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DEFENSINS PURIFIED FROM HUMAN GRANULOCYTES BIND C1q AND ACTIVATE THE CLASSICAL COMPLEMENT PATHWAY LIKE THE TRANSMEMBRANE GLYCOPROTEIN gp41 OF HIV-1

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Abstract—The transmembrane glycoprotein gp41 of HIV-1 contains a C1q binding domain (HIVenv 583–610) and activates the human complement system through the classical pathway. Based on structural and functional similarities between human defensins (human neutrophil peptide, HNP 1-3) and synthetic peptides representing the env 583–610 region of HIV-1, we found it interesting to investigate the C1q binding and complement activating ability of human defensins. Human defensins were purified and characterized by size exclusion chromatography, ultrafiltration, gel electrophoresis and HPLC. The complement activating ability of the purified peptides was assessed in a solid-phase immunoassay. Defensins, fixed to an ELISA plate, were able to bind the C1q subcomponent of the first complement component (C1), triggering the classical pathway of complement activation which led to C4b binding to the plate. Reduction and subsequent alkylation of disulfide bridges of defensins greatly decreased the C1q binding ability but complement activation (C4b binding) remained high. Further acetylation of the reduced defensin peptide resulted in a molecule which bound very little or no C1q but still activated the complement cascade. These phenomena indicate that defensins interact with the complement system via C1q-dependent and C1q-independent mechanisms, and extend the number of functional similarities between defensins and gp41 of HIV-1 to include C1q binding and complement activation.

Key words: defensins, C1q binding, complement activation, gp41.

1. INTRODUCTION

The three defensins HNP-1, 2 and 3 are a family of small (M_w 3500), cationic, natural peptide antibiotics highly abundant in the azurophil granules of granulocytes and in small intestinal Paneth cells (Ganz *et al.*, 1985, 1990; Lehrer, 1993; Ganz and Lehrer, 1994). The defensin molecule typically consists of 29–35 amino acids with a conserved pattern of disulfide linkage between pairs of the six cysteines of the molecule. The existence of analogs in certain invertebrates suggests that they are ancestral components of the host defense system. Defensins effectively kill a wide range of bacteria (*S. aureus*, *P. aeruginosa*, *E. coli*, *C. neoformans*; (Ganz *et al.*, 1985)) and directly inactivate herpes simplex virus type 1 (Daher *et al.*, 1986). Defensins cause lysis of many normal cells (lung-derived cells, endothelial cells; Okrent *et al.*, 1990) and tumour cell types (K562, Molt 4, Raji; Lichtenstein *et al.*, 1988); the lysis is inhibited by as low as 1% fetal calf serum present in the assay medium and by low temperature.

Human serum inactivates animal C-type RNA tumour viruses (Welsh *et al.*, 1975). These viruses activate the classical complement pathway, which leads to the lysis of the virions. The first step of this complement activation is the binding of the C1q component to its viral receptor, to the transmembrane protein (Bartholomew *et al.*, 1978). In the case of Moloney leukemia virus the viral C1q receptor was shown to be the p15E segment of the transmembrane protein.

The transmembrane glycoprotein gp41 of HIV-1 also binds C1q and activates complement (Ebenbichler *et al.*, 1991); the virus is, however, not inactivated and not lysed by human serum because of the viral-membrane-bound complement-restriction factors (Dierich *et al.*, 1993). The

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Abbreviations: NHS, normal human serum; PKC, protein-kinase C; VBS, veronal-buffered saline.

main C1q binding domain on the gp41 molecule was localized to the HIVenv 591–610 region (Thielens *et al.*, 1993; amino acids are numbered in this paper according to Ratner *et al.* (1985)). This investigation showed a crucial role for the disulfide bond and type I reverse turn maintained by the disulfide bridge in positions 605 and 611 in C1q binding ability. This C1q binding region of the transmembrane glycoprotein gp41 of HIV-1 is highly conserved among many type-C and type-D animal retroviruses (including Moloney leukemia virus) as well as human retroviruses (including different HIV-1 and 2 strains; Denner *et al.*, 1994). The HIVenv 583–620 domain was shown to be immunodominant (Gnann *et al.*, 1987) and primer enhancing (Mitchell *et al.*, 1995) and the synthetic peptide representing the domain to be immunosuppressive (Ruegg *et al.*, 1989).

