Immunology 1984 **53** 275

Antibody-dependent cell-mediated cytotoxicity against IBR-infected bovine kidney cells by ruminant neutrophils: the role of lysosomal cationic protein

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Accepted for publication 19th June 1984

Summary. Antibody-dependent cell-mediated cytotoxicity (ADCC) of infectious bovine rhinotracheitis (IBR)-infected bovine kidney cells (MDBK) by neutrophils was demonstrated. Neutrophils from bovine and sheep mammary exudate and peripheral blood, and also from human peripheral blood, were all active in the presence of anti-IBR antibody. The component of the ruminant neutrophil granules which was responsible for cytotoxicity appeared to be cationic protein since purified cationic protein lysed the virus-infected cells and heparin inhibited cytotoxicity. Human neutrophil cytotoxicity to herpes simplex virus (HSV)-infected human Chang liver cells was also inhibited by heparin. Human neutrophil cytotoxicity to IBR-infected bovine kidney cells did not appear to be mediated by cationic protein since it was inhibited by the chelators of oxidative intermediates DMSO, thiourea, tryptophane, benzoate and mannitol, and not by heparin.

INTRODUCTION

Three major types of cell-mediated cytolysis of virus-infected cells have been described: killing by cytotoxic T-lymphocytes (Ada, Leung & Ertl, 1981; Finberg & Benacerraf, 1981) NK cell-mediated cytolysis (Santoli & Koprowski, 1979) and ADCC (Sissons & Oldstone, 1980). A number of different effector cells have been implicated in ADCC. In the presence of specific antibody, K cells from human blood kill herpes simplex virus (HSV)-infected Chang liver cells (Kohl *et al.*, 1977; Melewica *et al.*, 1977) and probably varicella zoster-infected human embryonic lung cells (Gershon & Steinberg, 1981; Kamiya *et al.*, 1982). Mouse peritoneal macrophages lyse HSV- and influenza-infected syngeneic and xenogeneic cells (Kohl, Drath & Loo, 1982) while human blood monocytes lyse HSV-infected Chang liver cells (Kohl *et al.*, 1977). Bovine kidney cells (Georgia) infected with infectious bovine rhinotracheitis (IBR) virus are lysed by phagocytic adherent and non-adherent cells from bovine mammary exudate, and by phagocytic adherent cells from bovine blood (Rouse, Wardley & Babiuk, 1976a). In the present paper, we report that bovine kidney cells (MDBK) infected with IBR virus are lysed, in the presence of specific bovine antibody, by ruminant neutrophils from blood or mammary exudate and also by human peripheral blood neutrophils.

Both oxidative and non-oxidative cytotoxic reactions have been characterized in neutrophils. One important killing mechanism involves the release of toxic cationic proteins from the neutrophil granules. Neutrophil cationic protein can kill Gram-negative bacteria (Thorne, Oliver & Barrett, 1976; Weiss *et al.*,

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1978; Zeya & Spitznagel, 1968), Gran-positive bacteria (Weiss *et al.*, 1978), fungi (Drazin & Lehrer, 1977), helminths (Finberg & Benacerraf, 1981) and tumour cells (Clark, Olsson & Klebanoff, 1976). We have obtained evidence that the lysis of IBR-infected MDBK cells by ruminant neutrophils is mediated by a cationic protein which is release onto the target cell from the lysosomal granules. However, lysis of bovine target cells by human neutrophils appears to involve an oxidative pathway.

