

A frequent target of paraproteins in the sera of patients with multiple myeloma and MGUS

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Antigenic targets of monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) paraproteins might play a role in the pathogenesis of these neoplasms. We screened a human fetal brain-derived macroarray with the IgA or IgG containing sera of 192 consecutive MGUS and MM patients. Twenty-nine of 192 (15.1%) paraproteins reacted with paratarg-7, a protein of unknown function which is expressed in all human tissues. Paratarg-7 reactivity was similarly frequent among IgA and IgG paraproteins, but all paratarg-7 reactive IgG paraproteins belonged to the IgG3 subtype with 24/57 IgG3 (42.1%) paraproteins displaying this specificity. Sequence analysis of paratarg-7 derived from patients having a paraprotein with specificity for paratarg-7 revealed no differences to paratarg-7 derived from patients with paraproteins of other specificities or healthy controls, excluding mutations or polymorphisms as a reason for its autoimmunogenicity. Similarly, Western-blot analysis showed identical bands for paratarg-7 derived from patients and controls. The above-random frequency of paratarg-7 as a paraprotein target suggests that paratarg-7 might be involved in the development of the respective clonal proliferations. The identification of paratarg-7 as an antigenic target enables the more detailed analysis of tumor–host interactions in these patients and their role in the pathogenesis of MM and MGUS.

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Key words: multiple myeloma; monoclonal gammopathy of undetermined significance; paraprotein; antigenic stimulation; antigen identification

The identification of the antigenic stimuli of B-cell neoplasms might have considerable impact, because a causal relationship between these neoplasms and antigenic stimulation has been suggested.^{1,2} To date, antigenic targets of paraproteins were discovered accidentally due to clinical symptoms caused by the paraprotein (*e.g.*, chronic cold agglutinin disease or cryoglobulinemia³ or bleeding disorder⁴), because of interference of the paraprotein with laboratory tests ordered for the clinical work-up of the patient (*e.g.*, HIV-1 p24 antigen in an HIV-infected patient with myeloma⁵) and/or by screening paraproteins against predefined antigens (*e.g.*, anti-streptolysin, anti-DNA, anti-IgG³). One of the first systematic searches covering a broad spectrum of potential antigens used SEREX (serological identification of antigens by expression cloning) which allows for the systematic screening of putative antibody–antigen interactions, even if neither the antigen nor the antibody are known.⁶ Investigating expressed cDNA libraries derived from human testis, lung and breast cancer, bovine and porcine muscle and wheat germ by SEREX for reactivity with paraproteins in the sera from 114 patients with MGUS and MM and thus probing $> 6 \times 10^8$ paraprotein–antigen interactions, only four antigens were identified: an IgA paraprotein from a female patient binding to sperm-specific cyclin-2,⁷ and three IgG paraproteins binding to tripeptidyl-peptidase-II (TPP-2), insulin-like growth-factor binding-protein-2 (IGFBP-2), and porcine kinesin, respectively.⁸ The rare identification of antigenic targets together with the broad spectrum of auto-, allo-, and heteroantigens as targets of human paraproteins in patients without signs of chronic antigenic stimulation observed in that study suggested that a role of the antigenic stimulus in the pathogenesis of MGUS and MM is unlikely. Rather, the results supported the view that the development of clonal disease is a random event among the entire repertoire of B cells, irrespective of their antigenic specificity.⁸

We now report the results of a follow-up study where we screened a protein macroarray derived from human fetal brain for reactivity with paraprotein-containing sera and found a structure, which is the target of paraproteins at a frequency that is clearly above random.

Material and methods

Patients and controls

This study was approved by the local ethical review board (“Ethikkommission der Ärztekammer des Saarlandes”) and conducted according to the Declaration of Helsinki. Recombinant DNA work was performed with the permission and according to the regulations of local authorities (Government of Saarland). Human materials were obtained during routine diagnostic or therapeutic procedures after obtaining written informed consent and stored at -80°C .

