antigen subunits common to Mca_1 and A_2 are to be found in the glycoproteic allotypes already identified in other species of ruminants (goat and buffalo) and to carry out their physicochemical analysis, but already the present results are interesting in that they provide meaningful experimental evidence to support the general project (the monitoring of phylogenetic relationships by cross-reacting allotypes) under study.

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Destruction of virus infected cells by neutrophils and complement[1]

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Summary. The paper describes an antibody independent mechanism of cytotoxicity whereby virus infected but not uninfected cells are destroyed by the combined presence of neutrophils and complement.

The quest to understand immunity to viruses by studying in vitro models has revealed a diverse array of mechanisms by which virus infected cells may be destroyed. These mechanisms include antibody and complement lysis[3], T-cell-mediated cytotoxicity[4], antibody dependent cellular cytotoxicity (ADCC)[5], complement facilitated ADCC[6], activated macrophages[7,8], natural killer cells[9,10], and mitogen induced cellular cytotoxicity[11,12]. The present report adds another mechanism, namely complement dependent neutrophil mediated cytotoxicity (CDNC). Since this mechanism is nonimmune, it could represent an important early defense mechanism against virus infections.

Materials and methods. Bovine PMN and macrophages were isolated from the mammary gland and lymphocytes from the peripheral blood by methods described in detail elsewhere[13]. The PMN were 98–99% pure and were contaminated by macrophages. 4 different donor animals were used; 2 of which had antibodies (A and B) to infectious bovine rhinotracheitis virus (IBR) and 2 without (C and D). The methods used to detect antibody were virus neutralization and an antibody dependent cell cytotoxicity assay. Both of these methods were described previously[14]. The rabbit complement source was also checked for anti-IBR antibody. None of the 4 animal donors contained detectable antibodies to herpes simplex virus type 1 (HSV) or vescicular stomatitis virus (VSV).

Cytotoxicity assays. The methods used for performing cytotoxicities have been described previously[15]. Briefly Georgia Bovine Kidney cells (GBK) were infected 20 h prior to assay at a multiplicity of infection of 1 with IBR virus. These cells were labelled with $Na_2{}^{51}CrO_4$ and served as targets. Uninfected GBK cells served as control targets. In some experiments, GBK cells infected with HSV or VSV were used as target cells. Rabbit complement (ICN Pharmaceuticals, Cleveland) was freshly reconstituted in Hank's balanced salt solution and dilutions were made to give final complement concentrations of 1/50, 1/70 and 1/100 in the cytotoxicity assays. Heat inactivated (56 °C 30 min) complement was used in control assays. All assays were run for 8 h at 37 °C and were carried out in quadruplicate in microtiter plates[13]. At the end of the incubation period, 50% of the contents were harvested to compute the percent specific release values according to the following formula.

$$\frac{(\text{PMN}+\text{C})-(\text{C}+\text{medium})}{\text{Triton releasible}-(\text{C}+\text{medium})}\times 100$$

The medium used in the cytotoxicity assays was RPMI 1640 containing 10% heat-inactivated foetal calf serum. This serum source was free of antibodies to IBR, HSV, or VSV viruses. Treatment of PMN to exclude cytophilic antibody. PMN from antibody free donors were preincubated in serum free medium (RPMI 1640) at 37 °C for 30 min as described by Goldstein et al.[16], pretreated at pH 4.0 at 0 °C for 1 min[18] or pretreated with 1% trypsin for 30 min at 37 °C[17] to remove putative cytophilic antibody. After treatment, the PMN were washed 3 times prior to use in the cytotoxicity assays.

Results and discussion. Bovine mammary neutrophils (PMN), were incubated with uninfected or virus infected cells both in the presence and absence of rabbit complement. The results, shown in table 1, indicate that bovine kidney cells, upon infection by IBR virus became susceptible to destruction by PMN and complement. Low levels of cytotoxicity in the absence of PMN was sometimes observed at the highest concentrations of complement used, but PMN alone exerted no appreciable cytotoxic activity (always less than 1% specific lysis). The bovine kidney cells showed increasing susceptibility to CDNC with time after infection (data not shown). Other leukocyte types were also tested for their ability to cooperate with C to lyse virus infected target cells. Peripheral blood lymphocytes, depleted of adherent cells, were ineffective but mammary gland macrophages gave low levels to CDNC that varied between 20 and 50% of that expressed by PMN.