Monell and co-workers presented structural and functional similarities between human defensins and synthetic peptides representing the immunosuppressive/C1q binding domain of gp41 (HIVenv 583–610) of HIV-1 (Monell and Strand, 1994); 20% amino acid sequence homology and shared bioactive form (looped motif) could be shown. Furthermore, it was shown that both defensins and synthetic peptides derived from the HIVenv 583–610 region are able to associate directly with lipid bilayers and cell membranes.

Panyutich *et al.* (1994) described defensin binding to inactivated C1 subcomponents in human serum. Defensin binding to C1-inhibitor (C1inh)-complexed activated C1 forms was shown. To the best of our knowledge complement activating ability of defensin has not been studied until now.

We previously investigated the complement binding and activating properties of the gp41 molecule. We found that gp41 activates the classical pathway of the complement and the binding of the C1q subcomponent is sufficient to block the binding of specific anti-gp41 antibodies (Hidvégi *et al.*, 1993; Füst *et al.*, 1994). We investigated in the same ELISA system if defensins showing structural and functional homologies to gp41 are able to bind C1q and/or activate the complement cascade similarly to gp41.

2. MATERIAL AND METHODS

2.1. *Buffers and reagents*

Phosphate-buffered saline (PBS) contained 137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4 and 1.5 mM KH_2PO_4. The sPBS contained 1.1 mg% additional sucrose. Veronal-buffered saline (VBS) contained 5 mM sodium barbital, 0.15 mM $CaCl_2$, 1 mM $MgCl_2$ and 150 mM NaCl. The VBS containing Mg^{2+}-EGTA was prepared as follows: 1000 ml VBS (without Ca^{2+} and Mg^{2+}) plus 5 mM EGTA and 2 mM $MgCl_2$.

2.2. *Isolation of human granulocytes*

A total of 3×10^{10} granulocytes were isolated from Buffy Coats by dextran sedimentation of erythrocytes followed by Ficoll gradient centrifugation as described by Absolom (1986). Erythrocytes were removed by hypotonic lysis. Leukocytes were washed with sPBS (600***g***, 10 min, 20°C), resuspended in 15 ml PBS containing 0.34 M sucrose, and counted; cell viability estimated by trypan blue staining and found to be >95%.

2.3. *Isolation of defensins (HNP1–3)*

Human neutrophil peptides were isolated as described by Ganz *et al.* (1985). The whole process was carried out on ice. Cells were broken with MSE Type 1077 sonicator (8×10 sec with amplitude 10, 30 sec pause between cycles), unbroken cells and nuclei were removed by low-speed centrifugation (200***g***, 10 min, 4°C) to prepare the granule extract. The extract was centrifuged at 27 000***g***, 30 min, 4°C (Beckman L7 ultracentrifuge, Type 75 fixed-angle rotor) resuspended in 10 ml of 10% (v/v) acetic acid and extracted overnight at 4°C. The extract was cleared at 27 000***g*** and chromatographed on a BioGel P10 (BioRad, Hercules, CA, U.S.A.) column (72×2.5 cm, Pharmacia LKB, Uppsala, Sweden) preequilibrated with 10% (v/v) acetic acid and eluted with this solution at a flow rate of 0.5 ml/min at 4°C. Fractions (7 ml each) containing defensins were pooled, concentrated and desalted by ultrafiltration using a stirred cell apparatus with YM1 membrane (Amicon, Beverly, MA, U.S.A.) and stored in 0.5% (v/v) acetic acid at −20°C in aliquots.

2.4. *Reduction, alkylation and acetylation of defensins*

The freeze-dried defensins were dissolved at 1 mg/ml concentration in 6 M guanidine hydrochloride, 0.4 M Tris–HCl, pH 8.0, and incubated with 20 mM dithiothreitol at 37°C for 3 hr. After reduction, the peptide was incubated with 60 mM iodoacetamide at 4°C for 1 hr. The reduced and alkylated peptide was dialysed against distilled water and concentrated. Some aliquots of the reduced and alkylated defensins were then acetylated with acetic anhydride in 2 M excess. The reduced, alkylated and acetylated peptide was then thoroughly dialysed against distilled water, concentrated and kept at −20°C until use.