Serum screening on high density protein arrays

High density protein arrays of the protein expression set of the hEx1 library consisting of 37,200 independent clones derived from a human fetal brain cDNA expression library were obtained from the German Resource Center for Genome Research (RZPD, Berlin, Germany) and provided by Ulrich Mählknecht (Homburg, Germany). For serum screening, the filters were blocked in 10% (w/v) nonfat dry milk powder in TBST [TBS, 0.1% (v/v) Tween 20] at 4°C overnight, washed twice in TBST and incubated for 1 hr with the paraprotein-containing sera at a dilution of 1×10^6 . Following three 30-min TBST washes and subsequent incubation with the 1:2,500 (v/v) secondary antibody (biotinylated goat anti-human IgA or anti-human IgG, anti-human Fc, anti-human κ -light chain, anti-human λ -light chain; Dako, Copenhagen, Denmark) and Strep-POX (1:15,000) in 2% (w/v) milk/TBST, the filters were washed 3 times for 10 min in TBST. This was followed by detection using a Pharmacia’s ECL system. Positive signals were localized according to the manufacturer’s protocol. Corresponding clones were obtained from RZPD.

Preparation of Fab fragments

Sera containing IgG paraproteins were purified by protein-A and protein-G beads followed by digestion with papain (10 $\mu\text{g}/\text{ml}$) for 2.5 hr at 37°C in the presence of 0.1 mM cysteine. After inactivation with iodoacetamide, the supernatant containing the Fab fragments was dialyzed against PBS, concentrated, checked by PAGE and Western blotting and used in the ELISA.

Grant sponsor: Förderverein Krebsforschungs Saar-Pfalz-Mosel e.V.

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Received 8 January 2009; Accepted after revision 24 February 2009

DOI 10.1002/ijc.24427

Published online 19 March 2009 in Wiley InterScience (www.interscience.wiley.com).

Western blot

For Western-blot analyses, proteins were purified from 500 ml of bacterial cultures grown at 37°C. Expression of proteins fused with a His₆ tag was induced in the cultures with 1 mM isopropyl- β -thiogalactopyranoside at an A₅₇₈ of 0.6–0.7. After 4 hr, cells were pelleted, resuspended in 10 ml of buffer B (20 mM Tris-HCl pH 8.0, 0.05% Triton X100, 0.4 μ M PMSF, 10 μ M Leupeptin, 1 mg/ml Lysozyme) and incubated at 4°C for 20 min. Alternatively, total blood lysates from patients and controls were used as a source for paratarg-7.

Immunoblot screening

One hundred nanogram of each recombinant protein were separated by SDS-PAGE and transferred to a PVDF membrane (Millipore Immobilon, Eschborn, Germany) by semidry blotting. The membrane was blocked in TST/milk buffer (10% milk in 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% (v/v) Tween 20) overnight, washed and incubated for 1 hr with serum in TST (paraprotein-containing serum from patients at a dilution of 1×10^8 and from controls at a dilution of 1×10^3). After three washings in TST, the membranes were incubated for 1 hr at RT with goat anti-human IgA and IgG biotinylated antibody (Dianova, Hamburg, Germany) diluted 1:5,000 in TST, subsequently washed in TST and followed by AP development.

Serum screening on protein-spot arrays

His₆-tagged fusion proteins were spotted onto nitrocellulose membranes. After spotting, the protein arrays were blocked, incubated and treated as described above.

Absorption studies

Recombinant His₆-tagged paratarg-7 protein or total blood lysate was immobilized on HiTRAP-chelating columns (Pharmacia, Freiburg, Germany) containing 1 ml nickel-nitrilotriacetic acid-agarose (Qiagen, Hilden, Germany) following a published procedure⁹ served as a control. Patients' serum (100 μ l) was diluted 1:2 (v/v) in PBS and depleted by passing 3 times over the paratarg-7 column. The flow-through was checked by immunofixation and serum protein electrophoresis.

