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Analysis of the V genes coding for a monospecific human antibody to myosin and functional expression of single chain Fv fragments

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Abstract A monospecific human IgM monoclonal antibody (mAb), reactive with myosin from human heart, has been obtained by EBV transformation. This mAb may have a diagnostic potential in the imaging of myocardial necrosis. However, owing to the fact that the molecular mass of an IgM is 900 kDa, a poor diffusion and a slow penetration inside necrotic myocytes could reduce its capacity for scintigraphic detection. In order to alleviate these problems, we constructed the scFv by cloning the VH and VL domains into the pHOG21 vector. Analysis of the V genes proved an unmutated configuration showing that the immortalized B cell issued from the primary IgM repertoire. The expression product in *Escherichia coli* was a 35 kDa scFv fragment with the antigen-binding specificity of the parental mAb.

Key words: Human antibody; IgM; Immunoglobulin variable region; Single chain variable fragment; Myosin; B cell repertoire

1. Introduction

Human monoclonal antibodies (mAbs) are a new class of diagnostic and therapeutic agents which have made a substantial impact in the biomedical field. They are less likely to provoke the immune response which can severely impair the clinical efficiency of murine antibodies [1]. Indeed, human anti-mouse antibodies (HAMA) may accelerate the clearance of the injected mAb, block its binding to the antigen and/or trigger allergic reactions. A murine anti-myosin mAb has been described as a powerful probe for the imaging of myocardial necrosis [2]. A mAb of entirely human origin may offer considerable advantages over the murine antibody.

The EBV transformation of B lymphocytes from a patient with a bleeding disorder had led to the unexpected immortalization of a B cell secreting a monospecific human anti-myosin IgM (referred to as B7) [3]. By Western blotting, we previously showed that B7 recognized the myosin heavy chain of different sources (skeletal muscle, cardiac muscle and non-muscle cells) and of different species (rabbit, pig, human) [3]. Using immunohistochemical staining, we also showed that B7 bound avidly to the myosin of human ventricular myocardium, but not to that of human aortic smooth muscle cells [3]. Because of this high avidity for cardiac myosin, B7 may be useful in the imaging of myocardial necrosis after graft rejection [2], cardiomyopathy [4], suspected myocarditis [5] and cardiac drug toxicosis [6]. However, because of the large molecular mass of the IgM (900 kDa), its use in scintigraphic detection would appear compromised. The development of genetically engineered scFv (around 32 kDa) molecules, which consist of only the antibody variable regions held together by a flexible linker, was thus considered. ScFv have the advantage of rapid clearance from the circulation, good penetration within solid tissue and theoretically, low immunogenicity [5].

We constructed the recombinant scFv fragments of B7 into the expression vector pHOG21 [7]. They were expressed in a soluble form using *Escherichia coli*, enriched by immobilized metal affinity chromatography (IMAC) [8] and tested for their activity against human heart myosin by ELISA. The recombinant single chain variable fragments (scFv) expressed by *E. coli* showed myosin-binding capacity.

From a fundamental point of view, an interesting question was raised concerning the relationship of this monospecific mAb with the pathology of the patient from where the B cells were obtained. This patient possessed an inherited disorder of platelet function linked to the absence of the integrin αIIbβ3 [9]. One explanation for the emergence of this anti-myosin antibody could be that antigen-driven in vivo selection had occurred following the increased destruction of platelets during the bleeding episodes experienced by the patient and which resulted in an exposure of the myosin contained in the platelet cytosol. In such a response to an antigenic stimulus, positive selection of B cells operates within the B cell repertoire. The B cells therefore became part of the post-activated repertoire with a high replacement/silent mutation ratio within the V CDRs [10,11]. The other possibility is that we have immortalized an autoreactive natural B cell from the primary repertoire. These naive B cells mostly express unmutated V region genes [12]. Analyses of B7 V gene sequences establish that the mAb is encoded by virtually unmutated segments. Therefore, B7 is the product of a naturally occurring antibody-producing B cell from the primary repertoire with no relation with the bleeding syndrome.