MATERIALS AND METHODS

Target cells

(a) Virus-infected MDBK cells. IBR and bovine mammilitis virus (BMV) (from Leeds University through the generosity of Professor P. Wildy, Department of Pathology, University of Cambridge) and para-influenza 3 (PI3) (from the Central Veterinary Research Laboratories, Weybridge, Surrey, U.K.) were grown on the bovine kidney cell line MDBK using RPMI 1640 medium containing 25 mM hepes, 200 i.u. penicillin/ml 200 μg streptomycin/ml and 10% non-heat-inactivated fetal calf serum (MED/c). Virus was adsorbed onto confluent cells in roller flasks at 37° for 3 hr using 0·1 PFU/cell and, after adding more MED/c, incubated for 2 days at 37°. The infected cells were harvested using sterile glass beads, concentrated to 5 ml, sonicated and finally stored in 100 μl aliquots at −70°. MDBK was also used to assay virus preparation for the number of PFU/ml. To produce target cells for cytotoxicity experiments, confluent cultures of MDBK cells grown in 25-ml plastic flasks were infected with virus at a multiplicity of 1·0 PFU/cell. The MED/c was removed from the MDBK and the virus added in 500 μl of fresh MED/c; after 1 hr of adsorbtion at 37° with occasional gentle mixing, 15 ml of MED/c was added and the cells incubated at 37°, either overnight with IBR and BMV, or for 48 hr with PI3. Control cultures were sham-infected with 500 μl MED/c. After incubation, cells were removed by gentle trypsinization, washed twice with MED (as MED/c but with 10% heat inactivated serum), and counted.

(b) HSV-infected Chang liver cells. HSV Type I MDK (CL1001 TK$^-$) was a generous gift from Dr A. Nash, Department of Pathology, University of Cambridge. Target cells were produced by infecting confluent cultures of Chang liver cells, grown in 25-ml plastic flasks, with virus at a multiplicity of 20 PFU/cell using the procedure described for IBR-infection of MDBK cells.

Effector cells

(a) Ruminant exudate neutrophils. Neutrophils were isolated using a technique similar to that described by Rouse, Wardley & Babiuk (1976a). Westphal lipopolysaccharide (Difco) was dissolved at a concentration of 250 μg/ml in distilled water and stored at −20°. Subsequently, 100 μl of the lipopolysaccharide stock was diluted in 10 ml PBS and inoculated up the teat canal of nonlactating heifers and ewes using a syringe attached to plastic tubing with an i.d. of 0·75 mm. After 16−18 hr, 10 ml MED containing 10 i.u. heparin/ml was infused into the teat canal, the udder was briefly massaged and the contents were expressed into sterile containers. The neutrophil preparations were layered onto 10 ml gradients of 9% Ficoll 400 and 11% sodium hypaque (w/v), and centrifuged at 1000 *g* for 25 min. The enriched neutrophil pellet was then washed in MED and a cell smear prepared and assessed for purity. Only cell preparations of 95% were used in subsequent experiments.

(b) Ruminant blood neutrophils. Peripheral blood neutrophils were isolated using a similar technique to that described by Clarkson & Kaneko (1973). Briefly, 80-ml blood samples were collected using EDTA (disodium salt) as anticoagulant, at a final concentration of 2 mg/ml of blood. The blood was then centrifuged at 1000 *g* for 15 min, and the serum, buffy coat and top layer of the packed red blood cells were discarded; the remaining cells were then resuspended in 0·8% NaCl in 0·013 M phosphate buffer pH 6·8. The erythrocytes were lysed by gentle mixing with distilled water (20 ml for every 10 ml of original blood sample) and, after 30 seconds, isotonicity restored by adding 10 ml of 0·0132 M phosphate buffer containing 2·7% NaCl. The granulocyte-rich pellet was then washed twice with MED and centrifuged at 200 *g* for 10 min. Further enrichment of the granulocytes was achieved by Ficoll-Hypaque gradients as described above. Samples were checked for purity by centrifuging and staining with Giemsa. Purity exceeded 90% neutrophils.

(c) Human peripheral blood neutrophils. Heparinized blood (20 ml) from normal donors was incubated for 30 min with 4 ml 4·5% (w/v) dextran 150 (Fisons Scientific Apparatus, Loughborough, U.K.)

in phosphate-buffered saline (PBS) to sediment the erythrocytes. The supernatant was collected and the leucocytes harvested by centrifuging for 10 min at 400 *g* and washed twice with MED. Neutrophils were separated from eosinophils and mononuclear cells by centrifuging through a discontinuous Metrizamide gradient (Vadas *et al.*, 1979). They were then collected and washed twice with MED. Samples were checked for purity by cyto-centrifuging and staining with Giemsa. Purity exceeded 90% neutrophils, with the main contaminant being eosinophils.