Since IBR virus is a common viral pathogen of the bovine species, the possibility had to be considered that the cytotoxicity was attributable to cytophilic anti-IBR antibody adhering to the PMN, or present in the foetal calf serum (FCS), or complement source giving ADCC or complement facilitated ADCC[6, 19]. These possibilities were considered

Table 1. Cytotoxicity by neutrophils and complement against IBR virus infected bovine kidney cells[a]

Reactant	Percent specific lysis IBR GBK[a]			GBK		
	Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3
PMN 100:1	0.2	0.5	0.2	0.2	0.2	0.2
PMN 100:1 + C 1/50	35	30	31	0.4	1.2	0.2
PMN 100:1 + C 1/70	28	24	23	0.2	0.1	0.1
PMN 50:1 + C 1/70	12	16	11	0	0.1	0.2
PMN 25:1 + C 1/70	5	8	7	0.1	0.1	0
PMN 100:1 + C 1/100	18	17	18	0	0.1	0.2
PMN 100:1 + H 1[b] C 1/50	0.2	0.3	0.1	0.1	0.2	0.1
Media + C 1/50	5	6	3.5	1.0	1.2	0.1
Media + C 1/70	1	1.1	1.5	0	0	0.1
Media + C 1/100	0.1	0.2	0.2	0	0	0

[a] Bovine kidney cell targets infected with IBR virus and labelled with Na_2 $^{51}CrO4$. [b] Heat inactivated complement.

Table 2. The cytotoxicity of bovine mammary gland PMN and complement against IBR virus targets does not involve cytophilic antibody[a]

PMN donor No.	Immune status of donor		FCS batch	Percent Specific Lysis No pretreatment		Preincubated 37 °C, 30 min		Preincubated pH 4.0, 1 min 0 °C	
	Virus serum neutralizing Ab titer	Serum ADCC activity		100:1	50:1	100:1	50:1	100:1	50:1
16	1:128	> 1:100	A	30.0	21.0				
			B	32.0	20.0	34.0	20.0	31.0	18.0
			C	31.0	19.2				
			D	ND	ND				
1	1:32	> 1:100	A	33.9	19.2				
			B	28.6	19.0	30.0	18.6	28.0	18.0
			C	31.0	18.0				
			D	ND	ND				
3	< 1:2	< 1:5	A Exp. I	13.8	12.0				
			Exp. II	25.5	18.9		Not done (ND)		
			B Exp. I	15.0	8.0				
			Exp. II	25.5	21.0				
			C	ND	ND				
			D Exp. I	16.0	9.0				
			Exp. II	26.0	20.0				
26	< 1:2	< 1:5	A	32.5	22.0				
			B	31.0	21.0	29.0	20.6	30.0	20.0
			C	32.5	23.5				
			D	32.0	26.0				

[a] In experiments not shown pretreatment of PMN with 1% trypsin failed to destroy the CDNC activity.

Table 3. Cytotoxicity by PMN[a] and complement against different virus infected cells and uninfected cells

C Dilution	Experiment No.	^{51}Cr specific release[d] (%)							
		Uninfected GBK		IBR-GBK		HSV-GBK[b]		VSV-GBK[c]	
		C	PMN + C	C	PMN + C	C	PMN + C	C	PMN + C
1:50	1	2.2	2.2	5.0	61.6	18.9	52.6	20.4	20.7
	2	1.8	3.4	15.9	72.6	21.0	62.5	26.0	53.5
	3	0.1	2.0	6.0	40.5	8.0	30.0	9.0	20.5
	4	0.0	2.2	6.8	38.0	10.0	30.0	11.0	31.0
	5	0.7	3.6	16.5	45.0	ND	ND	ND	ND
1:70	1	0.0	0.4	1.6	46.3	10.0	37.6	8.8	9.0
	2	0.6	1.4	6.0	58.0	7.9	44.9	9.1	36.9
	3	0.0	0.5	4.2	26.0	ND	ND	ND	ND
	4	0.0	0.4	3.8	21.5	ND	ND	ND	ND
	5	3.1	2.5	13.3	46.0	ND	ND	ND	ND
1:100	1	0.0	1.2	0.0	26.0	1.0	23.0	1.5	4.9
	2	0.0	0.8	1.25	38.0	1.3	32.0	2.0	21.0
	3	0.0	0.4	1.50	13.0	1.0	33.0	2.5	18.0
	4	0.0	0.4	1.2	12.0	0.5	30.0	1.8	23.0
	5	1.2	1.5	4.6	12.6	ND	ND	ND	ND

