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Group B streptococci inactivate complement component C5a by enzymic cleavage at the *C*-terminus

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Incubation of recombinant human C5a (rC5a) with the 7360 strain of group B streptococci (GBS) destroyed the ability of rC5a to stimulate chemotaxis or adherence of purified human polymorphonuclear leucocytes (PMNs). Treatment of ^{125}I-labelled rC5a with GBS 7360 correspondingly decreased rC5a binding to human PMNs. This also resulted in an approx. 600 Da decrease in the molecular mass of rC5a as determined by SDS/PAGE. Incubation of rC5a with the GBS strain GW, which only minimally altered the ability of rC5a to activate human PMNs, did not affect rC5a binding to PMNs and did not alter the molecular mass of rC5a on SDS/PAGE. Plasma-desorption m.s. of rC5a inactivated by GBS 7360 showed that the GBS cleaved the rC5a between histidine-67 and lysine-68 near the *C*-terminus of rC5a. This mechanism of inactivation of C5a by proteolytic cleavage at the *C*-terminus of C5a is consistent with the known critical role of the *C*-terminus in C5a activation of human PMNs. This C5a-cleaving proteinase activity may contribute to the pathophysiology of GBS infections.

INTRODUCTION

Infections with group B streptococci (GBS) are a cause of considerable morbidity and mortality in the human newborn [1]. Such infections often result in overwhelming bacterial invasion in affected organs without significant accumulations of polymorphonuclear leucocytes (PMNs) [2]. The reason for this poor host inflammatory response is not well understood. We have previously reported [3] that the majority of strains of GBS inactivate the chemotactic activity of zymosan-activated serum. This would be most readily explained by an inhibitory effect on $C5a_{desArg}$, a proteolytically derived fragment of complement component C5, since $C5a_{desArg}$ is the major PMN chemoattractant generated when zymosan activates the complement cascade in serum. The availability of recombinant human C5a (rC5a) that possesses the PMN-activating properties of C5a isolated from human serum, and binds to the same PMN receptor as purified human C5a [4–8], allows a direct examination of this hypothesis. In the studies reported in the present paper we have investigated the effects of GBS on the ability of rC5a to activate PMNs. Our results show that GBS do inactivate rC5a, and that they do so by proteolytic cleavage of a heptapeptide from the *C*-terminus of rC5a.

MATERIALS AND METHODS

Human rC5a

Details of the synthesis, cloning and expression of the C5a gene in *Escherichia coli* have been previously described [4–6]. The rC5a was purified to homogeneity by reversed-phase h.p.l.c. [9]. Two-thirds of the rC5a used in most experiments retains a methionine residue at the *N*-terminus [6]. For experiments with plasma-desorption m.s., rC5a devoid of methionine at its *N*-terminus was prepared by further separation on a 21.2 mm Dynamax C8 reverse-phase column (Rainin Instruments Co., Wobern, MA, U.S.A.). The column was equilibrated in aq. 0.1 % (v/v) trifluoroacetic acid (eluent A). Eluent B consisted of 80 % (v/v) acetonitrile in aq. 0.1 % (v/v) trifluoroacetic acid. The separation was conducted at 60 °C with a gradient of 0.02 % eluent B/min.

Human PMN purification

PMNs were isolated from heparinized peripheral blood from normal adult donors by dextran sedimentation and density-gradient centrifugation on Ficoll/Hypaque cushions as previously described [3]. Contaminating erythrocytes were removed by hypo-osmotic lysis. These preparations were routinely more than 98 % viable by Trypan Blue exclusion and more than 95 % PMNs by Giemsa staining.

Treatment of rC5a with GBS

Frozen portions of type III GBS strains 7360 and GW were inoculated into Todd–Hewitt broth and grown overnight at 37 °C [3]. After 16 h the GBS were pelleted by centrifugation, washed with phosphate-buffered saline (0.145 M-NaCl/0.01 M-sodium phosphate buffer, pH 7.4) and resuspended in phosphate-buffered saline to $A_{620} = 0.9$ (5×10^8 colony-forming units/ml). In experiments measuring inactivation of rC5a function, a pellet of GBS (5×10^8 colony-forming units) was incubated at 37 °C with 1 ml of rC5a at 1 μg/ml in Hanks buffered salt solution containing 0.8 mM-Ca^{2+} and 0.8 mM-Mg^{2+} (HBSS) (M. A. Bioproducts, Walkersville, MD, U.S.A.) and 0.1 % human serum albumin (HSA) (Cutter Biologicals, Berkeley, CA, U.S.A.). After 30 min the GBS were pelleted by centrifugation and the supernatant was removed. Control tubes contained rC5a incubated under the same conditions without GBS. To prepare rC5a for the experiments involving m.s., rC5a was incubated at a concentration of 1.37 mg/ml with washed GBS in HBSS containing no carrier protein until functional C5a activity was no longer detectable.