Identification of the paraprotein-binding paratarg-7 epitope

The epitopes recognized by the patients' paraprotein were determined as described before.¹⁰ In brief, matrix-bound synthesized decamer peptides with 5 amino acids overlaps between every second consecutive decamer covering the entire paratarg-7 sequence were used to screen patient's sera. Paratarg-7 specific paraproteins binding to the corresponding paratarg-7 epitopes were visualized by ECL technique according to the supplier's instructions (Pierce, Rockford, IL) and photographed for documentation.

Paratarg-7 ELISA

Full-length paratarg-7 was recombinantly expressed in HEK293 cells under control of a CMV promoter introducing a FLAG tag at the C-terminus of the protein. Total cell extract was prepared and coated indirectly to Nunc maxisorb plates using anti-FLAG mAb (Sigma, Munich). ELISA was performed according to standard protocols with goat anti-human IgA, anti-IgG, anti-human Fc, anti-human κ - and anti human λ -light chain (Dako) or subclass-specific anti-human IgG₁, IgG₂, IgG₃ and IgG₄ (Binding Site, Birmingham, United Kingdom). For the determination of the antibody-binding epitope of paratarg-7, recombinant fragments of paratarg-7 were constructed and expressed as described for the full-length paratarg-7.

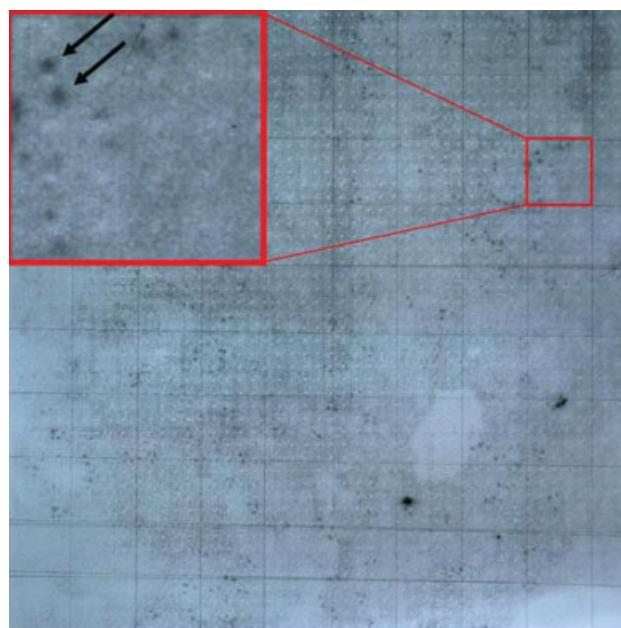


FIGURE 1 – Screening of sera for antibodies against proteins represented in a protein macroarray. A pool of 103 paraprotein containing sera, each diluted 1×10^6 , was used for immunoscreening of a human fetal brain derived recombinant protein macroarray. The autoradiography shows approximately 150 signals (double spots). After comparing with the results obtained by using a serum pool from healthy donors a specific signal was identified. The position of this protein was determined and correlated with the cDNA sequences encoding for this protein on the array. The arrow indicates the signal obtained for paratarg-7. The remaining <37,000 other proteins of the macroarray served as internal negative controls. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Results

Serum samples from 192 consecutive patients, which had been sent to the routine immunology laboratory because a monoclonal spike had been detected in the serum electrophoresis and were demonstrated to contain an IgA or IgG paraproteins by immunofixation were included in this study. The control group consisted of healthy blood donors with no paraprotein in their sera as demonstrated by immunofixation ($n = 30$).

Identification of paraprotein-binding clones

A first series of 103 paraprotein-containing sera (15 IgA, 88 IgG) diluted 1×10^6 were screened for reactivity with proteins represented in the fetal brain-derived macroarray. The signal pattern obtained from screening the paraproteins was compared with the pattern obtained with control sera from clinically healthy donors and controls without serum. From these data, 14 proteins reacting with highly diluted paraprotein-containing sera were identified (Fig. 1). 5'- and 3'-tag sequencing of these clones was performed, and their sequences were used for BLAST searches against public databases at NCBI. Of the positive clones, six were in reading frame. Sequence analysis identified five known proteins and one unknown gene product. For verification and assignment of the reactivity to the serum of an individual patient, the immunopositive clones were expressed in *Escherichia coli* and the corresponding denatured His₆-tagged proteins were spotted onto nitrocellulose membranes and incubated with the paraprotein-containing sera at a dilution of 1×10^6 . The observed reactions could be assigned to 3 paratarg (for paraprotein target) proteins (Table I), designated as paratarg-5, paratarg-6 and paratarg-7. The paraproteins of two patients each reacted with paratarg-5 and paratarg-6, respectively, and the sera from 11 patients (9.7%) reacted with

