Clin Exp Immunol 1996; **105**:504–510

Epitope mapping of anti-glomerular basement membrane (GBM) antibodies with synthetic peptides

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(Accepted for publication 30 April 1996)

SUMMARY

Autoantibodies to the non-collagenous (NC1) domain of the α3(IV)-chain of type IV collagen are found in sera from patients with anti-GBM nephritis. These antibodies have been shown to be pathogenic. In this study the antibody specificity has been investigated in patients with Goodpasture's syndrome and from a patient with atypical anti-GBM antibodies, recognizing the α1(IV)-chain only. Overlapping synthetic peptides, covering the complete NC1 domains of the α1(IV)- and α3(IV)-chains were used in sandwich ELISA and competitive ELISA. None of the Goodpasture sera showed reactivity to the synthetic peptides. However, antibodies from the patient with atypical anti-GBM antibodies recognized a 20 amino acid peptide from the α1(IV)-chain. The reactive peptide was further narrowed down with glycine substitution of the different amino acids. We have localized the epitope to the four last C-terminal amino acids of the α1(IV)-chain, with the sequence 1754-MRRT. The two arginine residues were found to be essential for antibody binding. Threonine is important, while methionine is of less importance. These four amino acids are also determined to be the smallest peptide that could inhibit the binding of the autoantibodies to the native α1(IV)-chain. This study shows that overlapping peptides can be used to map linear epitopes. However, for conformational epitopes such as the Goodpasture epitope, other methods must be used. It would be prognostically important to know the fine specificity of anti-GBM antibodies, since the patient with anti-α1(IV) antibodies had a mild disease, while the Goodpasture patients with anti-α3(IV) antibodies had a rapidly progressive disease.

Keywords anti-GBM antibodies Goodpasture syndrome type IV collagen synthetic peptides

INTRODUCTION

Basement membranes, which provide a continuous layer between the endothelial and the underlying connective tissue, comprise a variety of macromolecules, e.g. type IV collagen, laminin, entactin and heparan sulphate protoglycan. Type IV collagen is one of the main structural components of basement membranes, and the molecule is composed of three subunits, so called α(IV)-chains. In the N-terminal and middle region the three α(IV)-chains are intertwined to a triple helical structure, while in the C-terminal region each chain is folded into a globular domain [1]. The globular domain is called NC1 (non-collagenous). Normally, type IV collagen is composed of two distinct α(IV)-chains [2], the classical α1(IV) and α2(IV). However, in the GBM three additional α(IV)-chains have been identified, α3(IV), α4(IV) and α5(IV) [3,4] and the gene for a sixth, α6(IV), has recently been discovered [5]. It has been shown that α3(IV)- and α4(IV)-chains co-localize and are only present in specialized basement membranes, for example in the kidney, eye, cochlea, lung and brain, whereas the α1(IV)- and α2(IV)-chains are present in all basement membranes [6]. Results show that the α5(IV)- and α6(IV)-chains also have a limited distribution in different basement membranes [5,7,8].

Patients with the autoimmune disease Goodpasture's syndrome have autoantibodies to type IV collagen. The disease is characterized by a rapidly progressive glomerulonephritis, lung haemorrhage and a linear deposit of IgG along the GBM [9]. The autoantigen is localized to the NC1 domain of the α3(IV)-chain [10,11]. However, in most patients there are also autoantibodies present to the other α(IV)-chains, including the α1(IV)-chain [12,13]. We have found a patient with a mild form of glomerulonephritis even though he has a high titre of anti-GBM antibodies. This patient was found to have antibodies to the NC1 domain of the α1(IV)-chain, but not to the α3(IV)-chain [14] as the Goodpasture patients have. Furthermore, the autoantibodies were found to be more reactive with reduced and alkylated α1(IV)NC1 than native α1(IV). The antibodies were of the IgG3 subclass, with λ light chains exclusively. This suggests a MoAb reacting with a linear epitope.