Antisera. (a) A nine-month-old Jersey bullock was infected intranasally with 10^9 PFU of IBR virus. Serum was collected 20 days later, heat inactivated (56° for 30 min) and stored in aliquots at −20°.

(b) Anti-HSVI human serum was a generous gift from Professor P. Wildy. It was heat inactivated before use.

Lysosomal cationic protein. Neutrophils were washed with 0·25 M sucrose, resuspended in heparin (3 mg/ml) in 0·25 M sucrose containing 2 mM phenylmethylsulphonylfluoride, at a concentration of 10^7 cells/ml, and lysed by repeated passage through a 19G needle. Unbroken cells were removed by centrifuging at 400 *g* for 5 min, and DNA was hydrolysed by incubating for 5 min at 37° with DNAase I (10 μg/ml) and 5 mM $MgCl_2$. Lysosomal granules were collected by centrifuging at 20,000 *g* for 20 min and washed once with 0·25 M sucrose. The granules were stored at −20°. They were lysed by repeated freezing and thawing in 1 ml 10 mM acetic acid. Insoluble debris was removed by centrifuging at 2000 *g* for 5 min and the soluble extract dialysed against 50 mM phosphate pH 7·4. It was then fractionated on a CM-32 cellulose column (2·2 × 8 cm) and eluted with a linear gradient from 50 mM sodium phosphate pH 7·4 to 0·5 M sodium citrate pH 3·0. The column eluates were concentrated with Aquacide II-A (Calbiochem, Bishops Stortford, Herts, U.K.) and then dialysed against PBS. Protein concentration was measured by the Folin-Lowry method using BSA as standard.

Iso-electric focusing. Soluble extract from lysed lysosomal granules prepared from 5×10^8 human peripheral blood neutrophils as described above, was dialysed extensively against 0·1% (w/v) glycine. Preparative flat-bed electrofocusing in a granulated gel slurry of IEF sephadex was carried out under the manufacturer's instructions with an LKB 2117 multiphor. The gel slurry consisted of 125 ml of gel from which 15 ml of supernatant water was replaced with 8ml of lysosomal extract, 6 ml of ampholines pH 3–10 (Pharmalyte, Pharmacia) and 1 ml of ampholines pH 5–8. At the anode was 5% phosphoric acid and at the cathode 5% ethylene diamine. the gel was electrofocused for 6 hr at 60 W. The pH of each fraction was measured with a surface pH electrode. The fractions were then eluted into 1 ml PBS dialysed extensively against PBS to remove amopholines.

Cytotoxicity assay. Virus-infected or uninfected target cells were labelled with isotope by incubation of 10^6 cells for 1 hr in 0·1 ml of medium with 200 μCi of $Na_2{}^{51}CrO_4$ (Amersham International, Bucks, U.K.). They were washed repeatedly with MED until the count rate in the supernatant was less than 5% of that in the cells. Using LP2 tubes (0·5 × 3·5 cm) (Luckham Ltd) quadruple samples of 10^3 target cells in 100 μl MED were incubated for 1–17 hr at 37°, with or without diluted antiserum (final concentration anti-IBR 1:150, anti-HSV 1:60), effector cells and inhibitors or purified proteins; the final volume of all tubes was 300 μl. The tubes were then centrifuged at 200 *g* for 2 min to accelerate contact between the cells. At the end of the incubation period, the cells were resuspended by vortex mixing and centrifuged again; after this, the supernatant (150 μl) was removed to a second tube. Both pellet and supernatant tubes were counted in a 3 cm sodium iodide scintillation counter and the percentage of the isotope released from the target cells calculated. The logarithm of percentage isotope released was analysed by analysis of variance, and tests were then made to determine the least significant differences (l.s.d.) at 5% confidence level by multiple range testing (Thorne, Free & Franks, 1982). Specific cytotoxicity was calculated as:

$$\frac{\text{isotope release with neutrophils (with antibody} - \text{without antibody)}}{\text{total radioactivity} - \text{isotope release with neutrophils without antibody}} \times 100.$$