TABLE I – PARAPROTEIN TARGETS IDENTIFIED BY SCREENING RECOMBINANTLY EXPRESSED CDNA AND A HUMAN FETAL BRAIN-DERIVED PROTEIN MACROARRAY WITH IgG AND IgA PARAPROTEIN-CONTAINING SERA

Paratarg no	GenBank accession no	cDNA source	Name	Reactive paraproteins	Titer
1 ⁷	BC114547	Human testis	Cylicin 2	1/115	1×10^9
2 ⁸	NM_003291	Human lung cancer	Tripeptidyl-peptidase II (TTP-2)	1/115	1×10^{10}
3 ⁸	X16302	Human breast cancer	Insulin-like growth-factor binding-protein-2 (IGFBP-2)	1/115	1×10^9
4 ⁸	CO868665	Porcine muscle	Porcine kinesin	1/115	1×10^9
5 ¹	NM_022818	Human fetal brain	Microtubule-associated proteins 1A/1B light chain 3B precursor	2/103	1×10^9
6 ¹	NM_006762.1	Human fetal brain	Lysosomal-associated multi-transmembrane protein	2/103	1×10^{10}
7 ¹	AF190167	Human fetal brain	STOML2 (stomatin [EPB72]-like), HSPC108, stomatin-like 2, stomatin-like-protein	29/103	1×10^{10}

¹This study.

paratarg-7. Sequence analysis revealed that paratarg-5 was identical to microtubule-associated protein, paratarg-6 to LAPTM5 and paratarg-7 to STOML2 (stomatin [EPB72]-like), also known as HSPC108 or stomatin-like-protein, which is expressed in all human tissues and has been reported to be overexpressed^{11,12} and related to cell growth in several cancer,¹³ while other investigators found that it modulates T-cell activation.¹⁴

To confirm this relative high frequency of paratarg-7 recognition by paraproteins, a second series of additional 89 paraprotein-containing sera (24 IgA, 65 IgG) were not screened for reactivity with the entire spectrum of 37,200 clones represented on the macroarray, but were tested together with the first 103 paraprotein-containing sera at a dilution of 1×10^8 for antiparatarg-7 reactivity only using both the paratarg-7 immunoblot assay and paratarg-7 ELISA. The frequency of antiparatarg-7 reactivity was even higher in this second series of 89 patients (18/89 or 20.2%) resulting in an overall paratarg-7 reactivity of 29/192 (15.1%) paraproteins tested. The antiparatarg-7 reactivity of these sera was detectable up to a serum dilution of 1×10^{10} , while none of the sera from healthy controls reacted at a dilution of $\leq 1 \times 10^3$. Fab fragments of paratarg-7 reactive paraproteins obtained by papain digestion bound to paratarg-7 at a similar titer like the whole paraproteins, thus excluding unspecific binding via the Fc part of the respective immunoglobulins (Fig. 2).

To demonstrate that the antiparatarg-7 activity-containing sera reacted not only with recombinant paratarg-7 expressed in *E. coli*, but also with natural human paratarg-7, a full-length construct of human paratarg-7 was expressed in HEK-293 cells and used to coat ELISA plates. All sera that had been shown to contain a paratarg-7 specific paraprotein by the dot-blot assay were also positive in the ELISA, demonstrating that expression in *E. coli* and purification did not affect reactivity of paratarg-7 with its specific paraproteins (data not shown).