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There are several reports discussing specificity of Goodpasture antibodies and their epitopes. The epitope has been localized to the NC1 domain of the α3(IV)-chain, and it has been shown to be a conformational epitope depending on intact intrachain disulphide bonds [15]. The epitope recognized by the Goodpasture antibodies has further been studied with purified bovine or human antigens [12,13], with recombinant NC1 domains of the different α(IV)-chains [16], and recently two different studies have been published [17,18] in which synthetic peptides have been used to identify the epitope. It was shown that the last 36 amino acids are part of the epitope [18]. In summary, the α3(IV)-chain is the major antigen and probably contains one major epitope [12].

However, in these earlier studies using synthetic peptides only selected parts of the α3(IV)-chain were used in the search for the Goodpasture epitope. As we wanted to identify other parts of the epitope as well as to confirm the presence of reactivity to the last 36 residues, we synthesized overlapping peptides covering the complete NC1 domains of the α1(IV)- and α3(IV)-chains. The reactivity of anti-GBM antibodies in sera from Goodpasture patients has been compared with serum from a patient with anti-α1(IV) antibodies.

PATIENTS AND METHODS

Antibodies

Human autoantibodies were from serum of a patient with anti-α1(IV) antibodies [14], and sera from 10 selected patients with Goodpasture's syndrome, previously characterized [12] as having 1% of their IgG to the α3(IV)-chain, of which ≈10% were cross-reactive with the other α(IV)-chains. Sera from five healthy blood donors were used as controls.

Synthesis of peptides

Sixty-four peptides were synthesized from human sequences, using the Fmoc method, and cleaved from the resin. The quality was controlled by high-performance liquid chromatography (HPLC) and Fab mass spectroscopy. All peptides were free C-terminal amides and had free amino termini. The overlapping peptides from the α1(IV)-chain were 20 amino acids long, with an overlap of five amino acids, except peptide A117 that has an overlap of 16 amino acids. The peptides used in the glycine substitution experiments were 10 amino acids long and the peptides used to detect the shortest possible reactive peptide ranged from 18 to four amino acids. All peptides from the α1(IV)-chain are listed in Table 1 and the peptides from α3(IV) in Table 2. Peptides A31–A318 from the α3(IV)-chain were 20 amino acids long with an overlap of five amino acids. Peptide A3J4 is 36 amino acids long with an overlap of 22 amino acids. Peptides A3J5 and A3J7 are 19 and 15 amino acids long, respectively, and contain one disulphide bond each. Peptides A117L, A117C, A3J4, A3J5 and A3J7 were extensively purified using HPLC. One 20 amino acid-long peptide from the C-terminal end of the α5(IV)-chain, with the sequence -LKAGDLRTRISRCQVCMKRT, was also synthesized.

Purification of bovine α(IV)-chains

Bovine α(IV)NC1 domains were obtained as previously described [12,19]. Some of the NC1 hexamer was reduced and alkylated with DTT (1,4 dithiothreitol) and iodoacetamide [20].

Table 1. All the peptides from the α1(IV)-chain are listed.

Peptide number	Peptide Sequence
A11	FPGPPGPDGLPGSMGPPGTP
A12	PPGTPSVDHGFLVTRHSQTI
A13	HSQTIDDPQCPSGTKILYHG
A14	ILYHGYSLLYVQGNERAHGQ
A15	RAHGQDLGTAGSCLRKFSTM
A16	KFSTMPFLFCNINNVCNFAS
A17	CNFASRNDYSYWLSTPEPMP
A18	PEPMPMSMAPITGENIRPFI
A19	IRPFISRCAVCEAPAMVMAV
A110	MVMAVHSQTIQIPPCPSGWS
A111	PSGWSSLWIGYSFVMHTSAG
A112	HTSAGAEGSGQALASPGSCL
A113	PGSCLEEFRSAPFIECHGRG
A114	CHGRGTCNYYANAYSFWLAT
A115	FWLATIERSEMFKKPTPSTL
A116	TPSTLKAGELRTHVSRCQVC
A117	LKAGELRTHVSRCQVCMRRT
A117L	LKAGELRTHVSRCQVCMRRT
A117C	LKAGELRTHVSRCQVCMRRT
A1N1	AGELRTHVSRCQVCMRRT
A1N2	ELRTHVSRCQVCMRRT
A1N3	RTHVSRCQVCMRRT
A1N4	HVSRCQVCMRRT
A1N5	SRCQVCMRRT
A1N6	CQVCMRRT
A1N7	VCMRRT
A1N8	MRRT
A1C1	AGELRTHVSRCQVCMR
A1C2	AGELRTHVSRCQVC
A1C3	AGELRTHVSRCQ
A1C4	AGELRTHVSR
A1C5	AGELRTHV
A1C6	AGELRT
A1C7	AGEL
A1S1	SRCQVCMRRG
A1S2	SRCQVCMRGT
A1S3	SRCQVCMGRT
A1S4	SRCQVCGRRT
A1S5	SRCQVGMRRT
A1S6	SRCQGCMRRT
A1S7	SRCGVCMRRT
A1S8	SRGQVCMRRT
A1S9	SGCQVCMRRT
A1S10	GRCQVCMRRT