Abbreviations used: GBS, group B streptococci; rC5a, recombinant human C5a; PMN, polymorphonuclear leucocyte; HBSS, Hanks buffered saline containing 0.8 mM-Ca^{2+} and 0.8 mM-Mg^{2+}; HSA, human serum albumin.

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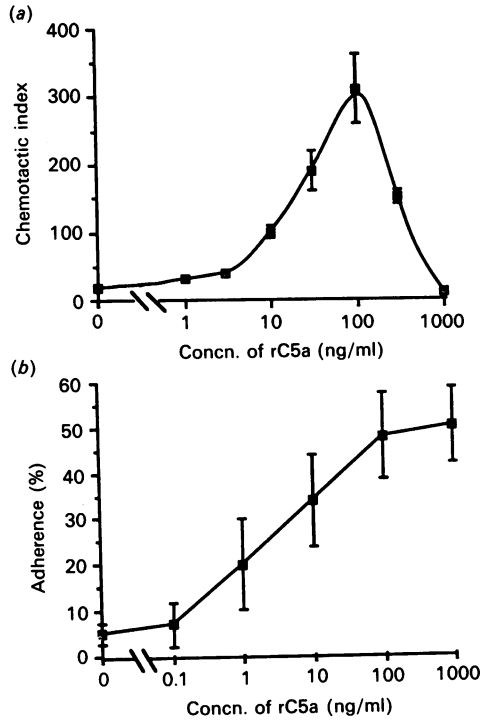

Fig. 1. Stimulation of PMN chemotaxis and adherence by rC5a is concentration-dependent

(*a*) Human rC5a stimulated maximum PMN chemotaxis in a modified Boyden chamber at a concentration of 100 ng/ml, with decreased chemotaxis observed at higher and lower concentrations. (*b*) rC5a stimulated rapid PMN adherence to gelatin-coated tissue-culture wells with maximal stimulation at concentrations greater than or equal to 100 ng/ml.

Chemotaxis assay

The chemotactic activity of control or GBS-treated rC5a was determined in 48-well micro chemotaxis chambers (Neuroprobe, Cabin John, MD, U.S.A.) with 5 μm-pore-size micropore filters (Millipore Corp., Bedford, MA, U.S.A.) between the upper and lower wells, as previously described [3,10]. Portions (50 μl) of PMNs (2×10^6/ml in HBSS containing 10 mM-Hepes and 1 % HSA) were added to the upper well (cell side) of the chamber, and rC5a, diluted in HBSS containing 1 % HSA, was added to the lower well. The chambers were incubated at 37 °C for 2 h in a humidified chamber with 5 % CO_2 in air. Filters were removed and stained with haematoxylin, and the number of PMNs that had migrated completely through ten random 400 × -power fields per filter (the chemotactic index) was determined by microscopy with a photographic reticle in the eyepiece. Under these conditions rC5a was found to stimulate PMN chemotaxis in a concentration-dependent manner (Fig. 1*a*).

PMN adherence assay

Adherence of stimulated and unstimulated PMNs to gelatin-coated surfaces was determined by the method of Zimmerman *et al.* [11]. Briefly, ^{111}In-labelled PMNs (225 μl at 5.5×10^6/ml in HBSS containing 0.5 % HSA) were added to gelatin-coated 16 mm tissue-culture wells. Various concentrations of rC5a in HBSS containing 0.5 % HSA were then added to each well and the wells were incubated at 37 °C. After 5 min non-adherent cells were removed by aspiration and one wash with 0.5 ml of HBSS. The percentage of adherent cells was calculated from the total radioactivity added to each well. Stimulation of PMN adherence by rC5a was found to be concentration-dependent (Fig. 1*b*).