To further demonstrate the specificity of the antiparatarg-7 reactivity of the respective paraproteins, recombinant His₆-tagged paratarg-7 protein was immobilized on a Ni²⁺ agarose. A mitofilin column served as a negative control. After three passages of the anti-paratarg-7 positive IgG₃ paraprotein-containing sera over the paratarg-7 column, the monoclonal spike in the electrophoresis of the flow-through electrophoresis and the clonal band in the immunofixation had disappeared, while this was not the case with a serum containing an IgG₃ paraprotein with a nonparatarg-7 reactivity (Fig. 3). These absorption studies were done with 3 IgG₃ paraproteins with anti-paratarg-7 reactivity and 2 IgG₃ paraproteins with nonparatarg-7 reactivity as controls.

Anti-paratarg-7 reactivity according to IgG subtype

To define the frequency of antiparatarg-7 reactivity in paraproteins of different Ig classes and IgG subclasses, subclass-specific anti-human IgG antibodies were used to demonstrate binding of the paraproteins to paratarg-7 (Table II). There were no signifi-

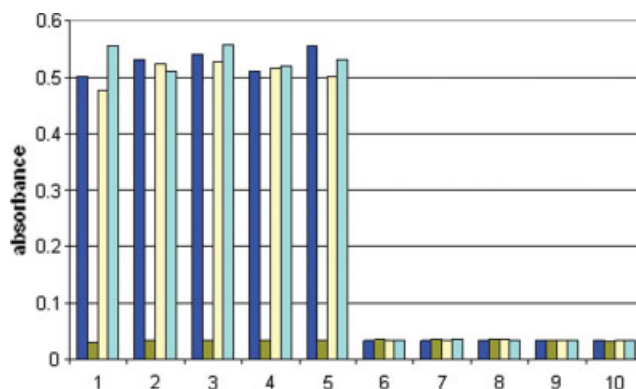


FIGURE 2 – Anti-paratarg-7 ELISA with whole IgG₃, Fc and Fab fragments. 1–5: Sera from five different patients containing IgG₃ paraproteins with antiparatarg-7 reactivity; 6–10: sera from five different patients containing IgG₃ paraproteins with nonparatarg-7 reactivity. Sera were used at a dilution of 10^8 , Fab fragments at a concentration of 10 µg/ml. Black columns: Fab fragments detected by a mix of labeled anti-human κ - and λ -light chain antibody; light-grey columns: Fab fragments detected by labeled anti-human Fc antibody; white columns: IgG₃ paraprotein detected by a mix of labeled anti-human κ - and λ -light chain antibody. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cant differences with respect to antiparatarg-7 reactivity between IgA and IgG paraproteins or paraproteins containing the κ or λ light chain, respectively. However, among the IgG subclasses, there was a remarkable difference with respect to antiparatarg-7 reactivity: none of the IgG₁, IgG₂ and IgG₄ paraproteins reacted with paratarg-7, while of the 57 IgG₃ paraproteins 24 (42.1%) were paratarg-7 reactive and 33 (57.9%) did not react with paratarg-7.

Antibody-binding epitope of paratarg-7

To determine the paraprotein-binding epitope of paratarg-7, peptide-spot analysis was performed by testing paratarg-7 derived decamer peptides with 5 amino acids overlaps covering the entire paratarg-7 sequence. A peptide spanning amino acids 16 to 35 was shown to bind the antiparatarg-7 paraproteins. All three paratarg-7 reactive paraproteins tested in the peptide-spot assay reacted with the same epitope (data not shown). Epitope specificity was also determined by ELISA using recombinant full-length paratarg-7 and recombinant fragments of paratarg-7 as a coat. All 29 paratarg-7 reactive paraproteins were tested by this ELISA and all reacted with recombinant paratarg-7₁₋₃₆ and paratarg-7₁₆₋₃₂ confirming the epitope specificity obtained with the peptide spot assay (Fig. 4).