In order to check the complete reduction and alkylation of the defensins, free SH groups were detected by standard protocol using Ellmann's reagent, 5,5′-dithiobis(2-nitrobenzoic acid) (Fluka, Switzerland).

2.5. *Gel electrophoresis*

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out in 15% acrylamide (thickness 0.8 mm) by the method of Laemmli (1970). The chromatogram was stained with Coomassie blue.

2.6. *HPLC*

Analytical RP–HPLC of purified defensins was performed on a Delta-Pak RP C_{18} column (3.9 mm × 30 cm) packed with spherical 15 μm silica of 300 Å pore size (Nixon Waters Ltd., Tokyo, Japan) using gradient

elution. Eluent A was 0.1% TFA in water and eluent B was 0.1% TFA in acetonitrile/water (80:20 v/v). After sample injection, a linear gradient from 25% eluent B to 40% eluent B was generated and applied over 30 min at room temperature with a flow rate of 2 ml/min.

2.7. *Proteins*

C1q, the first component of the classical complement pathway, was isolated using standard methods as described before (Arlaud *et al.*, 1979) and kindly provided by Dr G. Arlaud (Institute de Biologie Structurale, Grenoble, France).

HIV-1 recombinant gp41 (the N-terminal 185 amino acids) was produced in *Bacillus megaterium* and purified to homogeneity. The protein was a kind gift of Dr H. Hampl (Abbott Diagnostika, Delkenheim, Germany).

A peptide representing a 20-amino acid fragment (AA 21–40) of the antigen of M_w 16000 of *Mycobacterium tuberculosis* was used as control. The amino acid composition of this peptide (LFAAFPSFAGLRPTFD TRLM) (Bogdán *et al.*, 1997) is similar to the composition of defensins.

2.8. *Enzyme-linked immunosorbent assay*

ELISA tests were performed as described previously (Prohászka *et al.*, 1995). F-form ELISA plates (Greiner, Frickenhausen, Germany) were coated with different amounts of purified defensins or gp41. The peptides were dissolved in distilled water and left to dry in the wells. After washing the wells were incubated with 50 μl of normal human serum (NHS) or with heat-inactivated (56°C, 30 min) human serum, prediluted 1:1 with VBS containing Ca^{2+} and Mg^{2+} or with Mg^{2+}-EGTA serum or with purified C1q (starting concentration 7.5 μg/ml) in indicated cases for 30 min at 37°C. The amount of complement proteins fixed to the plate was determined with specific goat anti-C1q and anti-C4b (Atlantic Antibodies, Stillwater, MN, U.S.A.), and anti-goat peroxidase labeled antibodies (Atlantic Antibodies) using *o*-phenylenediamine (DAKO, Glostrup, Denmark) substrate. The optical density was measured at 490 nm (reference 620 nm).

2.9. *Statistics*

Data were analysed by two sample *t*-test analysis to evaluate the significance level, $p < 0.05$ was considered as significant.

3. RESULTS

3.1. *Purification and purity analysis of defensins*

The fractionation of the crude granule extract was accomplished by gel permeation chromatography on a long BioGel P10 column. Fractions containing defensins (as shown in Fig. 1) were pooled, concentrated and desalted yielding a stock solution of defensins in 0.5% acetic acid. The final concentration of the defensin solution was 0.33 mg/ml as determined by the method of Lowry.

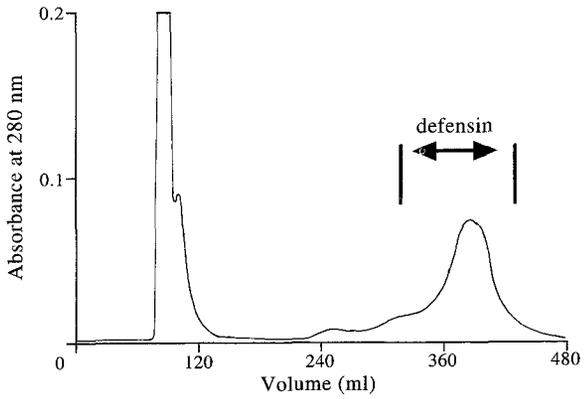

Fig. 1. BioGel P10 chromatography of a human neutrphil granule extract. Approximately 10 ml (equivalent of 3×10^{10} cells) of the extract was chromatographed on a long (72 cm × 2.5 cm, Pharmacia, Uppsala, Sweden) column of BioGel P10 (BioRad Inc, Hercules, CA, U.S.A.). The effluent was monitored at 280 nm. Defensins emerged as a late peak as shown. For details see Section 2.