Binding of radiolabelled rC5a to PMNs

rC5a was labelled with ^{125}I with the use of lactoperoxidase (Enzymobeads; Bio-Rad Laboratories, Richmond, CA, U.S.A.). After removal of the Enzymobeads from the reaction mixture, the rC5a was mixed with phosphate-buffered saline containing 0.1 % gelatin and 0.1 % NaN_3, and free $^{125}I^-$ was removed by dialysis against phosphate-buffered saline. The specific radioactivity of the ^{125}I-rC5a was 2.4×10^7 c.p.m./μg. Portions (50 μl) of PMNs (1×10^6/ml in HBSS containing 1 % HSA) were incubated with 300 ng of ^{125}I-rC5a in the presence or in the absence of a 100-fold excess of unlabelled rC5a for 20 min at room temperature. This concentration of ^{125}I-rC5a causes saturation of most of the PMN rC5a receptor sites under the conditions of this assay. Free rC5a was separated from rC5a bound to PMNs by centrifugation through silicone oil (Versilube; General Electric, Schenectady, NY, U.S.A.). The tube was quickly frozen in a 95 % ethanol/solid CO_2 bath, the tip was cut off the tube and cell-associated radioactivity in the tip was determined.

SDS/PAGE

^{125}I-rC5a was analysed by SDS/PAGE in an 8–18 % gradient of polyacrylamide according to the method of Laemmli [12]. Autoradiography was performed with X-Omat film (Eastman–Kodak, Rochester, NY, U.S.A.).

Plasma-desorption m.s.

The mass of purified rC5a or its various fragments was determined by plasma-desorption m.s. as described by Jardine and co-workers [13]. Plasma-desorption mass spectra were obtained on a Bio-Ion 20 plasma-desorption time-of-flight mass spectrometer (Bio-Ion Nordic, Uppsala, Sweden) with an acceleration voltage of 19 kV and a flight tube length of 14 cm. Spectra of peptides were accumulated over a 1–2 h period. The calculated molecular masses of peptides are the isotopically averaged molecular masses. All mass spectra shown are background-subtracted.

H.p.l.c. separation of rC5a fragments produced by cleavage with GBS

Purified rC5a inactivated by GBS for analysis by m.s. was resolved into two major peaks by using reverse-phase h.p.l.c. at 60 °C on a Vydac C4 column (The Separations Group, Hesperia, CA, U.S.A.) that had been equilibrated in aq. 0.1 % (v/v) trifluoroacetic acid (eluent A). Eluent B was 80 % (v/v) acetonitrile in aq. 0.1 % (v/v) trifluoroacetic acid. Fractions commencing with the start of a gradient of 1 % eluent B/min and eluted before regions where rC5a was found to be eluted were pooled and called fraction 1. Subsequent fractions were collected and pooled as fraction 2.

Treatment of rC5a fragment by endoproteinase Glu-C

Purified rC5a that had been inactivated by GBS was subjected to proteolytic cleavage by incubation at 37 °C with endoproteinase Glu-C (Boehringer–Mannheim, Indianapolis, IN, U.S.A.) [14] in 0.1 M-ammonium bicarbonate buffer, pH 8.0, at an enzyme/substrate ratio of 1:50 (w/w) for 3 h at 37 °C. After 3 h proteolysis was terminated by acidification with 1.0 M-trifluoroacetic acid and the resulting product was analysed by plasma-desorption m.s. as described above.

Table 1. Effect of incubation of GBS with rC5a on the ability of rC5a to activate PMNs

rC5a at 1 μg/ml was incubated with GBS (7360 or GW) for 30 min at 37 °C and then tested for its ability to stimulate PMN chemotaxis or adherence at a final concentration of 100 ng/ml. These data represent the means ± S.E.M. for the numbers of separate experiments indicated in the parentheses.

	Chemotactic index	Adherence (%)
Buffer	11 ± 2 (5)	5 ± 1 (8)
rC5a	345 ± 55 (5)	46 ± 2 (6)
7360-treated rC5a	26 ± 4* (5)	12 ± 3* (4)
GW-treated rC5a	260 ± 64† (4)	45 ± 11† (3)

* $P < 0.01$ compared with untreated rC5a by Student's t test.
† $P > 0.1$ compared with untreated rC5a by Student's t test.