[a] The PMN were from animals that lacked detectable antibody to all three viruses. [b] Bovine kidney cells infected with herpes simplex type 1. [c] Bovine kidney cells infected with vescicular stomatitis virus. [d] Computed by the following formula.

unlikely since PMN from animals judged free of IBR exposure (and antibody negative as measured by virus neutralization and ADCC), still expressed CDNC and, in fact, comparable degrees of cytotoxicity were observed with PMN from antibody positive and antibody negative animals (table 2). Furthermore, CDNC was apparent with 4 separate batches of FCS all of which lacked detectable antiviral antibody activity (table 2). In addition the complement source lacked detectable antibody to IBR and did not support CDNC following heat inactivation (table 1). Finally, the CDNC effect could be demonstrated against cells infected with HSV or VSV infected cells (table 3), viruses against which cattle are not normally exposed and do not have antibodies to. Finally, pretreatment of PMN to elute any putative cytophilic antibody, failed to ablate the cooperative PMN and complement cytotoxicity (table 2). Taken together, these results indicate that cells infected with certain viruses become susceptible to destruction by the combined presence of PMN and complement. If a similar effect occurs in vivo, it could provide a mechanism of recovery from infection before the time when specific components of immunity become active.

Since only virus infected cells acted as targets, it seems likely that some component on the surface of such cells, was activating complement. Candidate substances to cause this activation could be a viral antigen or perhaps a protease[20]. Thus some viruses, including the herpesvirus Epstein-Barr virus can activate complement by the alternative pathway[21]. In addition, it was recently shown that the G protein present on the viral envelope of VSV can activate the classical complement pathway without the participation of antibody[22]. Whether or not IBR and HSV viruses can activate complement has not been determined. The idea of a membrane bound protease activating complement was recently elaborated by Dierich and Landen[20] and it has been reported that certain virus infected cells may express increased protease activity[20,23]. Although complement activation alone may be sufficient to destroy virus infected cells[24] and did occur to a degree at the highest level of complement used in our study, our results showed a marked increase of cytotoxicity in the presence of PMN. It would be that these cells, which bear complement receptors[25], were being anchored to the target cells and as a result delivered a lytic signal to the targets. This lytic signal could in turn involve the elaboration of certain activated complement components as suggested by Mayer[26]. Alternatively, the PMN could be activating complement, perhaps by proteases present in their membrane. Those activated components generated could bind by different receptors to both PMN and virus infected cells with the binding to the latter cells being followed by the elaboration of the lytic unit. Clearly more work is needed to resolve the mechanism of CDNC and to clarify the possible in vivo significance of a defense mechanism which could operate before antibodies and sensitized T cells build up to protective levels.

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The concentration of high molecular weight kininogen antigen in homogenates of various human tissues[1]

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Summary. The concentration of high molecular weight kininogen, measured in human tissue homogenates, was 2–3 times higher in kidneys, adrenals and thyroid than in homogenates of lung, heart, liver and spleen. No measurable quantities of this protein were found in homogenates of brain and skeletal muscles.

Of the 2 plasma kinin precursors only high molecular weight kininogen (HMW-kininogen), but not low molecular weight kininogen (LMW-kininogen) plays a key role in Hageman factor-dependent activation of clotting, fibrinolysis and prekallikrein activation[3–6]. The site of synthesis of this protein, and its distribution in the organism, are unknown. The present report deals with the results of the measurement of HMW-kininogen antigen in human tissue homogenates.

Material and methods. Fragments of selected organs, obtained during routine postmortem examination of 40 bodies, maintained at 4 °C, were homogenized in a Potter