Peptides A11–A117C are used in the epitope mapping experiment. Peptides A1N1–A1S10 are from the sequence of peptide A117 where the antibody reactivity was found. The line under peptide A117C indicate a disulphide bond.

ELISA

Polystyrene microtitre plates (Nunc Immunoplate, Roskilde, Denmark) were coated with 100 μl of the bovine α(IV)-chains, diluted to 0·2 μg/ml in 50 mM Tris–HCl pH 7·4, 6 M guanidine–HCl. The

Table 2. All the peptides from the α3(IV)-chain are listed.

Peptide number	Peptide Sequence
A31	PCGPRGKPGKDGKPGTPGPA
A32	TPGPAGEKGNKGSKGEPGPA
A33	EPGPAGSDGLPGLKGKRGDS
A34	KRGDSGSPATWTTRGFVFTR
A35	FVFTRHSQTTAIPSCPEGTV
A36	PEGTVPLYSGFSFLFVQGNQ
A37	VQGNQRAHGQDLGTLGSCLQ
A38	GSCLQRFTTMPFLFCNVNDV
A39	NVNDVCNFASRNDYSYWLST
A310	YWLSTPALMPMNMAPITGRA
A311	ITGRALEPYISRCTVCEGPA
A312	CEGPAIAIAVHSQTTDIPPC
A313	DIPPCPHGWISLWKGFSFIM
A314	FSFIMFTSAGSEGTGQALAS
A315	QALASPGSCLEEFRASPFLE
A317	SNSYSFWLASLNPERMFRKP
A318	MFRKPIPSTVKAGELEKIIS
A3J4	LNPERMFRKPIPSTVKAGELEKIISRCQVCMKKRH
A3J5	ASPFLECHGRGTCNYYSNS
A3J7	FLFCNVNDVCNFASR

Peptides A3J5 and A3J7 contain one disulphide bond, each indicated with a line. Peptide A3J4 is previously reported [18] to contain reactivity with Goodpasture antibodies.

plates were incubated overnight at room temperature. The peptides were dissolved in F_3EtOH and transferred to the microtitre plate (0·5 μg/well) and dried out overnight as previously described [21]. The plates were then washed three times with 0·15 M NaCl, 0·05% (v/v) Tween 20. Human sera (100 μl), diluted 1:200 in PBS (1·5 mM KH_2PO_4, 8 mM Na_2HPO_4, 0·12 M NaCl, 2·5 mM KCl, 0·05% (w/v) NaN_3) containing 0·2% (w/v) bovine serum albumin (BSA) pH 7·3, were added to each well. The plates were incubated at room temperature for 1 h and after washing, alkaline phosphatase-conjugated swine anti-human IgG (Orion Diagnostica AB, Trosa, Sweden) diluted 1:500 in PBS–BSA was added and incubated for 1 h. *p*-nitrophenyl phosphate (1 mg/ml; Sigma Chemical Co., St Louis, MO) in substrate buffer (1 M diethanolamine, 0·5 mM $MgCl_2$ pH 9·8) was used as substrate and colour development was measured spectrophotometrically at 405 nm. All assays were made in duplicates and when standard errors >5% were found the sample was reanalysed.

Competitive ELISA

The plates were treated as described above, but the human sera were diluted 1:500 in PBS buffer pH 7·3 and preincubated overnight at 4°C in a preincubation plate (Nunc lowbinding) with the different inhibitors in concentrations from 0·0025 to 250 μg/ml. Bound antibodies were detected with alkaline phosphatase-conjugated secondary antibodies as described above.