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Abbreviations: EBV, Epstein–Barr virus; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; scFv, single chain variable fragment; mAb, monoclonal antibody; IPTG, isopropyl-β-D-thiogalactopyranoside

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2. Materials and methods

2.1. E. coli strains, primers and vector

E. coli strain XL1-Blue (Stratagene, La Jolla, CA, USA) was used as the cloning host. For cloning and sequencing hybridoma-derived V regions, we used the vector pHOG21 provided by Dr. Melvin Little (Recombinant Antibody Research Group (0445), German Cancer Research Center, Heidelberg, Germany).

For amplification of variable domains of Ig μ and λ chains, we used 5′ primers specific for each of the V_H and Vλ gene families and 3′ primers specific for the antisense sequences of Cμ1 and Cλ constant domains [13]. These primers were kind gifts from Dr. Diane Nugent (Pediatric Hematology Department, CHOC, Orange, CA, USA). We then used a second set of sense and antisense primers specific for variable regions, including the restriction sites appropriate for cloning in the vector pHOG21 [14].

2.2. Isolation and characterization of the human anti-myosin autoantibody

The human mAb B7 was obtained by EBV transformation followed by somatic cell hybridization of lymphocytes from a patient with a bleeding disorder, Glanzmann thrombasthenia [9]. The mAb B7 was extensively characterized by Western blotting, ELISA and immunohistochemistry [3].

2.3. Antigen-binding assay of the parental B7 mAb

B7 mAb was tested at 5 μg/ml for its binding to ssDNA and dsDNA by ELISA using the INOVA QUANTA Lite ssDNA and dsDNA kits (INOVA Diagnostics Inc, San Diego, CA, USA) according to the manufacturer's instructions. B7 was also tested for its binding to human cardiac myosin (Valbiotech, Paris, France), actin, elastin, keratin, light meromyosin (LMM), heavy meromyosin (HMM) (Sigma Chemical Co., St Louis, MO, USA), and IgGFc (RF, rheumatoid factor) (Jackson ImmunoResearch, West Grove, PA, USA). Supernatant from a polyspecific cell line obtained by EBV transformation which secretes an IgM antibody (unpublished data) was included in the test at 5 μg/ml as a positive control for all autoantigens tested. The ELISA was performed according to Clofent-Sanchez et al. [15]. Nunc 96-well microtiter plates were coated with 3 μg/ml of each antigen. Bound B7 and polyspecific IgM antibody were revealed using alkaline phosphatase-labeled goat antibodies to human IgM (Fc5μ fragment specific) (Jackson ImmunoResearch).

2.4. Isolation of variable heavy and light chain genes

Total RNA was extracted from about 5×10^7 hybridoma cells of five subclones of B7 using TRIzol LS Reagent, a solution of phenol and guanidine isothiocyanate (Gibco BRL, Eragny Pontoise, France), according to standard methodology.

Five separated first-strand cDNA preparations were synthesized using a constant heavy chain μ antisense primer (Cμ1 gene 3′ primer) or a constant λ antisense primer (Cλ gene 3′ primer), the Moloney Murine Leukemia Virus reverse transcriptase (BRL/Life Technologies) and RNA guard (Pharmacia Biotech, Saclay, Orsay, France).

The initial cDNA strand was amplified using primers specific for each human heavy and light gene families as provided by Dr. D. Nugent [13]. For the amplification of the V genes, we combined each of the six V_H family framework region FR1 sense primers and the antisense primer in separate reactions. For the amplification of the Vλ region genes, we combined six Vλ family framework region (FR1) sense primers with the antisense primer. PCR amplification was carried out in a total of 100 μl containing 5–50 ng cDNA, 25 pM of each primer, 300 μM dNTPs, 10 μl of 10×amplification buffer (100 mM Tris-HCl, pH 8.8, 15 mM $MgCl_2$, 500 mM KCl, 1% Triton X-100) and 5 U HITAQ DNA polymerase (Bioprobe Systems, Montreuil sous Bois, France); 12 separate reactions were performed. Each mixture was subjected to 30 cycles consisting of 94°C (1 min), 50°C (1 min), 72°C (1 min) using a Gene Amp PCR System 9600 (Perkin-Elmer, Foster City, CA, USA). At the end of the last cycle, the mixture was incubated at 72°C for 5 min. Amplified DNA was analyzed by electrophoresis on a 1.2% agarose gel stained with ethidium bromide. Specific amplified fragments of the expected base pair size (630 bp for the light chain, 680 bp for the heavy chain) were purified from the agarose gel and subsequently used for cloning.