Lysis by fractions from CM-cellulose and from the electrofocusing gel was measured by the same method, except that incubations were in calcium-free Hanks' balanced salts solution (HBSS) containing 0·5% w/v bovine serum albumin, and the target cells were not centrifuged before incubation. All experiments were done at least twice, with reproducible results.

Inhibitors and enzymes. Heparin, protamine, catalase, xanthine oxidase (XO) (1 unit/mg), horseradish peroxidase (HPO) (165 units/mg), superoxide dismutase (SOD) (2800 units/mg) and glucose oxidase (GO) (125 units/mg) were obtained from the Sigma Chemical Co. (Poole, Dorset, U.K.). Myeloperoxidase (MPO) was obtained from human neutrophil granules by gel filtration on Sephacryl S200 (Pharmacia, Uxbridge, U.K.).

RESULTS

ADCC of IBR-infected MDBK cells by neutrophils

Incubation of neutrophils from both ruminants and humans with IBR-infected MDBK cells and specific antibody led to an increasingly rapid release of ^{51}Cr from the target cells, with increasing effector cell: target ratios. With ratios of 1000:1, over 80% of the radioactivity was released in 2 hr, while with ratios of 320:1, 80% was released after 5 hr incubation. In the absence of antibody, neutrophils had little or no effect on the target.

Inhibitors of neutrophil-mediated ADCC

ADCC to IBR-infected bovine cells, mediated by bovine and sheep neutrophils, from blood or from exudate, was inhibited by heparin (Table 1). In contrast, the activity of human neutrophils to IBR-infected bovine cells was only slightly sensitive to heparin.

When human blood neutrophils were tested against HSV-infected human cells, ADCC was inhibited by heparin (Table 1). Scavengers of hydroxyl radicals, DMSO, thiourea, tryptophane, benzoate and mannitol all inhibited the cytotoxic activity of human blood neutrophils to IBR-infected cells, but *not* that of neutrophils from bovine blood or sheep, or bovine exudate to IBR-infected cells, or human neutrophils to HSV-infected cells (Table 2). None of the agents tested was toxic to the target cells.

Sensitivity of IBR-infected cells to purified neutrophil cationic protein

Cationic protein was purified from neutrophil granules by ion exchange chromatography on carboxymethyl cellulose (Table 3). Cationic protein with activity against IBR-infected MDBK cells could be obtained from bovine exudate and from human blood neutrophils. Activity was only eluted from CM-cellulose when the concentration of citrate exceeded 0·375 M. Activity was inhibited by heparin. The purified protein was considerably more active against virus-infected cells than against uninfected cells. MDBK cells infected with the viruses BMV and PI3 were also lysed by cationic protein purified from bovine neutrophils (Table 4).

Table 1. Inhibition by heparin of cytotoxic activity of neutrophils to IBR-infected MDBK cells and to HSV-infected Chang liver cells by heparin

	Percent specific cytotoxicity							
	IBR-MDBK							HSV-Chang
Heparin (mg/ml)	Sheep exudate 320:1	Sheep exudate 100:1	Bovine exudate 320:1	Bovine exudate 100:1	Bovine blood 320:1	Bovine blood 100:1	Human blood 100:1	Human blood 320:1
0	52	28	60	38	82	68	42	35
1	28*	8*	54	20*	85	61	36	46
2	14*	5*	42*	15*	80	58*	34*	42
5	10*	4*	35*	10*	81	40*	32*	11*
Statistical analysis								
l.s.d.	7	3·5	7	5	8	7	5	7

Neutrophils were incubated with IBR-infected MDBK cells and with HSV-infected Chang liver cells at ratios of 320:1 or 100:1, with and without antiserum, in the presence of increasing concentrations of heparin, for 5 hr.