RESULTS

Antibody specificity

The conformational nature of the epitopes was investigated by testing Goodpasture antibodies and antibodies from the patient with atypical anti-GBM antibodies for reactivity against native, denaturated and reduced and alkylated NC1 hexamers in a sandwich ELISA. The Goodpasture antibodies did bind the native and denaturated antigen, but no reaction was found to the reduced and alkylated antigen, whereas the atypical anti-GBM antibodies increased their reactivity when the antigen was reduced and alkylated. In a competitive ELISA we were able to calculate the reactivity to the different antigens. Fifty percent inhibition of the Goodpasture antibodies was reached with 0·8 μg/ml of the denaturated antigen (NC1 hexamers boiled for 5 min in 1 M Gu-HCl and then diluted), while 60 times more had to be used of the native antigen, that is 50 μg/ml. When using the reduced antigen we could not inhibit the reaction to 50%. However, to reach 20% inhibition we needed over 2000 times more of the reduced and alkylated antigen compared with the denaturated antigen (Fig. 1). Antibody to the α1(IV)-chain increased its reactivity by 100 times compared with the denaturated antigen, when the antigen was reduced.

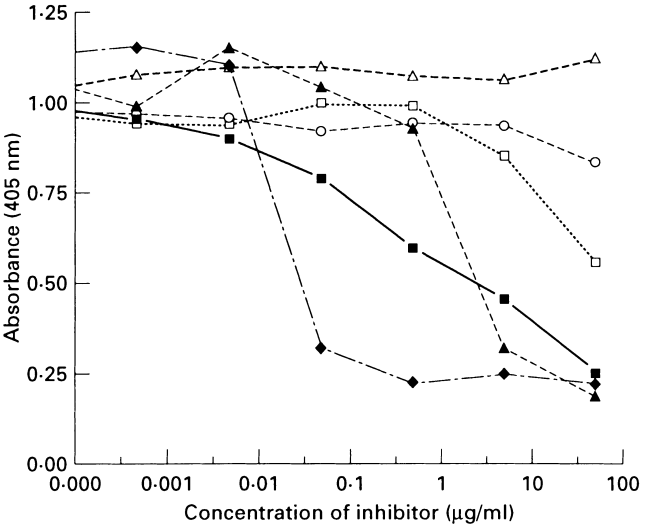

Fig. 1. A competitive ELISA where bovine NC1 hexamers are used as antigen. The Goodpasture antibodies and the atypical anti-GBM antibodies were inhibited with native, denaturated and reduced and alkylated NC1 hexamers from bovine kidneys. The Goodpasture antibodies prefer a denaturated antigen, whereas the atypical anti-GBM antibodies prefer a reduced antigen. □, Goodpasture sera inhibited with native antigen; ■, Goodpasture sera inhibited with denatured antigen; ○, Goodpasture sera inhibited with reduced and ankylated antigen; △, atypical anti-GBM antibodies inhibited with native antigen; ▲, atypical anti-GBM antibodies inhibited with denatured antigen; ◆, atypical anti-GBM antibodies inhibited with reduced and ankylated antigen.

Epitope mapping of the α3(IV)-chain

All the peptides from the α3(IV)-chain (see Table 2) were coated in ELISA plates and tested against patients' sera. All the Goodpasture sera reacted with peptide A3J7 and peptide A312 (data not shown). There was no significant difference between antibodies from normal healthy blood donors, the patient with anti-α1(IV) antibodies and patients with Goodpasture antibodies. The reactivity must therefore be explained as a non-specific binding to the peptides. Furthermore, the peptides could not inhibit antibodies from binding to the α3(IV)-chain in a competitive ELISA. Since peptide A3J4 is reported to contain a reactive epitope, this peptide was resynthesized together with peptide A312 and tested. No reactivity could be found. Thus no specific interaction to any of the peptides from the α3(IV)-chain could be found with this technique.