2.5. Cloning of hybridoma genes

For Ig heavy and light chain cloning, we used the pHOG21 vector and a second set of sense and antisense primers containing the suitable restriction sites [14]. Cycles (30) were 95°C for 30 s, 55°C for 1 min, 72°C for 1 min, followed by 5 min at 75°C to allow the final extension. Specific fragment amplification of the expected base pair size (400 bp) was confirmed by 1.2% agarose gel electrophoresis on a 10 μl sample of the final PCR product. For light chain cloning into the vector pHOG21, Vλ DNA was digested with *Mlu*I and *Not*I (restriction enzymes obtained from Boehringer Mannheim, Meylan, France), separated on an agarose gel and purified. These DNA products were then ligated to *Mlu*I/*Not*I digested pHOG21. The ligation product was purified on Geneclean (BIO 101 Inc., La Jolla, CA, USA). Transformed *E. coli* XL1-Blue cells (Stratagene, La Jolla, CA, USA) were grown on LB agar plates containing 100 μg/ml ampicillin and 100 mM glucose (LB_{GA}). Clones were tested for the presence of correctly assembled Vλ by PCR screening using oligonucleotides Vλ (backward) and Vλ (forward). The DNA from 25 positive clones (five from each cDNA preparation) was extracted with the Qiaquick kit (Qiagen, Courtaboeuf, France).

2.6. Sequencing and analysis of hybridoma genes

Double-stranded sequencing of the cloned insert was determined according to the dideoxy method [16]. Insert-carrying clones were sequenced on both strands. The sequence reaction was performed with the sequenase sequencing kit (Amersham, Courtaboeuf, France) using a DNA sequencer (LI-COR DNA Sequencer 4000, ScienceTec, Les Ulis, France). Sequence identity was confirmed from all 25 separate clones. Before inserting the V_H DNA into a clone containing the Vλ gene, it was purified on 1.2% agarose, digested with *Nco*I and *Hin*dIII, purified again on agarose and ligated to the vector of the previous construct digested with the same enzymes. The transformation, the selection, and the sequencing of the clones containing the V_H gene were performed as described for the Vλ gene.

The Ig V region sequences of the heavy and light chain genes were submitted to the IMGT [17] and V BASE [18] databases to determine the germline gene sequences.

2.7. Expression of recombinant single chain antibody fragments

XL1-Blue *E. coli* bacteria transformed with the pHOG21 vector containing the B7 scFv fragment were grown overnight in LB medium (Invitrogen, San Diego, CA, USA) with 50 μg/ml ampicillin and 100 mM glucose (LB_{GA}) at 37°C. The overnight culture was diluted (1/20) and the culture continued in 5 l flasks at 37°C with shaking at 280 rpm. When the culture reached $OD_{600} = 0.7$, bacteria were pelleted by centrifugation at $1500\times g$ for 10 min at 20°C and resuspended in the same volume of fresh LB medium containing 50 μg/ml ampicillin and 0.4 M sucrose. The expression of the scFv was induced by the addition of 1 mM IPTG. The culture was allowed to grow for 18–20 h at 37°C.