Table 2. Inactivation of ^{125}I-rC5a binding to PMNs by GBS

^{125}I-rC5a was incubated with GBS (7360 or GW) or without GBS for 30 min at 37 °C and tested for its ability to bind to PMNs. Each point was performed in triplicate. These data are the means ± S.D. for one experiment performed in triplicate, and are representative of three separate experiments with similar results.

Treatment of radioligand	^{125}I-rC5a bound to PMN (c.p.m.)
None	24678 ± 941
None (+excess of unlabelled rC5a*)	462 ± 103
7360	4294 ± 220
GW	25243 ± 481

* 100-fold excess of unlabelled rC5a added to measure non-specific binding of ^{125}I-rC5a to PMNs.

RESULTS

rC5a is inactivated by GBS

rC5a induced PMN chemotaxis at 100 ng/ml, and decreased chemoattractant activity was observed at higher and lower concentrations of rC5a (Fig. 1*a*). The chemotactic activity of rC5a that had been exposed to GBS was therefore determined at an rC5a concentration of 100 ng/ml. Incubation of rC5a with the 7360 strain of GBS caused a 96% decrease in stimulated PMN migration in response to the rC5a when compared with PMN migration in response to control untreated rC5a (Table 1). In contrast, PMN migration towards rC5a previously incubated with the GW strain of GBS was not significantly decreased (Table 1).

It seemed likely that the GBS was directly inactivating the rC5a, rather than adversely affecting the ability of PMNs to undergo directed migration. To test this hypothesis, we examined whether GBS could inactivate the ability of rC5a to stimulate another PMN function. rC5a stimulated adherence of PMNs to gelatin-coated surfaces in a concentration-dependent manner with a plateau at a concentration of 100 ng/ml (Fig. 1*b*). Treatment of rC5a with the 7360 strain of GBS strongly inhibited the adherence-promoting activity of the rC5a in comparison with untreated rC5a (Table 1). The amount of adherence stimulated by 7360-treated rC5a is equivalent to the amount of adherence stimulated by 1 ng of native rC5a/ml, indicating that approx. 99% of the functional activity of rC5a had been destroyed. In contrast, treatment of rC5a with the GW strain did not inhibit the adherence-promoting activity of the rC5a (Table 1).

These data are consistent with the hypothesis that the 7360 strain inactivated rC5a by destroying its capacity to interact with the C5a receptor on human PMNs. We tested this by incubating ^{125}I-labelled rC5a with the 7360 strain of GBS, and then examining the binding of rC5a to PMNs. Approximately equal amounts (90%) of ^{125}I-rC5a were recovered from tubes containing GBS strains 7360 or GW or no GBS. This shows that the inactivating strain of GBS does not deplete the chemotactic activity of serum by simply adsorbing $C5a_{desArg}$ on their surface. Incubation of ^{125}I-rC5a with strain 7360 significantly decreased the specific binding of ^{125}I-rC5a to PMNs to approx. 20% of control ^{125}I-rC5a binding (Table 2). In contrast, binding of GW-treated ^{125}I-C5a to PMNs was approximately equal to that of untreated ^{125}I-rC5a. Thus inactivation of rC5a by the 7360 strain correlated with destruction of its ability to bind to PMNs.

GBS cleave a *C*-terminal heptapeptide from rC5a

We next analysed GBS-treated ^{125}I-rC5a by autoradiography after SDS/PAGE to determine if the loss of biological activity was associated with a change in its mobility. rC5a that had been exposed to the 7360 strain had a slightly smaller molecular mass (approx. 600 Da lower) under reducing conditions on SDS/PAGE than did rC5a treated with either strain GW or native rC5a (Fig. 2). These data suggest that the 7360 strain contains an enzyme that cleaves a small fragment from the rC5a.