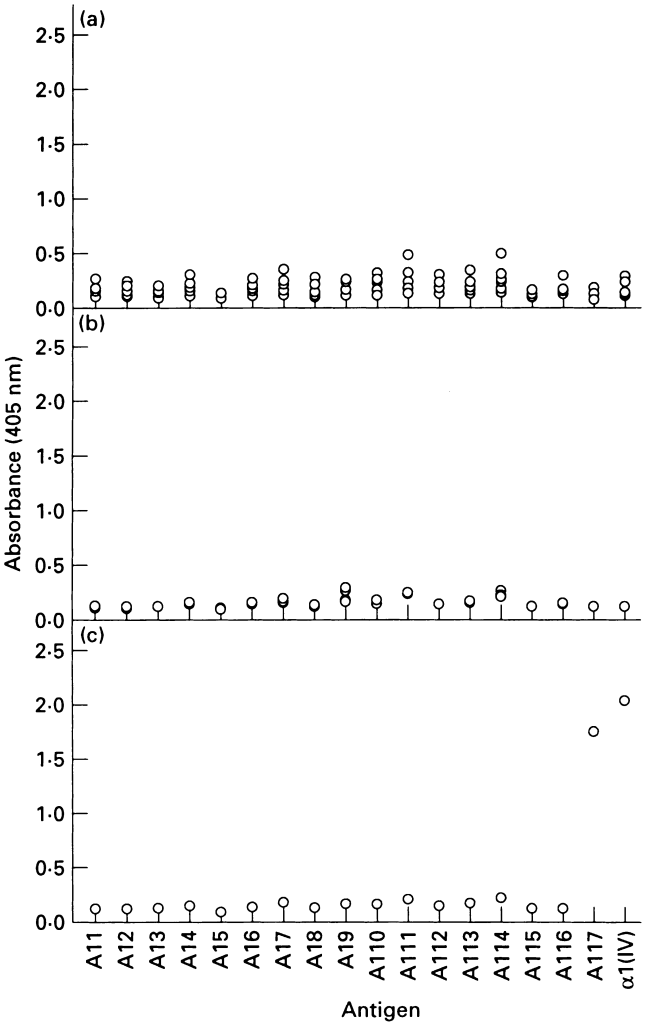

Fig. 2. Human sera are tested with all the 17 overlapping peptides from the α1(IV)-chain and bovine α1(IV) used as antigens in direct ELISA. (a) Goodpasture patients. (b) Healthy blood donors. (c) Atypical anti-GBM antibody.

Epitope mapping of the α1(IV)-chain

All 17 overlapping peptides from the α1(IV)-chain were coated in ELISA plates and tested against patients' antibodies. In sera from the patient with anti-α1(IV) antibodies, all reactivity was found against peptide number A117 (Fig. 2c). No reactivity to the other peptides was found, neither by sera from healthy blood donors (Fig. 2b) nor from patients with Goodpasture's syndrome (Fig. 2a). Reactivity to the peptide A117 could be inhibited with the α1(IV) and peptide A117. Reactivity to the bovine α1(IV)NC1 was inhibited by the purified α1(IV)-chain and peptide A117, indicating that peptide A117 contains the linear epitope to which the patient has autoantibodies. However, to avoid false-positive results arising from impurities or unwanted polymerizations, we synthesized two additional peptides. The first is called A117 L and is identical to peptide A117 except that the cysteine residues are blocked. The second is called A117C and this peptide is a cyclic peptide, with a disulphide bond between the two cysteine residues. The peptides A117 L and A117C were extensively purified by HPLC. Both peptides and the original peptide A117 reacted with the antibodies.