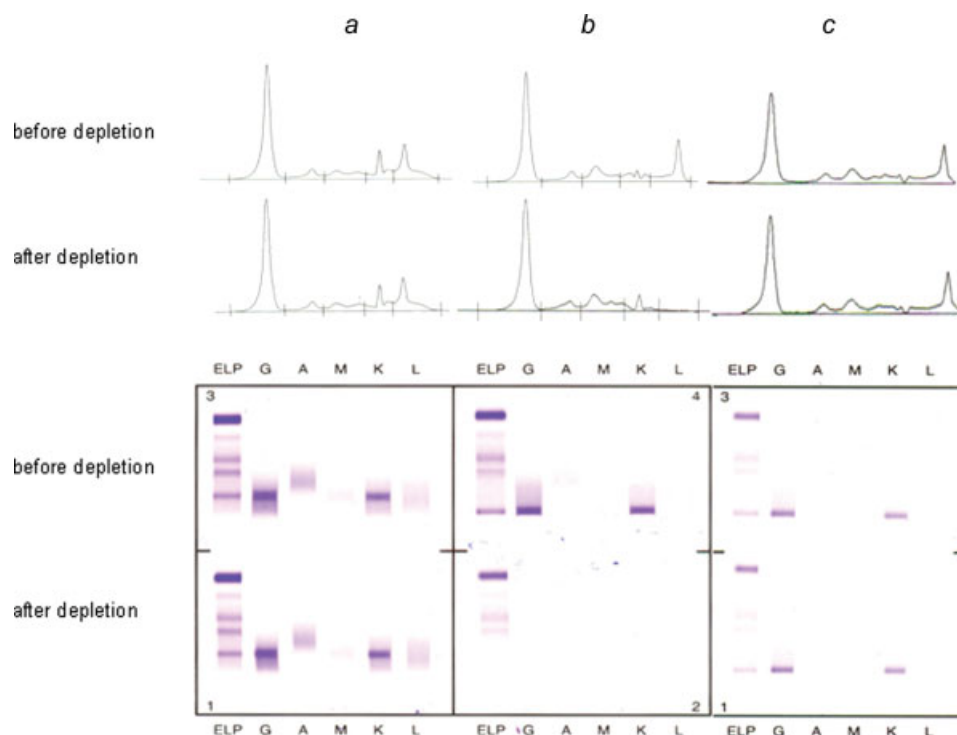


FIGURE 3 – Absorption of a serum containing an IgG₃ paraprotein with antiparatarg-7 reactivity. (a) IgG₃ paraprotein with nonparatarg-7 reactivity and absorption by a paratarg-7 column (control). (b) IgG₃ paraprotein with paratarg-7 reactivity and absorption by a paratarg-7 column. (c) The same IgG₃ paraprotein as in b, but absorption by mitofilin column (control). Upper panels: serum electrophoresis before and after absorption, lower panels: immunofixation before and after depletion. The absorption studies of 3 sera containing a paratarg-7 reactive IgG₃ paraprotein and 2 IgG₃ control paraproteins with paratarg-7 nonreactivity yielded identical results. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II – SUMMARY OF PARAPROTEINS TESTED FOR ANTI-PARATARG-7 REACTIVITY

Type	Paraproteins analyzed (n)	Antiparatarg-7 positive
IgA	39	5 (12.9%)
IgA _λ	17	1 (5.9%)
IgA _κ	22	4 (18.1%)
IgG	153	24 (15.6%)
IgG _λ	57	10 (17.5%)
IgG _κ	96	14 (14.7%)
IgG ₁	83	0
IgG ₂	6	0
IgG ₃	57	24 (42.1%)
IgG ₄	7	0
Total	192	29

Analysis of human paratarg-7

Paratarg-7 is a protein that is expressed in all human tissues and hematopoietic cells including erythrocytes (data not shown). Flow cytometry of mononuclear bone marrow cells showed that paratarg-7 is also expressed by all subpopulations of cells in the bone marrow tested (data not shown). To check whether the patient-derived paratarg-7 proteins reacting with the respective paraprotein were different from paratarg-7 proteins in healthy controls or from patients with paraproteins displaying a reactivity other than antiparatarg-7, paratarg-7 from different patients and healthy controls was sequenced. The DNA sequence of paratarg-7 from all individuals analyzed ($n = 6$) was identical, excluding the possibility that mutations or polymorphisms are responsible for the observed autoimmunogenicity of paratarg-7 in the respective patients. In addition, total blood lysates from patients and controls were subjected to Western-blot analysis using a paraprotein with