2.8. Preparation of periplasmic extract and culture supernatant containing the soluble scFv

Bacteria pellet and culture supernatant were harvested by centrifugation at $5000\times g$ for 15 min at 4°C. To isolate soluble periplasmic proteins, the pelleted bacteria were resuspended in 5% of the initial volume of ice-cold 50 mM Tris-HCl, 20% sucrose, 1 mM EDTA, pH 8.0. After a 1 h incubation on ice with occasional stirring, the spheroplasts were centrifuged at $30\,000\times g$ for 30 min at 4°C, yielding a soluble periplasmic extract in the supernatant and insoluble periplasmic material in the pellet. The B7 culture supernatant and the soluble periplasmic extract were combined, clarified by additional centrifugation ($30\,000\times g$, 4°C, 30 min) and passed through a 0.2 μm 1 l filter (Nalgene, Poly Labo, Strasbourg, France). The clarified supernatant was concentrated and dialyzed against 50 mM Tris-HCl, 1 M NaCl, pH 7.0 using a Prep/Scale-ultrafiltration system (Millipore, St. Quentin Yvelines, France) incorporating a cartridge with a molecular weight cut off of 5 kDa.

The concentration of scFv was calculated by determining the total protein concentration using the Micro BCA Protein Assay Reagent and the NanoOrange protein quantification kit (Pierce, Interchim, Montluçon, France) and by measuring the percentage of scFv by gel scanning (Image Station 440 CF, Kodak, Rochester, NY, USA).

2.9. SDS-PAGE and immunoblot analysis

Analysis of the fractions containing scFv was performed by electrophoresis on 12% SDS-polyacrylamide gels under reducing conditions. The gels were Coomassie blue or silver stained (Silver Stain Kit) (Bio-Rad, Ivry Sur Seine, France). The proteins were also transferred by electrophoresis to a nitrocellulose membrane for immunoblotting using the mAb, 9E10 recognizing the c-myc peptide tag. This mouse mAb was produced in our laboratory from the CRL-1729 clone purchased from ATCC (Rockville, Canada). Bound antibody was detected using peroxidase-labeled sheep anti-mouse IgG antibody (Jackson Immunoresearch) and chemiluminescence ECL Western blotting detection reagents (Pierce).

2.10. Antigen-binding assay of the produced scFv

Myosin-binding activity of the recombinant scFv from a fraction enriched by IMAC (immobilized metal affinity chromatography) (manuscript in preparation) was assayed using the ELISA technique described above with the mAb, 9E10 as the first and HRP-conjugated goat anti-mouse IgG as the second antibody. A volume (100 μl) of 17% IMAC-enriched recombinant scFv (17 μg/ml) was incubated in the presence of 1% of Triton X-100 (Sigma Chemical Co.) on plates coated with the total myosin, LMM or HMM fragments at 4°C overnight. As controls, proteins not recognized by the parental mAb (actin, elastin, keratin, IgGFc, ssDNA, dsDNA) and BSA from Sigma Chemical Co. (St. Louis, MO, USA) were coated at 5 μg/ml. A monospecific scFv fragment (kindly provided by Melvin Little) directed against Fab fragments was included in the test as a negative control.

3. Results

3.1. ELISA

In a previous publication [3], we have described how B7 reacted specifically with the myosin heavy chain after separation of platelet proteins by SDS-PAGE and with no cross-reactivity with other platelet proteins. In order to confirm the monospecificity of this mAb, we have now verified that B7 did not react with autoantigens such as ssDNA, dsDNA, actin, elastin, keratin and RF. As shown in Table 1, OD values below 0.05 were obtained with all these autoantigens, whereas the polyspecific IgM gave positive values with an OD above 1.6. The only positive value for B7 was obtained with myosin. We further determined that the epitope recognized by B7 is located on the LMM (light meromyosin: the C-terminal half of the rod) [19]. All of these results showed that we had immortalized, by EBV transformation, a B cell which secreted a monospecific IgM anti-myosin autoantibody.