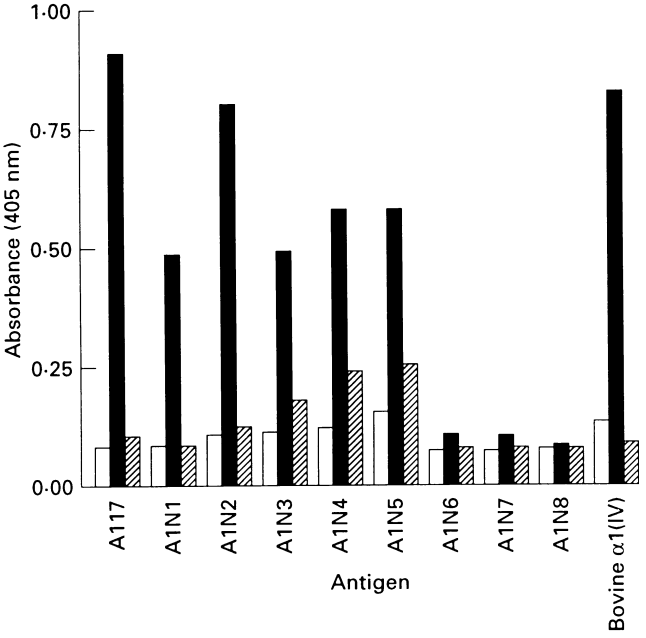

Fig. 3. The result from the direct ELISA in which the shortest reactive fragment of peptide A117 was determined. Peptides A1N1–A1N8, peptide A117 and bovine α1(IV) were bound to the ELISA plate and bound anti-α1(IV) antibodies were measured spectrophotometrically. □, Goodpasture serum; ■, atypical anti-GBM patient; ▨, healthy blood donor.

Identification of the crucial amino acid residues

In order to determine the shortest peptide that still could bind the atypical anti-GBM antibody, eight new peptides, A1N1–A1N8 (Table 1) from the sequence of peptide A117, were synthesized. Each new peptide lacks two N-terminal amino acids. The peptides A117, A1N1–A1N8 and bovine α1(IV) were bound to a microtitre plate, and in sandwich ELISA we found that the shortest peptide that bound the anti-α1(IV) antibodies was peptide A1N5, that is composed of 10 amino acids (Fig. 3). On the other hand, in competitive ELISA even the shortest peptide, A1N8, was able to inhibit the binding of the anti-α1(IV) antibodies to bovine α1(IV). The concentration of the peptides to reach 20% and 50% inhibition is calculated and shown in Table 3. Note that reactivity increased when the peptide became shorter, except for the shortest peptide. Still, the intact reduced protein reacted 200 times better, in molar equivalents, than the most reactive peptide, A1N7. This indicates that the antibodies only need the four last residues to bind.

We tried to shorten the peptide A117 from the C-terminal end, peptides A1C1–A1C7 (Table 1), but all reactivity disappeared in both sandwich and competitive ELISA when the two most C-terminal amino acids where removed (peptide A1C1) (data not shown).

We next synthesized 10 peptides to identify critical amino acids, A1S1–A1S10 (Table 1), of the 10 last amino acids of peptide A117. We choose 10 amino acid-long peptides, to be able to detect the reactivity in sandwich ELISA, as the 10 amino acid-long peptide A1N5 was the shortest peptide that was recognized in the sandwich ELISA. In each peptide, one amino acid was substituted with a glycine residue. The peptides were tested using sandwich ELISA (Fig. 4) and competitive ELISA. The results further support the results that the four last amino acids contain the epitope, and that none of the other residues is of any

Table 3. The concentrations of the peptides A1N1–A1N8, A117 and bovine α1(IV) that are needed to reach 50% and 20% inhibition of the binding of the atypical anti-GBM antibodies to bovine α1(IV) in a competitive ELISA, are calculated and the values are presented (nM).

Peptide	Fifty percent inhibition (nM)	Twenty percent inhibition (nM)
A117	6000	200
A1N1	4400	550
A1N2	3800	430
A1N3	1200	180
A1N4	1000	130
A1N5	280	20
A1N6	250	40
A1N7	170	30
A1N8	2200	750
α1(IV) chain	0·8	0·15

Table 4. The concentrations of the peptides A1S1–A1S10, A1N5 and bovine α1(IV) that are needed to reach 50% and 20% inhibition of the binding of the atypical anti-GBM antibodies to bovine α1(IV) in a competitive ELISA