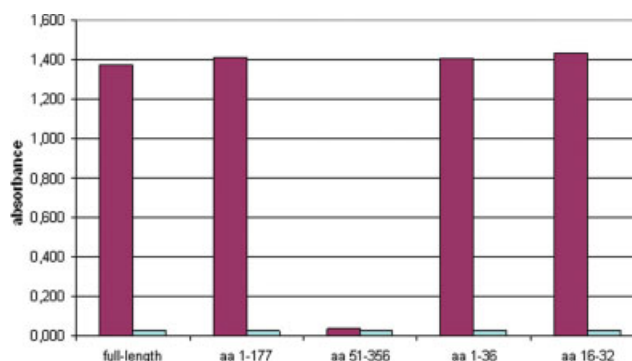


FIGURE 4 – Determination of the anti-paratarg-7 binding epitope of paratarg-7. For this ELISA, recombinant full-length and recombinant peptide fragments containing amino acids 1–177, 51–356 ($\Delta 50$), 1–36 and 16–32, respectively, were used as a coat. The amino acids 16–32 are identified as the antiparatarg-7 antibody binding epitope. All 29 paraproteins with antiparatarg-7 reactivity bound to the same peptide epitope. Black columns: patients serum; gray columns: healthy donors serum. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

antiparatarg-7 activity. All lysates from patients with paraproteins with antiparatarg-7 or nonparatarg-7 specificity and healthy controls showed identical bands in the Western-blot (Fig. 5).

Discussion

Several previous reports have identified myeloma paraproteins directed against various infectious agents, including bacteria³ and the p24 antigen of the human immunodeficiency virus-1.⁵ In

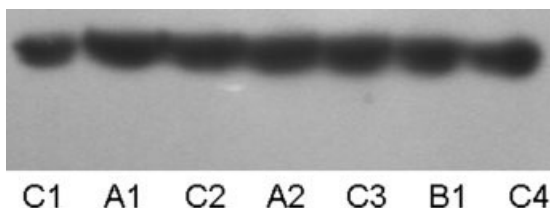


FIGURE 5 – Western-blot analysis of paratarg-7 derived from total blood lysates of patients with paratarg-7-reactive paraproteins, paraproteins with specificity other than for paratarg-7 and healthy controls. Blood samples were centrifuged and washed with PBS. The pellet was resuspended in 8 M urea/0.1 M phosphate/10 mM Tris-HCl pH 8/0.1% NP-40). After gel electrophoresis and blotting onto nitrocellulose membrane, the membrane was incubated with a 1×10^6 diluted serum containing an antiparatarg-7 paraprotein. The numbers indicate different patients with paratarg-7 reactive paraproteins (A1, A2), with a non-paratarg-7 reactive paraprotein (B1) and healthy controls (C1–C4).

