct from that of cytotoxic T cells[29]. This is not immediately compatible with the view that they are 'plug inserters' and give identical lesions to those produced by cytotoxic T cells. This conflict remains to be resolved. □

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Virology

Molecular biology of the coronaviruses

from Brian W.J. Mahy

THE rapid advances being made in understanding the molecular biology of the coronaviruses took centre stage at a recent international workshop* on these viruses, overshadowing studies of the pathogenesis of the diseases they cause: respiratory infections and colds in man, and numerous acute and chronic diseases in animals. Of particular interest were studies revealing novel mechanisms employed by coronaviruses for the synthesis of glycoproteins and mRNA. These studies have concentrated mainly on mouse hepatitis virus (MHV) and avian infectious bronchitis virus (IBV).

Glycoproteins

Coronaviruses contain two envelope glycoproteins, termed E_1 and E_2 in MHV. E_1, the matrix protein, is a transmembrane glycoprotein with its N-terminus exterior to the envelope and its C-terminus associated internally with the nucleocapsid. E_2 or spike protein which forms the peplomers on the virion surface attaches to host cells and elicits neutralizing antibodies during infection. Virions are formed by budding into intracytoplasmic vesicles from membranes of the rough endoplasmic reticulum and the Golgi apparatus, and not from the plasma membrane where most enveloped viruses bud. Coronaviruses thus provide a unique opportunity to study protein structural features which influence the site of budding.

Budding is determined by restricted intracellular migration of the E_1 membrane glycoprotein; incorporation of E_2 is a late event, not necessary for budding to occur (K. Holmes, NIH; H. Neimann, Justus-Liebig Universität, Giessen). The carbohydrate moiety of E_1 is O-glycosydically linked in murine and bovine coronaviruses; in the presence of tunicamycin (which blocks the N-linked glycosylation of E_2), E_1 continues to be synthesized and spikeless particles bud intracellularly. O-glycosylation of E_1 occurs late, in the Golgi apparatus, and is not essential for virus maturation. Niemann reported that addition of the glycoprotein transport inhibitor monensin to murine coronavirus-infected cells blocked glycosylation of E_1 but allowed accumulation of enveloped virions in the endoplasmic reticulum. Similar results were reported for human coronavirus 229E (M.C. Kemp, University of Colorado). Glycosylation of E_1 is therefore not co-translational, in contrast with E_2 which is formed by synchronous membrane insertion and glycosylation of the nascent polypeptide chain, involving recognition and cleavage of an N-terminal signal sequence.

E_1 has been synthesized *in vitro* (P. Rottier, University of Utrecht) by translation of MHV mRNA in the presence of dog pancreatic microsomes, and the disposition of the protein in the membrane determined by accessibility to proteases and selective N-terminal labelling. The bulk of the E_1 protein is buried in the membrane, with only small portions from the N- and C-termini expressed in the lumenal and cytoplasmic domains respectively. Addition of microsomes at different times after synthesis shows that the protein can enter the membrane at any stage during synthesis of the first 150 amino-acid residues. O-glycosylation does not occur *in vitro,* and there is no evidence for a cleavable signal sequence. The nucleotide sequence of the E_1 gene (J. Armstrong, EMBL, Heidelberg) confirms the strongly hydrophobic nature of the protein, and the N-terminal sequence (Met-Ser-Ser-Thr-Thr-Glu . . . . .) reveals four potential O-glycosylation sites. From a preliminary sequence of the matrix protein gene of IBV (M. Boursnell and T. Brown, Poultry Research Station, Houghton), the avian virus protein appears similarly hydrophobic, but the sequence predicts no serine or threonine residues near the N-terminus. In agreement with this, IBV matrix protein has been found to have N- rather than O-linked carbohydrate side chains (D. Stern, MIT and D. Cavanagh, Poultry Research Station, Houghton). Further comparative studies of coronaviruses would be of interest in this respect, but clearly the existence of this unusual O-linked glycoprotein in murine and bovine Coronaviridae does not provide a hallmark for all members of the family.

The availability of cloned DNA copies of the E_1 protein gene has opened the way for site-directed mutagenesis which may soon reveal, at least *in vitro,* which features of the protein govern its interaction with membranes of various cellular compartments.

Generation of coronavirus mRNAs

The coronavirus genome is a linear single-stranded RNA molecule about 20 kilobases (kb) long which is polyadenylated at the 3′ end, capped at the 5′end and infectious. During replication, mRNA of genome length is synthesized together with six subgenomic mRNA molecules which range in size from 2 to 10 kb. Previous T_1-oligonucleotide mapping studies showed that these mRNAs form a 3′-co-terminal nested

*The second international workshop on coronaviruses, organized by P. Rottier, B. van der Zeijst, W. Spaan and M. Horzinek, was sponsored by EMBO and held in Zeist, The Netherlands, 8–10 June 1983.

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