* Significantly different from Cr release without heparin.

Table 2. Inhibition of cytotoxic activity by scavengers of hydroxyl radicals

	Percent specific cytotoxicity							
	IBR-MDBK							HSV-Chang
	Bovine exudate		Sheep exudate		Bovine blood		Human blood	Human blood
Inhibitors	320:1	32:1	320:1	32:1	320:1	32:1	320:1	320:1
no additions	84	35	81	44	79	34	32	35
DMSO								
0·07 M	84	22*	81	39	83	35	21*	27
0·14 M	78	27*	80	34*	83	31	6*	23*
Thiourea								
10 mM	–	–	–	–	–	–	8*	24
20 mM	85	42	85	51	85	47	7*	19*
Tryptophane								
10 mM	84	29*	79	47	84	52	5*	–
Sodium benzoate								
10 mM	–	–	–	–	–	–	9*	31
20 mM	84	21*	76	40	76	30	0*	29
Mannitol								
10 mM	–	–	–	–	–	–	13*	33
20 mM	84	29*	76	38*	77	31	9*	34
50 mM	82	25*	73	31*	78	31	5*	33
Statistical analysis								
l.s.d	9	4	8·5	5	8·5	4	4	12

Neutrophils were incubated with IBR-infected MDBK cells or HSV-infected Chang cells and specific antiserum for 5 hr in the presence of inhibitors.

* Significantly less than Cr release without added inhibitor

Table 3. Cytotoxic activity of fractions from neutrophil granules, separated by ion exchange chromatography on carboxy methyl cellulose

		Percent Cr release			
Source of neutrophils	Concentration of sodium citrate	IBR	IBR + heparin	MDBK	Protein (μg/ml)
Bovine exudate	0	15	17	9	270
	0–0·125 M	17	17	8	14
	0·125 M–0·25 M	17	20*	11*	12
	0·25 M–0·375 M	21*	21*	13*	27
	0·375 M–0·5 M	23*†	16	8	51
PBS control		16	14	9	0
Human blood	0	16	18	5	25
	0–0·125 M	17	18	4	35
	0·125 M–0·25 M	14	14	6	12
	0·25 M–0·375 M	19*	19*	8*	14
	0·375 M–0·5 M	25*†	20*	9*	60
PBS control		12	15	6	0
Statistical analysis					
Bovine exudate	l.s.d.	5	5	2	
Human blood	l.s.d.	3	4	1	

IBR-infected MDBK cells were incubated in HBSS and albumin for 4 hr with fractions eluted from CM32 cellulose columns, with and without heparin (1 mg/ml). Neutrophil granule contents were put on the column in 50 mM sodium phosphate pH 7·4 and eluted with a linear gradient of increasing concentration of sodium citrate pH 3.

* Significantly greater than PBS alone.

† Significantly greater than IBR + heparin.

Table 4. Sensitivity of virus-infected MDBK cells to cationic protein

	Percent Cr release		
	Bovine cationic protein (55 μg/ml)		Statistical analysis
Virus	+protein	−protein	l.s.d.
None	28*	7	2
IBR	77*	26	5
PI3	64*	18	4
BMV	52*	28	6

Cationic protein was purified from human and bovine neutrophil lysosomal granules by ion exchange chromatography on CM-32 cellulose. MDBK cells, infected with different viruses, were incubated for 4 hr with cationic protein in HBSS with albumin.

* Significantly greater than without cationic protein.