Peptide	Fifty percent inhibition (nM)	Twenty percent inhibition (nM)
A1N5	250	20
A1S1	2000	400
A1S2	—	—
A1S3	—	—
A1S4	300	50
A1S5	290	40
A1S6	80	15
A1S7	270	40
A1S8	20	4
A1S9	300	50
A1S10	300	45
α1(IV) chain	0·8	0·1

significance. The concentration of peptide to reach 20% and 50% inhibition in the competitive ELISA is calculated and shown in Table 4. When the threonine was substituted in peptide A1S1 the reactivity decreased 10 times compared with the original 10 amino acid-long peptide, A1N5. Moreover, if one of the two arginine residues in the C-terminal end, that is peptides A1S2 and A1S3, was substituted all reactivity was lost. Surprisingly, reactivity to peptides A1S6 and A1S8, where a valanine and a cysteine residue, respectively, is substituted, increased despite the fact that these two peptides had a lower reactivity in the sandwich ELISA. It strongly indicates that the two arginine residues are essential for antibody recognition as well as the threonine, whereas the methionine is less important for antibody recognition.

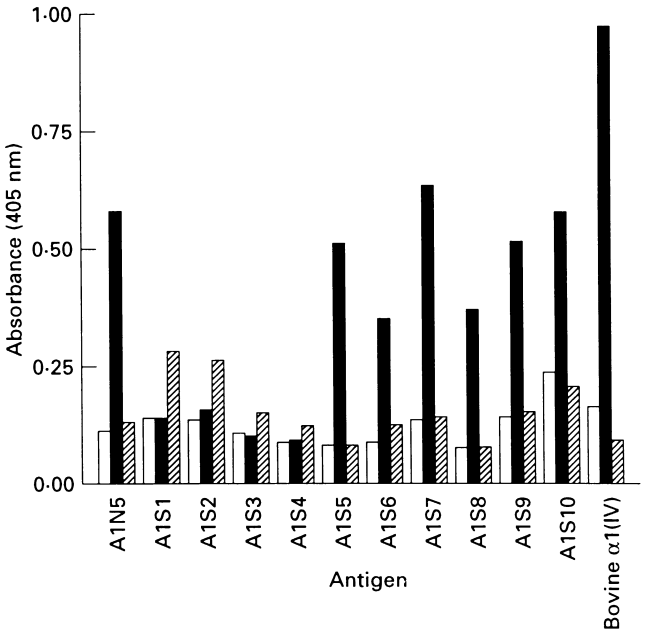

Fig. 4. Direct ELISA with the A1N5, containing the 10 C-terminal amino acids, and the peptides A1S1–A1S10 in which each one of the amino acids is substituted with a glycin residue. Bovine α1(IV)-chain was used as positive control. □, Goodpasture serum; ■, atypical anti-GBM patient; ▨, healthy blood donor.

In spite of the large homology beween the α1(IV) and α3(IV), the anti-α1(IV) antibody did not react with the peptide covering the same area on the α3(IV)-chain (A3J4). Since homology to the α5(IV)-chain is even larger, we also synthesized a peptide of the last 20 C-terminal amino acids from the α5(IV)-chain. The epitope was found to be -MRRT and in the α5(IV) sequence the corresponding residues are -MKRT. The antibodies reacted almost as much with the α5(IV) peptide as with the α1(IV) peptide in sandwich ELISA. In competitive ELISA the small difference was confirmed, and only about 15% of reactivity was lost.

In short, we characterized the epitope for the anti-α1(IV) antibodies from a patient with an atypical anti-GBM nephritis. The antibodies recognized an epitope that is composed of the four last amino acids in the C-terminal end of the α1(IV)-chain. Furthermore, we could not localize the epitope for the anti-α3(IV) antibodies, using synthetic peptides. This further supports the theory that the Goodpasture epitope is conformational and that intact disulphide bonds are essential.

DISCUSSION

Anti-GBM antibodies are found in patients with rapidly progressive glomerulonephritis. However, there are differences in clinical features such as progression rate of the renal disease and severity of pulmonary involvement. There have earlier been indications that the fine specificity of the antibodies could have prognostic value. With this background we wanted to identify epitopes involved in anti-GBM nephritis.

The Goodpasture antibodies recognize an epitope that is situated on the α3(IV)-chain that depends on intact disulphide bonds. Therefore, it is not surprising that Goodpasture antibodies did not show reactivity to the synthetic peptides. In an earlier investigation it was proposed that the last 36 C-terminal amino acids of the α3(IV)-chain [18], identical to our peptide A3J4, are involved in the Goodpasture epitope. However, in this study we could not confirm this.