3.2. Amplification and identification of Igμ and λ chain cDNA from B7

Ig mRNA was specifically converted to cDNA using either Cμ1 or Cλ specific primers and reverse transcriptase. Heavy chain cDNA was separately amplified using primers corresponding to all six V_H gene families. Similarly, light chain cDNA was separately amplified using primers corresponding to all six Vλ gene families. Using the V_H3 specific 5′ primer, a single intense DNA band of approximately 680 bp was obtained from B7 mRNA. No DNA bands of the size anticipated for a Fd segment (V_H+D+J_H+Cμ1) were produced using any of the five other V_H primers. Using the $V_{\lambda 3}$ specific 5′ primer, a DNA fragment of about 630 bp, corresponding to the complete λ light chain was amplified from B7 mRNA (data not illustrated). No DNA was amplified from B7 mRNA when 5′ primers corresponding to the remaining five sequences were employed.

3.3. Molecular analysis of the heavy and light chain genes from the B7 cell line

After cloning, we sequenced the Ig V region genes of the mAb B7. The nucleotide sequences of the B7 V_H3 and Vλ3 genes termed HAb B7-H3 and HAb B7-L3 are shown in Fig. 1A and B respectively. In order to address the question as to whether B7 cDNA could be related to a germline sequence and had accumulated somatic mutations, a computer search for sequence identity was undertaken against the Ig germline sequences contained in the V BASE and IMGT database. Sequence analysis of the B7 V_H and V_L genes revealed that the nucleotide sequences HAb B7-H3 and HAb B7-L3 were most homologous to the p1 germline V_H3 [20] and the DPL23 germline Vλ3 genes [21] respectively.

The nucleotide sequence HAb B7-H3 differs from that of the germline p1 by two nucleotides (99.3% nucleic acid homology (292/294) and 98.9% amino acid homology (97/98)) (Fig. 1A). The nucleotide sequence HAb B7-H3 has one silent mutation (codon 63) and one replacement (a valine substituted for a leucine at the 81 codon) compared to the germline p1. The HAb B7-H3 heavy chain employs DN1 (D6-13) and J_H5b genes; the D region deviates from a DN1 sequence by one replacement (a glycine substituted for an alanine at the first codon of DN1) [22]. The J_H region is identical to the germline J_H5b [23].

The nucleotide sequence HAb B7-L3 was 100% (282/282) homologous with the DPL23 germline gene [21] and was rearranged with a Jλ2 gene [24]. The Jλ2 of the HAb B7-L3 differed from the Jλ2 germline at the first codon (a valine substituted for an alanine) (Fig. 1B).

3.4. Expression of the scFv fragments

The anti-myosin scFv fragments were analyzed both from the periplasmic fraction and supernatant of IPTG-induced *E. coli* XL1-Blue transfected with the scFv recombinant pHOG21 plasmid. After induction, a scFv band with a molecular weight of 35 kDa was found on SDS-PAGE for the combined periplasmic and supernatant fraction (Fig. 2A, lane b) and confirmed on immunoblots using the mAb 9E10 (Fig.

Table 1
Monospecificity of the human mAb B7

	Autoantigens								
	Myosin	HMM	LMM	Actin	Elastin	Keratin	RF	ssDNA	dsDNA
B7[a]	1.5	0.03	0.95	0.04	0.01	0.01	0.05	0.02	0.01
Polyspecific IgM[a]	1.8	n.d.[b]	n.d.	1.9	1.8	2	1.9	1.6	1.7

Supernatant from the B7 culture was tested for antibody binding in ELISA against different autoantigens. A polyspecific IgM is used as a positive control.
[a]Optical density readings at 405 nm.
[b]n.d., not detected.