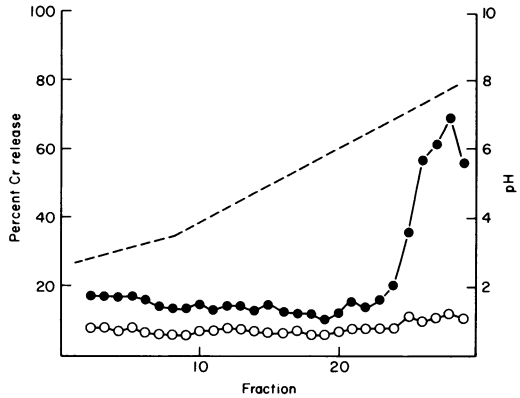

Figure 1. Iso-electric focusing of neutrophil lysosomal protein. Soluble extracts from lysed neutrophil granules were subjected to electrofocusing at a constant 60 W for 6 hr in IEF sephadex, with ampholine (pH 3–10). After separation, the pH of each fraction was measured with a surface electrode (– – – –). The fractions were then eluted into 1 ml PBS and assayed for their cytotoxic activity against MDBK cells (●) with and (○) without IBR infection.

Further confirmation that the toxic activity resided in cationic components of the lysosomal granules was obtained by iso-electric focusing (Fig. 1). The activity was recovered from the basic end of the focusing gel, in the region of pH 8. This material was also only active against virus-infected cells.

The activity of the purified cationic protein was inhibited 20% by 3 mM calcium chloride, and 75% by 9 mM calcium chloride. For this reason, its activity was always measured in calcium-free HBSS.

Sensitivity of IBR-infected cells to oxidative intermediates

The direct toxic effect of reactive oxygen metabolites was also measured (Table 5). Neither superoxide

Table 5. Sensitivity of MDBK cells to oxidative intermediates

	Percent Cr release	
Incubated with	+IBR	−IBR
HBSS+BSA	5	6
+Xanthine (0·1 mM)	20*	16*
+XO (0·2 units/ml)	8	5
+Xanthine+XO	22*	20*
+Xanthine+XO+SOD (56 units/ml)	22*	18*
+GO (1·25 units/ml)	11	7
+GO+MPO (0·4 units/ml)	58*	48*
+MPO	9	6
+H_2O_2 (1 mM)	17*	16*
+H_2O_2+MPO	40*	70*
+H_2O_2+HPO (3·3 units/ml)	20*	10
Statistical analysis l.s.d.	6·5	4

MDBK cells were incubated in HBSS and BSA for 4 hr.

* Significantly greater than HBSS and BSA alone.

(generated from xanthine and xanthine oxidase) nor hydrogen peroxide (generated from glucose oxidase and glucose, or added directly) lysed MDBK cells substantially, with or without virus. However, substantial lysis was obtained when myeloperoxidase, purified from human neutrophils, was added together with hydrogen peroxide. A small toxic effect of xanthine alone was observed, but no explanation for this has been found. Horseradish peroxidase did not replace myeloperoxidase.

DISCUSSION

Neutrophils from both ruminant and human blood, as well as from ruminant mammary exudate, prove to be very active against IBR-infected MDBK cells at high effector:target ratios in the presence of specific antibody, but show no activity in its absence. Bovine neutrophils are now well established as one of the major effector cells in ADCC assays involving herpes virus-infected targets (Grewal & Rouse, 1979; Rouse, Wardley & Babiuk, 1976b). In contrast, the major human effector cell in ADCC against herpes-infected targets is the classical K lymphocyte (Melewica *et al.*, 1977). The human neutrophil will, however, kill herpes virus-infected targets as reported by Oleske *et al.* (1977), by Fujimiya, Rouse & Babiuk (1978) and by Siebens, Tevethia & Babior (1979).

Cytolysis of IBR-infected MDBK cells by ruminant neutrophils appears to involve cationic protein. The virus-infected target is susceptible to cationic protein isolated from lysosomal granules by ion exchange chromatography or by iso-electric focusing. The cytotoxic activity of the intact neutrophil is inhibited by heparin.