The atypical anti-GBM epitope is localized to the last four C-terminal amino acids on the α1(IV)-chain with the sequence -MRRT. There is a large homology between the α1(IV) sequence and the sequences of the α3(IV)- and α5(IV)-chains. The last C-terminal amino acids on the α3(IV)-chain are -CMKKRH and on the α5(IV)-chain -CMKRT. The region from all three chains is positively charged at neutral pH, and the fact that the antibodies do not react with the peptide from the α3(IV)-chain is a good control that we are not measuring a false reactivity, due to the positive charge. The C-terminal end of the α5(IV)-chain differs from the α1(IV)-chain by only one single amino acid—an arginine is substituted with a lysine—and since both arginine and lysine have basic side chains that fill approximately the same space, it is not surprising that it cross-reacts with a peptide from the same area of the α5(IV)-chain. However, the cross-reactivity is much lower when using the native purified bovine α5(IV)-chain. This may indicate a different folding of the C-terminus of the α5(IV)-chain than the α1(IV)-chain. It could also be due to a different C-terminal sequence in the bovine protein from the human.

This study shows that results reached by short or middle sized synthetic peptides depend on which type of assay is used. Peptide A1N8 is recognized in a competitive ELISA when it is free in solution, but it is not recognized when it is bound to the microtitre plate in the direct ELISA. Though we used synthetic peptides of very high quality and extensively purified, we nonetheless had problems with non-specific binding of antibodies. This is clearly shown with peptides A3J7 and A312 from the α3(IV)-chain. All sera tested both from humans and rabbits have shown reactivity to this peptide, indicating a non-specific interaction. Thus it is important to confirm found recognition sites with additional peptides, for example with glycin substitution.

The epitopes that are found with synthetic peptides differ from those that are found with other techniques, such as x-ray diffraction of crystallized complexes. With the x-ray diffraction technique often more than 15, sometimes up to 30, amino acid residues take part in the antibody–antigen interaction. We have, however, shown that a four residue peptide can completely block the antibody binding. This difference is due to a different conception of an epitope as discussed by Laver and co-workers [22]. The residues that are recognized by the binding site on the antibody are $\approx$4–8 amino acids. These amino acids bind very tight, and are those that one could expect to find by using synthetic peptides. However, when an antibody binds to a native protein it covers an area of 7 nm^2, and on this surface several additional bonds between the antibody and the antigen occur. If all these bonds are counted as part of the epitope, then of course, synthetic peptides are not the tool to use for epitope mapping, but if the epitope is the smallest part of the protein that can bind and block the antibody binding site, synthetic peptides are the substance of choice. Synthetic peptides have, however, a disadvantage if the epitope is conformational. It could then be very difficult to find the amino acids that are involved, and one may have to use a peptide library to search for mimotopes.

We have studied two categories of anti-GBM antibodies. Both types are antibodies to the NC1 domain of type IV collagen: first, the Goodpasture antibodies that recognize a conformational epitope on the α3(IV)-chain that is destroyed if the disulphide bonds are broken, and leading to a rapidly progressive glomerulonephritis; second, atypical anti-GBM antibodies that react with a linear epitope on the α1(IV)-chain, determined to the four last amino acids in the C-terminal end in this investigation, from a patient with a mild and stable glomerulonephritis. The difference in the progression rate of the disease between these two patients could be ascribed to the specificity of the antibodies. The harmlessness of the anti-α1(IV) antibody is probably because these antibodies do not bind very easily to the GBM, since first, they prefer a more denaturated molecule, and second, there is less α1(IV) in the GBM than α3(IV). Nevertheless, the α1(IV)-chain is found in all basement membranes, and when tissue damage appears the epitope will be exposed and the anti-α1(IV) antibody could recognize the epitope and initiate an inflammatory response.

ACKNOWLEDGMENTS

This work was supported by the Swedish Medical Research Council no. 16X-09487, Riksförbundet för Njursjuka, Sweden, the Crafoord Foundation, Sweden, and the Thelma Zoégas Foundation, Sweden. The authors thank Barbro Persson for skilful technical assistance.

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