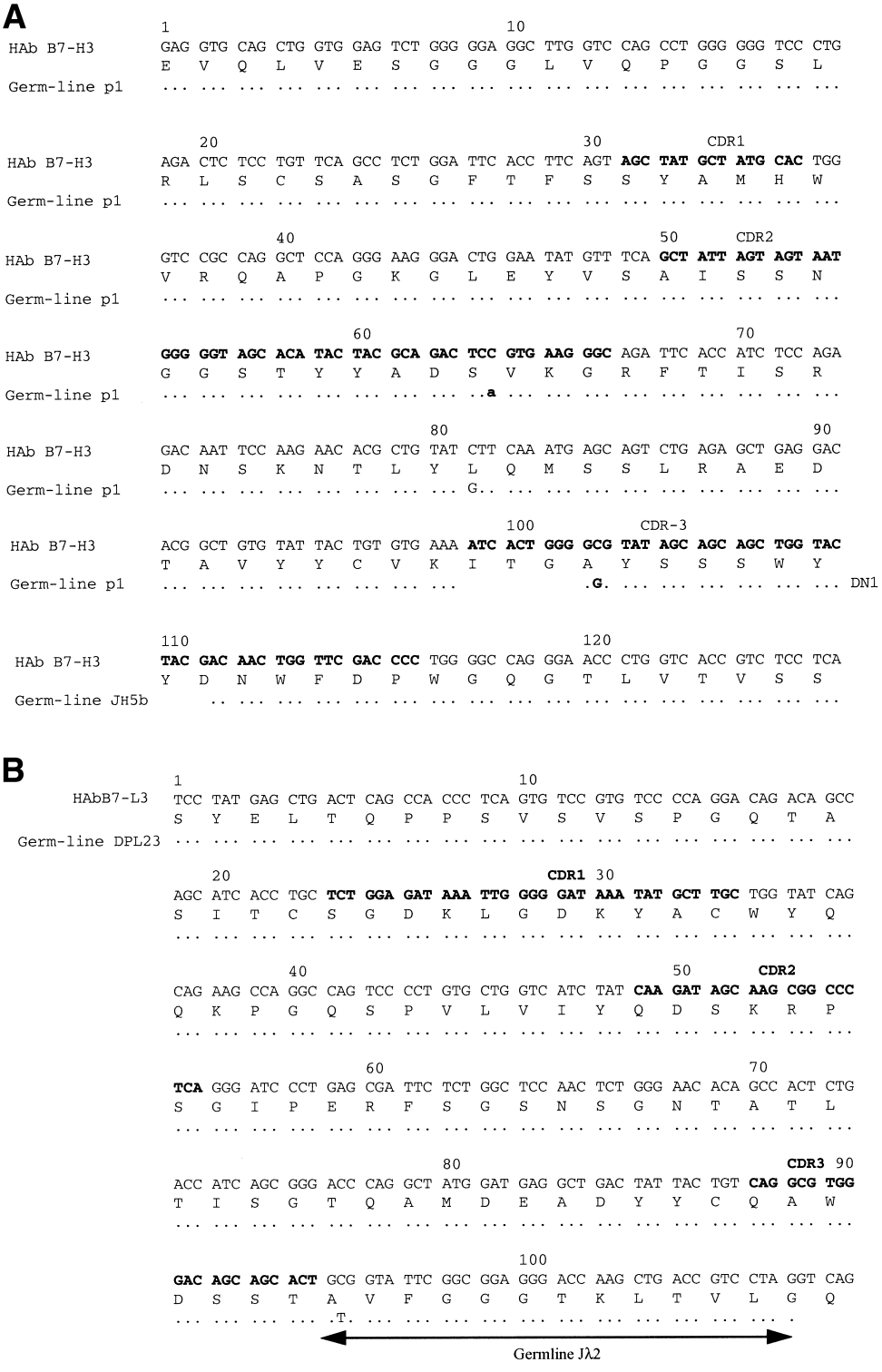

Fig. 1. A: Nucleotide sequence and translated amino acid sequence of the B7 Ig heavy chain variable region. The VH sequence of B7 is compared with the most homologous germline gene (p1) in the V BASE and IMGT database. Dotted lines signify coidentity of the two sequences and base changes inducing amino acid replacement are indicated in capital. The CDRs are highlighted in bold. The B7 VH sequence is available from the EMBL bank (accession number: AJ224899). B: Nucleotide sequence and translated amino acid sequence of the B7 Ig light chain variable region. The VL sequence of B7 is compared with the most homologous germline DPL23 segment in the V BASE and IMGT database. Dotted lines signify coidentity of the two sequences and base changes inducing amino acid replacement are indicated in capital. The CDRs are highlighted in bold. The B7 VL sequence is available from EMBL bank (accession number: AJ224900).