Cationic proteins from neutrophil lysosomes have been found which are toxic to Gram-negative bacteria (Zeya & Spitznagel, 1968; Thorne, Oliver & Barrett, 1976; Weiss *et al.*, 1978), to Gram-positive bacteria (Zeya & Spitznagel, 1968), to fungi (Drazin & Lehrer, 1977), to helminths (McClaren *et al.*, 1981) and to tumour cells (Clark, Olsson & Klebanoff, 1976). Cationic proteins appear to act non-enzymically (Odeberg, Olsson & Venge, 1975; Thorne, Oliver & Barrett, 1976). The protein isolated from neutrophils studied by Elsbach *et al.* (1979) increases the permeability of the Gram-negative bacterial outer membrane and activates a bacterial phospholipase A to do further damage. It can be displaced by addition of Ca^{2+} or Mg^{2+} (Weiss *et al.*, 1976) but not by monovalent cations.

Cytolysis of HSV-infected human cells by human neutrophils may also be mediated by cationic protein, since it is inhibited by heparin but not by chelators of reactive oxygen metabolites. Siebens *et al.* (1979) reported that ADCC of HSV-infected cells by human neutrophils is independent of the respiratory burst; but cytolysis of IBR-infected bovine cells by human neutrophils appears to involve an oxidative pathway, possibly utilizing hydroxyl radicals. Perhaps in this unnatural situation, the antibody-coated alien targets activate the neutrophil surface oxidase non-specifically. In the natural situation where bovine neutrophils are killing virus-infected bovine cells, or human neutrophils are killing virus-infected human cells, it is lysosomal cationic protein which plays the major role.

ACKNOWLEDGMENTS

We are grateful to the Nuffield Trust, to the Agricultural Research Council and to the Wellcome Foundation for financial support.

REFERENCES

ADA G.L., LEUNG K.N. & ERTL H. (1981) An analysis of effector T cell generation and function in mice exposed to infleunza A or sendai virus. *Immunol. Rev.* **58,** 5.

CLARK R.A., OLSSON R. & KLEBANOFF S.J. (1976) Cytotoxicity for tumour cells of cationic proteins from human neutrophil granules. *J. Cell Biol.* **70,** 719.

CLARKSON G.P. & KANENKO J.J. (1973). Isolation of leucocytes from bovine peripheral blood. *Proc. Soc. exp. Biol. Med.* **142,** 853.

DRAZIN R.E. & LEHRER R.I. (1977). Fungicidal properties of a chymotrypsin like cationic protein from human neutrophils: adsorption to *Candida parapsilosis. Infect. Immun.* **17,** 382.

ELSBACH P., WEISS J., FRANSON R.C., BECKERDITE-QUAGLISTA S., SCHEICK A. & HARRIS L. (1979) Separation and purification of a potent bactericidal/permeability increasing protein and a closely related phospholipase A_2 from rabbit polymorphonuclear leucocytes. *J. biol. Chem.* **254,** 11000.

FINBERG R. & BENACERRAF B. (1981) Induction, control and consequences of virus specific cytotoxic T cells. *Immunol. Rev.* **58,** 157.

FUJIMIYA Y., ROUSE B.T. & BABIUK L.K. (1978) Human neutrophil-mediated destruction of antibody sensitized herpes simplex virus type I infected cells. *Canad. J. Microbiol.* **24,** 182.

GERSHON A.A. & STEINBERG S.P. (1981). Inactivation of varicella zoster virus *in vitro*: effect of leukocytes and specific antibody. *Infect. Immun.* **33,** 507.

GREWAL A.S. & ROUSE B.T. (1979). Characterization of

bovine leucocytes involved in antibody-dependent cell cytotoxicity. *Int. Archs Allergy appl. Immunol.* **60,** 169.

KAMIYA H., STARR S.E., ARBETER A.M. & PLOTKIN S.A. (1982) Antibody-dependent cell-mediated cytotoxicity against varicella zoster virus-infected targets. *Infect. Immun.* **38,** 554.