another case, a patient developed a serum M-component with specificity for horse α_2 -macroglobulin 30 years after receiving passive serotherapy with horse antiserum to tetanus.¹⁵ Even though few of these studies convincingly proved that the observed phenomena were indeed caused by binding of the respective paraprotein to its specific antigenic target (*e.g.*, by “reverse Western” blotting and/or sequencing of the target antigen as done in this study), these selective observations are often cited to support a causal relationship between the development of an MGUS or MM clone and chronic antigenic stimulation.^{1–5,16–23} Similarly, doubts are raised about the paraprotein-mediated reactions with antigens predicted by epitope reconstruction and reported in a recent paper,²⁴ because the reactions were observed at concentrations of the paraprotein-containing serum of only 1:2,500. In contrast to these reports, our first systematic studies^{7,8} did not only convincingly prove the paraprotein-mediated reactivity against the identified antigens, it also revealed a broad spectrum of antigenic targets of paraproteins. Of $> 6 \times 10^8$ paraprotein/target–antigen interactions screened which represented a large proportion of the expressed human genome as possible sources for autoantigens as well as the major sources for food allergens, only four were identified as paraprotein-mediated, suggesting that these antigens represented only a small part of the entire antigenic target spectrum of human paraproteins. The observed diversity and the fact that the respective targets were detected by paraproteins from patients who did not have any signs of chronic antigenic stimulation, let us conclude that the role of chronic antigenic stimulation in the pathogenesis of MGUS and MM had probably been overestimated in the past. The view that many—if not any—auto-, allo- and heteroantigen-reactive B-cell clones can become the random target of malignant transformation was also supported by the fact that there is no convincing evidence for the preferential use of V genes in MGUS and MM,^{25,26} which contrasts with the selective immunoglobulin V gene use in other B-cell malignancies, *e.g.*, marginal zone lym-

phomas associated with chronic infection and/or autoimmunity.²⁷ However, in this follow-up study using a fetal-brain derived protein macroarray that had not been included in our first studies, we are now confronted with a paraprotein target that reacts with 15% of all paraproteins analyzed. Why paratarg-7 was not detected during our previous analysis⁸ even though paratarg-7 cDNA was represented in the cDNA library used for our SEREX analysis (data not shown) is unclear; most likely the protein was not expressed or not expressed at its full length.

The cloning of the B-cell receptor expressed by the respective plasmacytoma cells as well as the analysis of the V_H and V_L gene usage are currently underway. While these tools will be invaluable for the investigation of the mechanisms underlying the pathogenesis of plasmacytomas secreting paraproteins with anti-paratarg-7 reactivity, the results obtained so far definitely prove the specificity of the paraprotein-mediated reactivities with anti-paratarg-7. This specificity was confirmed by the fact that of the first 103 paraproteins tested, 92 did not react with paratarg-7 and that the 11 paratarg-7 reactive paraproteins detected by macroarray screening reacted only with paratarg-7, but not with any other of the 37,200 proteins spotted on the macroarray. Similarly, 42.1% of all IgG₃ paraproteins reacted with paratarg-7, while 57.9% of the IgG₃ paraproteins did not. While already the fact that IgA and IgG paraproteins reacted with a similar frequency against paratarg-7 made an Fc-mediated binding rather unlikely, this possibility was definitely excluded by the demonstration that the anti-paratarg-7 reactivity of Fab fragments derived from paraproteins with anti-paratarg-7 reactivity reacted with a similar titer as the complete paraprotein to recombinant paratarg-7. Furthermore, there was no reactivity of the paratarg-7 reactive paraproteins with structurally related proteins such as slp-1 and stomatin (data not shown). Finally, anti-paratarg-7 reactivity was specifically absorbed by paratarg-7 coated beads, but not by beads loaded with mitofilin which was used as a control (Fig. 3). All these control experiments together with the fact that all paratarg-7 reactive paraproteins bound to the same epitope definitely prove the specificity of the paratarg-7 binding paraproteins.

This clearly above-random frequency of paratarg-7 as a paraprotein target suggests that paratarg-7 is directly or indirectly involved in the pathogenesis of the respective clonal proliferations, *i.e.*, MGUS and multiple myeloma, supporting the notion that at least a significant proportion of MGUS and MM might arise as a result of (chronic) antigenic stimulation. The restriction of this target to the IgG₃ subclass of the IgG paraproteins indicates that additional (genetic) factors might be necessary to render this protein, which is not different in patients and controls with respect to its DNA sequence and pattern in Western blots, autoimmunogenic in the respective patients. The identification of paratarg-7 as a frequent antigenic target and/or stimulus for paraprotein-producing clones now enables the more detailed analysis of tumor–host interactions in the presence and absence of this antigen in the respective patients, and to study more specifically the role of immunoregulatory deficiencies, such as the recently reported dysfunction of regulatory T cells²⁸ in patients with MGUS and multiple myeloma.

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