2A, lane c: periplasmic fraction; lane d: supernatant). The concentrated *E. coli* supernatant combined with the periplasmic extract was further passed through a Cu^{2+}-charged chelating Sepharose column (IMAC, manuscript in preparation). The concentration of proteins in this IMAC eluted fraction was calculated with the NanoOrange protein quantification kit and found to be 100 μg/ml. SDS-PAGE analysis of material purified by IMAC showed several major proteins of $M_r = 30$ kDa, 35 kDa, and 70 kDa (Fig. 2B, lane a) with only the 35 kDa band reacting with the anti-c-myc antibody on immunoblot analysis (Fig. 2B, lane b). The percentage of the scFv in the IMAC preparation was estimated by gel scanning to be 17%. We had thus obtained a concentration of 17 μg/ml of scFv.

3.5. Biological activity of B7-derived scFv fragments to myosin

The immunoreactivity of the IMAC-enriched B7 scFv preparation was analyzed for its ability to bind to purified human myosin in an ELISA assay. Fig. 3 demonstrates that the scFv at 17 μg/ml reacted most strongly with human myosin and consistently generated values well above those obtained with a control preparation of scFv. The level of reactivity on light meromyosin (LMM) was comparable to that obtained on total myosin (OD = 0.3; data not shown). The non-ionic detergent Triton X-100 was included in the preparation of scFv tested in order to avoid the formation of aggregates. Results for two other antigens (actin and BSA) were shown on Fig. 3 to verify the immunospecificity of B7 scFv fragments. They were also tested for their binding to HMM, elastin, keratin, ssDNA, dsDNA, and IgGFc (RF, rheumatoid factor) and gave the same background values as for actin (OD < 0.08; data not shown). These values were similar to those obtained with the control scFv preparation.

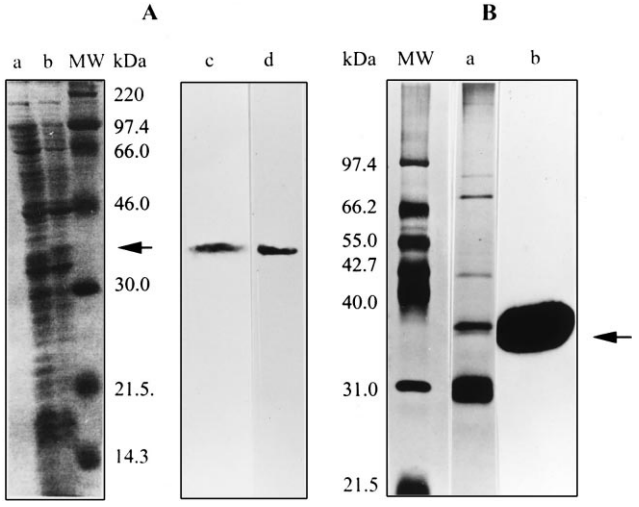

Fig. 2. A: 12% SDS-PAGE analysis of concentrated culture medium added to periplasmic extract (20 μl) of *E. coli* transfected with the plasmid encoding B7 scFv. Lanes: a, without IPTG; b, induced with 1 mM IPTG; c, MW (molecular mass markers) on a Coomassie blue stained gel. No scFv were detected in the concentrated culture medium and periplasmic extract from non-induced bacteria. Immunoblot analysis of soluble periplasmic content (lane c) and culture medium (lane d) of *E. coli* transfected with pHOG21 and induced with 1 mM IPTG. The scFv were detected using the mAb, 9E10, recognizing the C-terminal c-myc epitope. The arrowhead indicates the position of the scFv. B: 12% SDS-PAGE (lane a on a silver stained gel) and immunoblot using anti-c-myc antibody (lane b) analyses of 80 μl scFv (1.36 μg scFv) enriched by IMAC (immobilized metal affinity chromatography). lane MW, molecular mass markers.