To characterize the final purity of our purified protein, SDS–PAGE and reverse-phase HPLC were carried out. As shown in Fig. 2 using a 15% SDS gel only one band of protein could be seen in the expected low-molecular-weight (3000–4000) zone (Fig. 2, lanes 1 and 2). We have further analysed the purity of our defensin preparation by reverse-phase HPLC using an acetonitrile–water gradient. The chromatogram is shown in Fig. 3. The peptide was found to emerge as one peak by this method and was substantially free from any contaminant protein.

3.2. *Binding of the C1q subcomponent of C1 to defensins*

To assess the C1q binding capacity of solid-phase defensins, wells of ELISA plates precoated with defensins

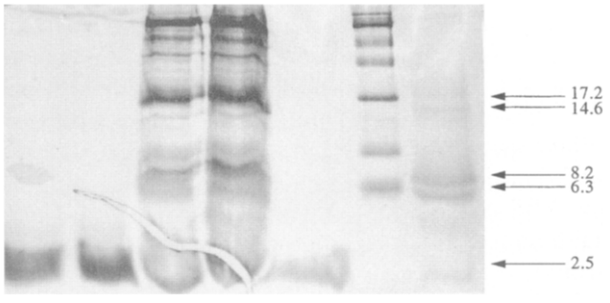

Fig. 2. SDS–PAGE of human neutrophil granule extract and purified defensins. Gel containing 15% polyacrylamide was stained with Coomassie Brilliant Blue. Lanes from the left: 1–2, two batches of defensins (20–20 μg); 3–4, two batches of crude granule extracts; 5, a third batch of defensins (5 μg); 6, molecular weight markers; 7, molecular weight markers (digested myoglobin, Pharmacia, Uppsala Sweden, Code: 17-0551-01).

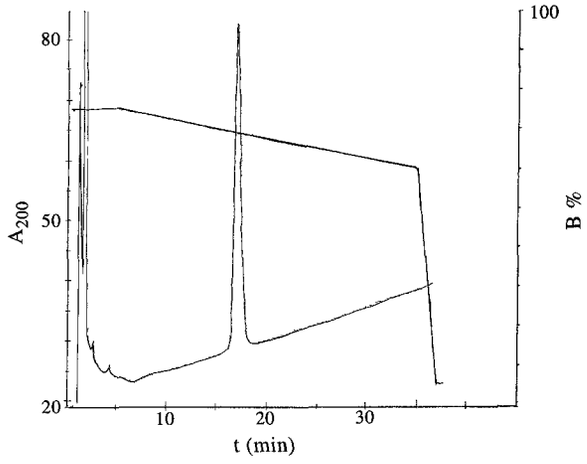

Fig. 3. RP–HPLC Chromatogram of purified human defensins. The concentrated preparation after P10 chromatography was loaded on a Delta-Pak RP-C18 column packed with spherical 15 μm silica of 300 Å pore size (Nixon Waters Ltd., Tokyo, Japan) using gradient acetonitrile–water elution from 25 to 40% eluent B (indicated on the right y-axis) over 30 min at room temperature with a flow rate of 2 ml/min. For details see Section 2. The absorbance of effluent at 220 nm is indicated on the left y-axis.