KOHL S., DRATH D.B. & LOO L.S. (1982) Murine cellular cytotoxicity to syngeneic and xenogeneic herpes simplex virus-infected cells. *Infect Immun.* **38,** 1231.

KOHL S., STARR S.E., OLESKE J.M., SHORE S.L., ASHMAN R.B. & NAHMIAS A.J. (1977) Human monocyte-macrophage mediated antibody-dependent cytotoxicity to herpes simplex virus infected cells. *J. Immunol.* **118,** 729.

MCLAREN D.J., MCKEAN J.R., OLSSON I., VENGE P. & KAY A.B. (1981) Morphological studies on the killing of schistosomula of *Schistosoma mansoni* by human eosinophil and neutrophil cationic proteins *in vitro*. *Parasite Immunol.* **3,** 359.

MELEWICA F.M., SHORE S.L., ADES E.W. & PHILLIPS D.J. (1977) The mononuclear cell in human blood which mediates ADCC to virus-infected target cells. II. Identification as a K cell. *J. Immunol.* **118,** 567.

ODEBERG H., OLSSON I. & VENGE P. (1975) Cationic proteins of human granulocytes. IV. Esterase activity. *Lab. Invest.* **32,** 86.

OLESKE J.M., ASHMAN R.B., KOHL S., SHORE S.L., STARR S.E., WOOD P. & NAHMIAS A.J. (1977) Human polymorphonuclear leukocytes as mediators of antibody-dependent cell-mediated cytotoxicity to herpes simplex virus infected cells. *Clin exp. Immunol.* **27,** 446.

ROUSE B.T., WARDLEY R.C. & BABIUK L.A. (1976a) Antibody-dependent cell-mediated cytotoxicity in cows: comparison of effector cell activity against heterologous erythrocyte and herpes-virus infected bovine target cells. *Infect. Immun.* **13,** 1433.

ROUSE B.T., WARDLEY R.C., BABIUK L.A. (1976b) The role of antibody-dependent cytotoxicity in recovery from herpes virus infections. *Cell Immunol.* **22,** 182.

SANTOLI D. & KOPROWSKI H. (1979) Mechanisms of activation of human NK cells against tumour and virus infected cells. *Immunol. Rev.* **44,** 125.

SIEBENS H., TEVETHIA S.S. & BABIOR B.M. (1979) Neutrophil-mediated antibody-dependent killing of Herpes-simplex-virus-infected cells. *Blood,* **54,** 88.

SISSONS J.G.P. & OLDSTONE M.B.A. (1980) Antibody-mediated destruction of virus infected cells. *Adv. Immunol.* **29,** 209.

THORNE K.J.I., FREE J. & FRANKS D. (1982) Role of sulphydryl groups in T lymphocyte mediated cytotoxicity. *Clin. exp. Immunol.* **50,** 644.

THORNE K.J.I., OLIVER R.C. & BARRETT A.J. (1976) Lysis and killing of bacteria by lysosomal proteinases. *Infect. Immun.* **14,** 555.

VADAS M.A., DAVID J.R., BUTTERWORTH A.E., PISANI N.T. & SIONGOK T.A. (1979) A new method for the purification of human eosinophils and neutrophils, and a comparison of the ability of these cells to damage schistosomula of *Schistosoma mansoni*. *J. Immunol.* **122,** 1228.

WEISS J., ELSBACH P., OLSSON D. & ODEBERG H. (1978) Purification and characterization of a potent bactericidal and membrane active protein from the granules of human polymorphonuclear leukocytes. *J. biol. Chem.* **253,** 2664.

WEISS J., FRANSON R.C., SCHMEIDLER K. & ELSBACH P. (1976) Reversible envelope effects during and after killing of *Escherichia coli* W by a highly purified rabbit polymorphonuclear leukocyte fraction. *Biochim. biophys. Acta,* **436,** 154.

ZEYA H.I. & SPITZNAGEL J.K. (1968) Arginine-rich proteins of polymorphonuclear leukocyte lysosomes. *J. exp. Med.* **127,** 927.