We then used plasma-desorption m.s. to determine the exact mass of the rC5a and the cleavage fragments induced by the GBS. Fig. 3(*a*) shows the mass spectrum of control rC5a, which is a single-chain polypeptide of 74 amino acid residues, with a glutathione moiety bound by a disulphide bond to the cysteine residue at position 27 (Fig. 4) [4–8]. The predicted mass of the protonated molecular ion with a single positive charge, $(M+H)^+$, of rC5a is 8573. The observed mass was 8572 (Fig. 3*a*). The

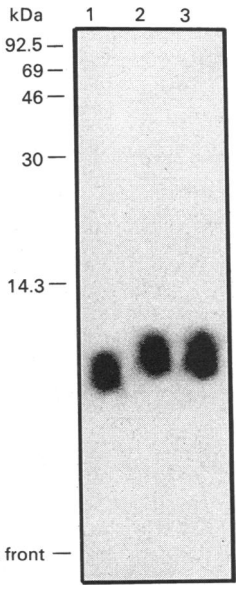

Fig. 2. Autoradiogram of an 8–18%-gradient SDS/PAGE of ^{125}I-rC5a performed under reducing conditions after exposure to GBS strain 7360 (lane 1) or strain GW (lane 2) or in its native state (lane 3)

The rC5a incubated with strain 7360 migrates about 600 Da smaller than native rC5a or rC5a exposed to strain GW. Molecular-mass standards: phosphorylase *b*, 92.5 kDa; BSA, 69 kDa; ovalbumin, 46 kDa; carbonic anhydrase, 30 kDa; lysozyme, 14.3 kDa.