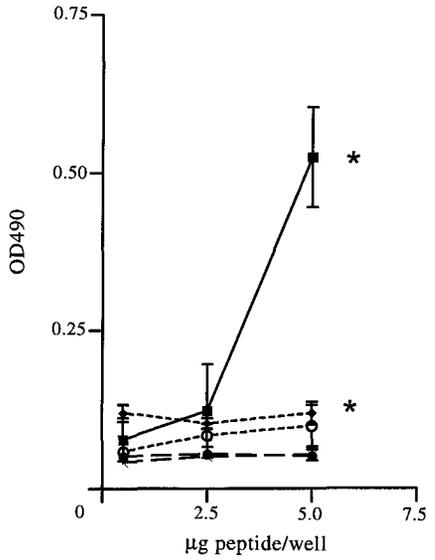

Fig. 4. Binding of the C1q subcomponent of C1 from normal human serum to purified human defensins. Wells of ELISA plates were coated with different amounts of peptides (indicated on the x-axis) followed by washing and incubation with 50 μl normal human serum prediluted 1:1 with VBS containing Ca^{2+} and Mg^{2+} ions for 30 min at 37°C. The amount of C1q fixed to the plate was determined as described in Section 2. – ■ –, defensin-; – ◆ –, reduced and alkylated defensin-; – ▽ –, reduced, alkylated and acetylated defensin-; – • –, control peptide-coated wells; – × –, uncoated wells. The figure shows means and standard deviations of duplicate results of one representative experiment out of four identical ones. The difference between uncoated wells and wells coated with defensins ($p=0.03$), reduced-alkylated defensins ($p=0.01$) is significant whereas in the case of acetylated defensins ($p=0.06$) is statistically not significant as calculated by Student's t-test.

were incubated with normal human serum diluted 1:1 in VBS containing Ca^{2+} and Mg^{2+}; then the amount of plate-bound C1q was determined as described above. As shown in Fig. 4, defensins bound significant amounts of C1q from NHS in a dose-dependent manner. The reduction of the disulfide bridges and alkylation of free SH groups as well as further acetylation of the charged side chains of the reduced and alkylated peptide resulted in a marked decrease of C1q binding, but detectable C1q binding was also seen in both cases. The complete reduction of the peptide was checked by a commercial detecting method using Ellmann's reagent. No free SH groups could be detected in the case of the reduced and alkylated peptide (data not shown). No detectable C1q binding was observed in the case of the control peptide (Fig. 4.)

The C1q binding by native and reduced-alkylated defensin peptides and gp41 was characterized by a further test using purified C1q. As shown in Fig. 5 dose-dependent binding of C1q to defensin-coated wells was detected after incubation with purified C1q diluted in VBS containing Ca^{2+} and Mg^{2+}. This binding is comparable to the C1q binding of gp41. Reduction of the cysteine bridges of the defensin molecule resulted in a marked, but only partial, inhibition of C1q binding; the reduced and alkylated peptide retained significant C1q binding ability.

3.3. *Complement activation by purified human defensins*

Complement activating ability of purified human defensins was determined in an ELISA system. Wells of ELISA plates were coated with different amounts of defensins and incubated with normal human serum. Complement activation was detected through the measurement of the plate-bound C4b fragments, the generation of which is characteristic for the activation of the classical pathway. As shown in Fig. 6, a significant amount of C4b bound to the defensin-coated plate in our solid-phase system as compared to uncoated and control-peptide coated wells. This C4b binding is due to specific complement activation since heat-inactivation (56°C, 30 min) of the serum (Fig. 6C) and the absence of Ca^{2+} and the presence of EGTA (Mg^{2+}-EGTA serum, Fig. 6B) markedly inhibited C4b binding, therefore complement activation triggered by purified human defensins takes place through the classical pathway. This type of complement activation is comparable to that of gp41 as shown in several previous studies. However, in the case of native defensins there are probably at least two ways to activate and bind C4, since Mg^{2+}-EGTA serum and heat inactivation resulted only in a partial impairment of C4b binding (Fig. 6B,C). Reduced and alkylated defensins retained some C4b binding only in the case of the heat-inactivated serum, whereas reduced-alkylated and acetylated peptides did not bind any C4b from heat-inactivated serum or from Mg^{2+}-EGTA serum. The control peptide had no complement activating or C4 binding ability (Fig. 6).

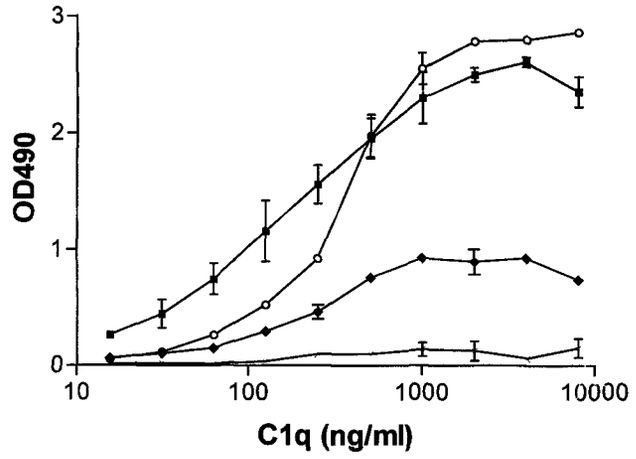

Fig. 5. Direct binding of purified C1q to human defensins. Wells of ELISA plates were coated with defensins (1 μg/well), with reduced and alkylated defensins (1 μg/well) or with gp41 (1 μg/well) and incubated with purified C1q (diluted in VBS containing Ca^{2+} and Mg^{2+} ions by twofold steps, starting at 7.5 μg/ml) as indicated on the *x*-axis (– ■ –, defensin-; – ◆ –, reduced defensin-; – ○ –, gp41-coated wells; – × –, uncoated wells) for 30 min at 37°C. The amount of fixed C1q was determined as described in Section 2. The figure shows means and standard deviations of duplicate results of one representative experiment out of two identical ones. All the three peptides bound significant ($p < 0.01$) amounts of C1q as calculated by Student's *t*-test.

4. DISCUSSION

In this paper we describe the purification and characterization of complement activating ability of human neutrophil peptides (HNP 1–3), known as defensins. Our preparation is comparable to that of Ganz *et al.* (1985) in terms of molecular weight (approximately 3 500 in both studies) and purity (Figs 1–3).

The aim of this study was to assess the complement activating ability of purified human defensins and compare it to the transmembrane glycoprotein gp41 of HIV-1 that was previously shown to activate the classical complement pathway through the binding of the first complement component C1q (Ebenbichler *et al.*, 1991). Since structural and functional similarities between gp41 and human defensins were reported (Monell and Strand, 1994), we tested if there is another functional similarity, i.e. complement activation and binding between these two molecules. In the case of gp41 the existence of a specific C1q binding domain was shown and the binding of C1q through this domain is responsible for the classical pathway complement activating ability of the protein. We investigated the C1q binding and complement activating ability of human defensins because of the sequence, structural and functional homology between the C1q binding domain of the gp41 (HIVenv 583–610) and the defensins.

Here we show that defensins cause similar complement activation to that of gp41; defensins fixed to ELISA plates