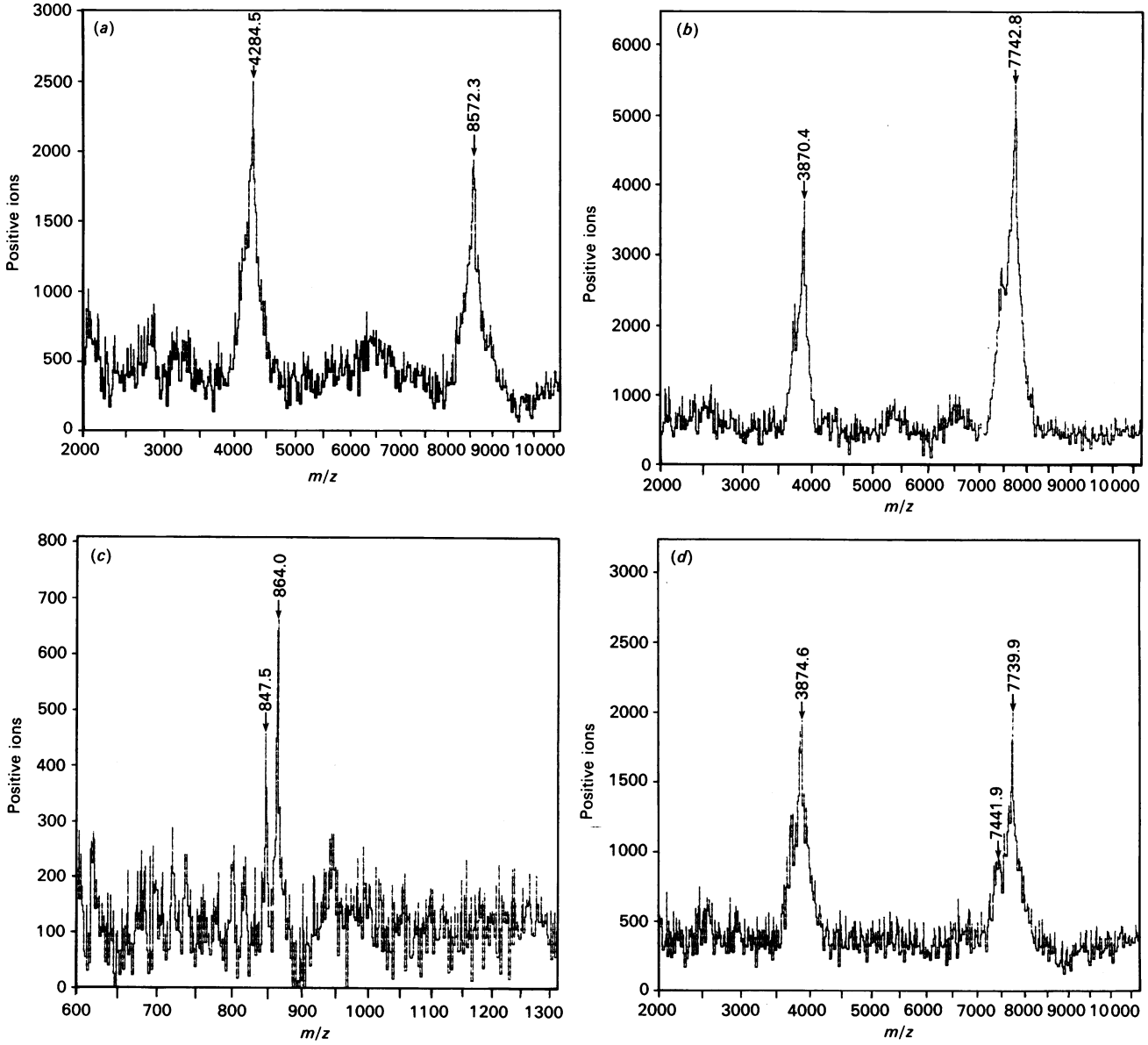

Fig. 3. Plasma-desorption mass spectra of rC5a

(*a*) Native rC5a. The peaks at m/z 8572.3 and 4284.5 are in the predicted locations of the $(M+H)^+$ and $(M+H)^{2+}$ ions of rC5a. (*b*) rC5a inactivated by GBS strain 7360. The peaks at m/z 7742.8 and 3870.4 are in the predicted locations of the $(M+H)^+$ and $(M+H)^{2+}$ ions of a fragment of rC5a missing seven amino acid residues from its *C*-terminus. (*c*) Fraction 1 from the h.p.l.c. separation of the GBS-generated fragment of rC5a. The peak at m/z 847.5 is in the predicted location for the $(M+H)^+$ ion of a heptapeptide from the *C*-terminus of rC5a, and the peak at m/z 864.0 is in the predicted location of the $(M+H)^+$ ion of the same heptapeptide with an oxidized methionine residue. (*d*) Fraction 2 from the h.p.l.c. separation of the GBS-generated fragment of rC5a. The peaks at m/z 7739.9 and 3874.6 correspond to the same $(M+H)^+$ and $(M+H)^{2+}$ ions as in (*b*). The small peak at m/z 7441.9 is in the predicted location of the $(M+H)^+$ ion for the same fragment of rC5a (residues 1–67) with the glutathione moiety removed.

second peak at 4284 results from the doubly charged ion $(M+H)^{2+}$, and, as predicted, has almost exactly one-half the mass-to-charge ration (m/z) of the $(M+H)^+$ ion.

Purified rC5a was incubated with the 7360 strain of GBS and its inactivation was confirmed by functional assay (stimulation of PMN adherence). rC5a that had been exposed to the 7360 strain of GBS had an observed m/z of 7743 for its $(M+H)^+$ ion (Fig. 3*b*). This mass corresponds to the predicted mass of 7744 Da of a fragment of rC5a consisting of amino acid residues 1–67 of the rC5a molecule. rC5a cleaved by the strain 7360 proteinase was subjected to reverse-phase h.p.l.c. and the two major fractions were examined by m.s. Fraction 1 contained two major peaks at m/z 847.5 and 864.0 (Fig. 3*c*). These are approximately equal to the predicted masses of the $(M+H)^+$ ions of the seven-amino-acid-residue *C*-terminal fragment in the unoxidized (847.9 Da predicted) and oxidized (at the methionine residue) forms (863.9 Da predicted). Fraction 2 (Fig. 3*d*) contained a molecule with an observed m/z of 7740 for its $(M+H)^+$ ion. This corresponds to the fragment seen in Fig. 3(*b*). A minor peak with an m/z of 7442 in Fig. 3(*d*) is interpreted to result from limited loss of the glutathione moiety from the same rC5a fragment because this mass difference is not consistent with the calculated change that would result from any other residue losses.

rC5a cleaved by GBS was treated with 0.1 mM-dithiothreitol for 3 h at 37 °C to reduce intra-chain disulphide bridges and to