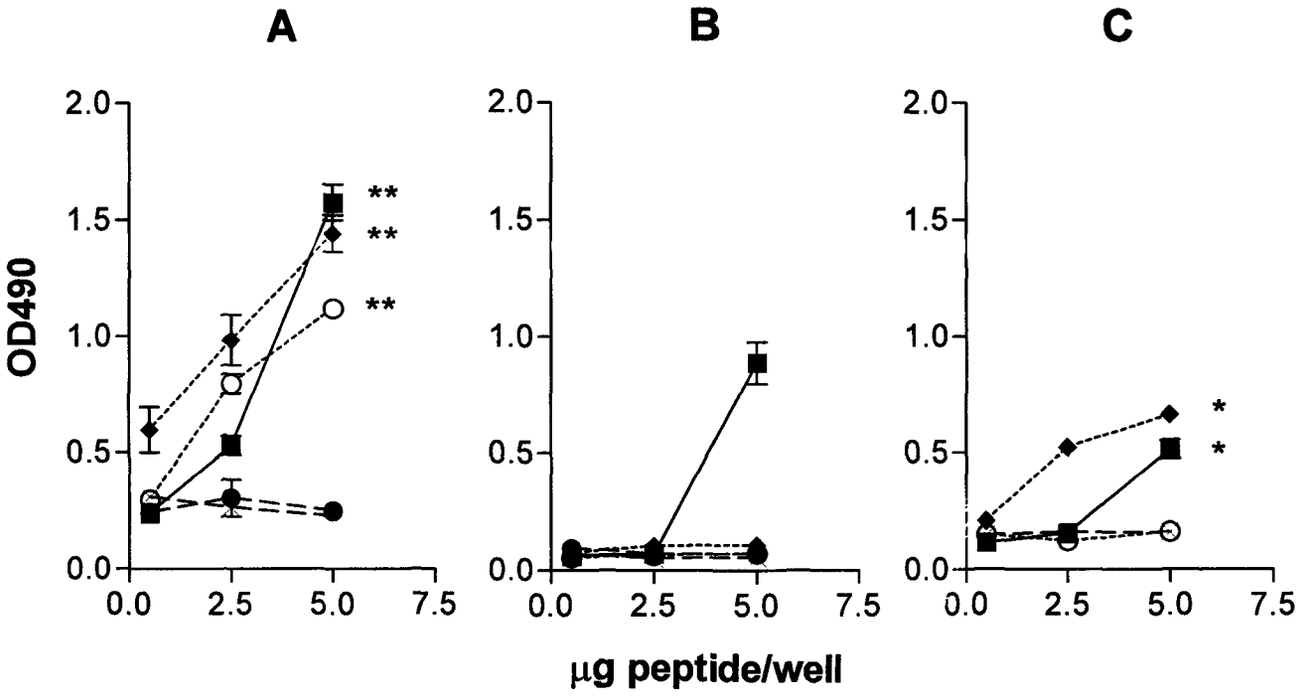

Fig. 6. Complement activation by purified human defensins in ELISA system. Defensin-coated wells (indicated on the *x*-axis) were incubated with normal human serum (panel A), heat-inactivated human serum (panel C, both diluted 1:1 with VBS containing Ca^{2+} and Mg^{2+} ions) or with NHS diluted 1:1 with VBS containing Mg^{2+} and EGTA (panel B) for 30 min at 37°C. The amount of fixed C4b was estimated as described in Section 2. – ■ –, defensin-; – ◆ –, reduced and alkylated defensin-; – ○ –, reduced, alkylated and acetylated defensin-; – · –, control peptide-coated wells; – × –, uncoated wells. The figure shows means and standard deviations of duplicate results of one representative experiment out of three identical ones. Statistically significant C4b binding were seen in the indicated cases ($*p < 0.05$, $**p < 0.01$).

are able to bind the C1q subcomponent of the first component of the classical pathway from macromolecular C1 present in normal human serum and trigger the complement activation through the classical pathway (Figs 4–6). Thus, C1q binding triggers complement activation, which results in C4b binding to the plate. C4b binding is due partially to classical pathway activation since markedly decreased C4b fixation was observed in the case of heat-inactivated and Mg^{2+}-EGTA serum. However, the incomplete reduction of C4b binding from Mg^{2+}-EGTA serum in the case of defensins indicates that some non-specific C4b might also occur. The reduced and alkylated defensins bind much less C1q, whereas the C4b binding ability is even stronger, as compared to the native (non-reduced) form. Furthermore, reduced, alkylated and acetylated defensins bind very few but detectable C1q, but cause similar C4b binding to the plate. The reason for this phenomenon might lie in the altered structure of the peptide, which results in an increased ability to covalently bind activated, nascent C4 forms. On the other hand, we have previously shown (Füst *et al.*, 1978) that C1q binding and C4 activation are not parallel phenomena. We have seen with different immunoglobulin preparations measurable complement activation without C1 fixation and, in contrast, with other preparations strong C1 binding but no complement activation.

According to our opinion native defensins bind significant amounts of C1q (Fig. 4) and they are able to trigger the classical complement pathway after the binding of very few C1q or even after dissociation of C1q (Figs 4 and 6A). Native defensins also possess non-specific C4b binding ability (Fig. 6B,C). Reduction and alkylation of defensins results in impaired C1q but increased C4b binding (Figs 5 and 6A). Reduced defensins retain the capacity to bind heat-destroyed C4b in a non-specific manner (Fig. 6C). Acetylated defensins bind very few or no (or probably dissociate quickly) C1q (Fig. 4), but activate C4 and bind C4b (Fig. 6A) only specifically (Fig. 6B,C).

The reason for this shared functional property of the two molecules might be the partial sequence and secondary structure homology. Although defensins are lytic peptides, their secondary structure is quite different from the structure from several other lytic or membrane-permeabilizing peptides with known secondary structure (such as melittin, cecropin, magainin, alamethicin and δ-hemolysin): these are all amphiphilic α helices containing no β-sheets whereas defensins are all-β-sheet proteins (Hill *et al.*, 1991; White *et al.*, 1995). The C1q binding domain of gp41 is folded as a β-sheet with a type I reverse turn maintained by a disulfide bond, which is a very similar structure to that of the defensins. Taken together, the sequence and partial structural homology may explain the lower level of C1q binding and complement activation by defensins compared to gp41 in our experiments.