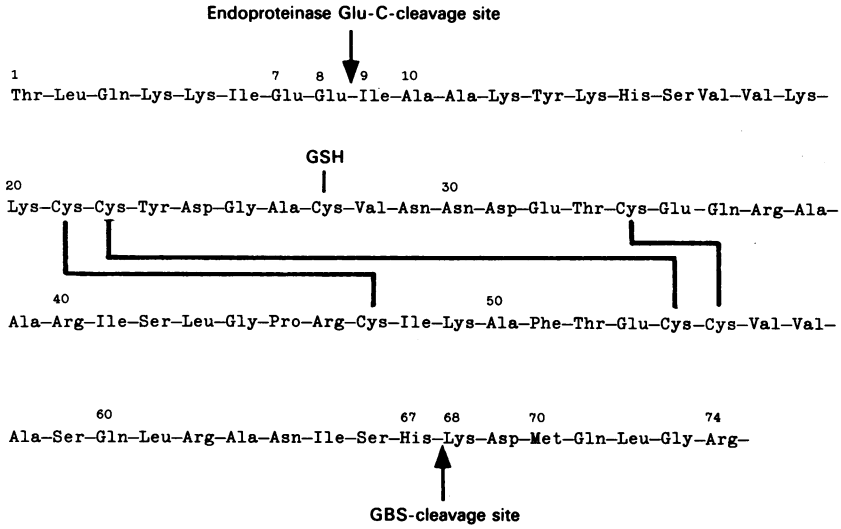

Fig. 4. Schematic structure of rC5a, showing intra-chain disulphide bonds, glutathione side chain and the endoproteinase Glu-C-cleavage site at glutamic acid-8

The proposed GBS-cleavage site is represented between histidine-67 and lysine-68.

Table 3. Plasma-desorption m.s. analysis of rC5a treated with GBS strain 7360

rC5a	Fragment	$(M+H)^+$ calculated (Da)	$(M+H)^+$ observed (m/z)	Figure no.
Native	1–74	8573	8572	3(*a*)
Treated with 7360	1–67	7744	7743	3(*b*)
Treated with 7360, h.p.l.c. fraction:*				
1	68–74	847.9	847.5	3(*c*)
	68–74, with oxidized Met	863.9	864.0	3(*c*)
2	1–67	7744	7740	3(*d*)
Treated with 7360 +dithiothreitol†	1–67 reduced, GSH removed	7445	7445	
Treated with 7360 +endoproteinase Glu-C‡	9–67	6774	6777	

* Treated rC5a fragments separated by h.p.l.c. (see the Materials and methods section for details).
† Treated rC5a was incubated with 0.1 mM-dithiothreitol for 3 h at 37 °C before analysis.
‡ Treated rC5a was incubated with endoproteinase Glu-C before analysis.

remove the glutathione moiety bound to the cysteine residue at position 27. Analysis of this reduced sample revealed a fragment with an m/z of 7445 for its $(M+H)^+$ ion. The predicted mass of this fragment is 7445 Da. Further fragmentation of the rC5a treated with the GBS was not observed after reduction whether analysed by SDS/PAGE (Fig. 2) or m.s. This is consistent with a single cleavage occurring outside the central core of the rC5a, which is held together by internal disulphide bonds (Fig. 4).

We predicted that cleavage would occur at the *C*-terminus since it is known that enzymic cleavage of short sequences from the *C*-terminus of C5a causes inactivation of C5a [15–19]. Removal of six, seven or eight amino acid residues from the *N*-terminus would have yielded residues with $(M+H)^+$ ions with masses of 7860, 7731 and 7602 Da respectively, none of which was observed on m.s. analysis. This supports the proposal that truncation of the rC5a has occurred at the *C*-terminus. Furthermore, analysis of fragments derived from endoproteinase Glu-C treatment of the GBS-derived rC5a fragment was inconsistent with GBS-mediated cleavage at the *N*-terminus. Endoproteinase Glu-C, under the conditions used in the present study, cleaves the *C*-terminal side of glutamic acid residues [14]. Thus rC5a has four potential cleavage sites, at residues 8, 32, 35 and 53. Endoproteinase Glu-C cleavage of the GBS-derived fragment resulted in a fragment with the observed m/z of 6777 for its $(M+H)^+$ ion when analysed under non-reducing conditions. This is very close to the predicted mass of 6774 Da for a fragment containing amino acid residues 9–67 of rC5a. Such a fragment would result from endoproteinase Glu-C cleavage at position 8 of a GBS-derived fragment containing residues 1–67 of rC5a. Further cleavage by endoproteinase Glu-C at positions 32, 35 and 53 does not yield fragments detectable under non-reducing conditions because of the disulphide bonds between the various fragments (Fig. 4). If the GBS proteinase had cleaved a peptide from the *N*-terminus of the rC5a, we would have detected an $(M+H)^+$ ion with an m/z of approx. 7604 after endoproteinase Glu-C digestion corresponding to a polypeptide containing amino acid residues 9–74 of the rC5a molecule. The data from the various spectra are summarized in Table 3.