There is one more similar point in the C1q binding ability of defensins and gp41: the C1q binding domain in both molecules contains two (or more) neighbouring charged amino acids (ER and KD in gp41 and ERR in defensins) in a looped structure. This situation is similar to immune complexes, the known C1q binding domain consists of the ExKxK motif on Cγ2 of IgG (Duncan and Winter, 1988). These amino acid similarities might explain why acetylation of the positively charged side chains (R) in the defensin molecule results in a sharp drop of C1q binding.

The defensin-complement interaction has also been investigated from another point of view (Panyutich *et al.*, 1994). Defensins, if released from activated or damaged cells, may be very dangerous (Okrent *et al.*, 1990), unless inactivated, defensins may injure host tissues. On the one hand, it was shown that target cell lysis by defensins was inhibited by heat inactivated serum (Lichtenstein *et al.*, 1988; Okrent *et al.*, 1990), the albumin content of the serum could account for the inhibitory effect. On the other hand, Panyutich *et al.* (1994) have shown Ca^{2+}-dependent defensin binding to C1-inhibitor (C1inh) complexed activated C1 forms. Human serum albumin, the major anionic protein of human blood, did not compete with defensin binding to purified C1 subcomponents or inactivated C1, not even in the 100-fold excess in their study. Comparison of our present findings with those of Panyutich *et al.* (1994) point out the difference between the surface-bound and liquid-phase defensins. Surface-bound defensins may serve as opsonins by binding and

Table 1. Known similarities between human defensins and peptides derived from the C1q binding/immunosuppressive domain of gp41 of HIV-1 (HIVenv 583-610)

	Defensins	gp41	Reference
C1q binding	Yes*	Yes, Ca^{2+} dependent[1]	*This study [1]Ebenbichler *et al.*, 1991
Complement activation	Yes, classical pathway*	Yes, classical pathway[2]	[2]Hidvégi *et al.*, 1993 and
Active config.	Cyclic, multimeric[3]	Unknown but critical	[3]Monell and Strand, 1994
Temperature	37°C active, 4°C inactive[4]	37°C active, 4°C inactive[5]	[4]Lichtenstein *et al.*, 1988
Inhibition of PKC	Yes ($IC_{50} = 2\,\mu M$)[6]	Yes ($IC_{50} = 3\text{-}6\,\mu M$)[5]	[5]Ruegg and Strand, 1990
Inhibition of proliferation of	HL-60 promyeloid lymphoid lines, not: L929 murine fibroblasts[7]	Lympkocytes, NK cells, macrophages, not: NIH3T3 murine fibroblasts[8]	[6]Charp *et al.*, 1988 [7]Bateman *et al.*, 1991 [8]Ruegg *et al.*, 1989

activating complement (our study), whereas released defensins must be adsorbed by serum proteins (such as inactivated C1-C1inh forms and α2 macroglobulin, Panyutich's study). The opsonic activity of defensins was shown in the case of rabbit macrophage neutrophil defensins NP-1 and NP-2 by showing increased ingestion of bacteria and fungi by rabbit alveolar macrophages in the presence of these peptides (Fleischmann *et al.*, 1985).

The evolutionary conserved C1q binding property of several different retroviruses as well as the conserved feature of the immunosuppressive-C1q binding-immunodominant domain of gp41(HIVenv 583–610) together with the known similarities between defensins and gp41 prompted us to investigate the complement activation by peptide defensins. As a result of our study the number of known functional similarities between human defensins and gp41 of HIV-1, such as activity at 37°C, PKC-inhibition, inhibition of cell proliferation, could be extended in terms of C1q binding and complement activation (Table 1). Defensins are membrane interacting, complement activating peptides showing similarity to a conserved region of gp41. Therefore, it is very probable that interaction of defensins with complement and subsequent complement activation has a role in defensin action to the host cells on one hand and, on the other hand, the HIVenv 583–610 region of gp41 may play a role in the membrane interactions required for viral fusion and infectivity. Further experiments are needed to determine the role of gp41-C1q interaction in the viro-immunopathogenesis of HIV-1. These experiments are under way in our laboratories.

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