DISCUSSION

The present studies were undertaken to determine whether GBS can inactivate purified rC5a, and to determine the molecular basis for this inactivation. We found that the 7360 strain of GBS inactivated the capacity of rC5a to stimulate PMNs whereas the GW strain did not. This ability to inactivate rC5a correlated exactly with the previously reported ability of these two strains of GBS to inactivate the chemotactic activity of zymosan-activated serum [3]. We also found that inactivation of rC5a correlated with destruction of the binding of ^{125}I-labelled rC5a to human PMNs. Furthermore, these studies document the exact site of cleavage of the recombinant molecule.

Previous studies have shown that proteolytic cleavage at the *C*-terminus of the C5a molecule inactivates the ability of C5a to stimulate PMNs and decreases C5a binding to its receptor on PMNs [15–19]. The critical involvement of the *C*-terminus of the C5a molecule in PMN activation and receptor binding has been further demonstrated by using site-directed mutagenesis of recombinant C5a [8]. Since the inactivation of rC5a by the 7360 strain was profound, and since SDS/PAGE analysis of rC5a inactivated by GBS strain 7360 migrated with a slightly smaller molecular mass than control rC5a or rC5a exposed to the GW strain, we hypothesized that the 7360 strain possessed a proteinase that cleaves the C5a molecule near the *C*-terminus. This hypothesis was confirmed by plasma-desorption m.s. analysis of rC5a that had been exposed to the 7360 strain. This technique, which is capable of determining the mass of ionized molecules with an accuracy of $\pm 0.1\%$ in the 10000 Da region [20], demonstrated that the product of the treatment of rC5a with the GBS was a molecule with a mass of 7743 Da, which corresponds to the predicted mass of the rC5a molecule lacking its seven *C*-terminal amino acid residues. As a check on whether the *N*-terminus was still intact, susceptibility of the GBS-derived fragment to digestion by endoproteinase Glu-C was examined. Indeed, a further change was detectable under non-reduced conditions by m.s. The mass of the resulting fragment corresponds to the predicted mass of a polypeptide consisting of amino acid residues 9–67 of the rC5a molecule. Such a fragment would be predicted to result from the combined effects of endoproteinase Glu-C cleavage of the first eight *N*-terminal residues of rC5a and the prior removal of its seven *C*-terminal residues. This result supports the contention that the GBS-mediated cleavage occurs at the *C*-terminus of the rC5a molecule, since GBS-mediated cleavage at any site in the *N*-terminus would have resulted in a fragment whose mass would differ significantly from that observed. Moreover, such a fragment would give a markedly different mass change than was seen upon further digestion with endoproteinase Glu-C.

On the basis of these studies, the cleavage site for this GBS proteinase is between the histidine and lysine residues at positions 67 and 68 in the C5a molecule. The C5a-cleaving proteinase activity present in M-protein-positive strains of group A streptococci has been reported to cleave between the lysine and aspartic acid residues at positions 68 and 69 [19]. These data demonstrating a different cleavage site in C5a by the GBS C5a-cleaving proteinase are consistent with our inability to detect the group A streptococcal C5a-cleaving proteinase in GBS by either immunochemical or DNA hybridization techniques [3,21].

The role that such a C5a-cleaving proteinase plays in the pathogenesis of GBS infections is unclear. GBS infections in the human newborn are often associated with inadequate accumulation of PMNs in the infected tissue [2], and thus destruction of the chemotactic factors C5a and $C5a_{desArg}$ by the infecting organism could quite conceivably restrict PMN accumulation in the affected organs. However, other factors may contribute to the poor host response, such as low concentrations of specific opsonizing antibody directed against GBS in newborn babies with systemic GBS infection [22–24]. These factors as well as the potential for the GBS strain to inactivate C5a directly could result in a profoundly altered acute inflammatory response to invasion by these bacteria.

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