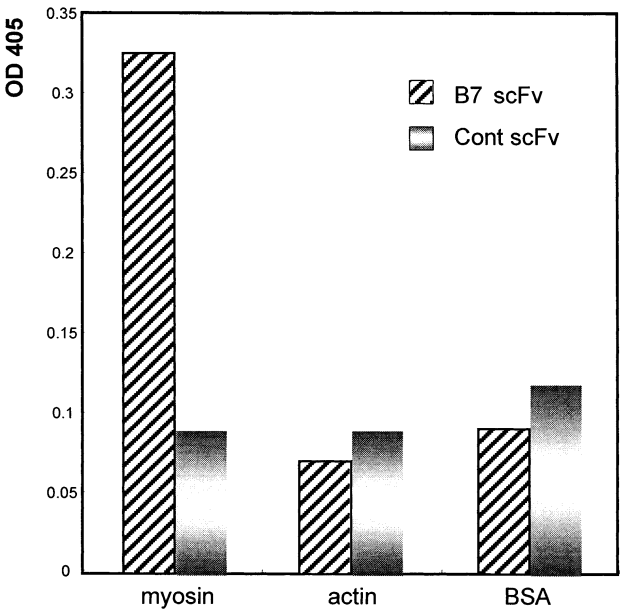

Fig. 3. Binding of recombinant B7 scFv at 17 μg/ml to purified myosin of human heart as determined by ELISA. The results showed significant myosin specific binding of the recombinant B7 scFv fragments compared to the binding to actin and BSA antigens. A monospecific scFv fragment directed against Fab fragments was used as a negative control.

4. Discussion

The EBV transformation of B lymphocytes from a Glanzmann thrombasthenia patient led to the immortalization of a monoclonal anti-myosin mAb, named B7, which does not cross-react with total platelet proteins and other self antigens (see Table 1). We had previously hypothesized that the production of an anti-myosin antibody may have resulted from an immune response to intracellular proteins exposed during platelet destruction [3]. Somatically mutated IgM+ memory B cells are known to circulate in peripheral blood [25,26]. Molecular studies were therefore performed to determine whether the immortalized B cell was an antigen-selected B cell or a naturally occurring autoimmune B cell. The nucleotide sequences of the heavy and light chain variable domain genes of the B7 anti-myosin mAb were analyzed for the closest germline V_H/V_L gene usage. The presence of somatic mutations in the CDRs and FRs was also evaluated to gain information on the cell origin in the B repertoire. Indeed, high numbers of amino acid replacement (R) mutations in the V_H segment CDRs compared to relatively unchanged FRs is consistent with the exertion of a positive antigenic pressure to mutate the structure of the CDRs and to preserve that of the FRs [11,27].

The alignment of V, D and J segments with known germline genes showed that B7 mAb was encoded by poorly mutated genes. Only two mutations with one amino acid substitution in the FR3 region were detected when we compared the B7 V_H sequence with the germline p1 (99.6% identity). The locations of these point mutations were not characteristic of those

resulting from an antigen-dependent selection process. Moreover, as two other antibodies reported in databases expressed a V_H gene sequence 100% homologous to that of B7 (data not shown), the question as to whether these changes actually represent mutations or are simply allelic polymorphisms of the p1 germline gene remains to be answered. Such polymorphisms have been reported by Olee et al. [20] for hv3005 and 1.9III germline genes. As for the B7 V_L segment, it was found to be 100% homologous with the DPL23 germline gene (V_L3r) [21].

Overall, the sequence analyses of the B7 V region genes are in line with it being the product of the primary repertoire with 'anti-self' specificity. A considerable proportion of cells in normal individuals secrete natural IgM and IgG antibodies which react with a variety of self antigens and are encoded by unmutated Ig V genes [28,29]. A large majority of them are polyspecific antibodies [30]. Here, we have a B cell of the primary repertoire able to produce a monospecific anti-myosin antibody.

Because of its specificity, B7 may have a potential for the in vivo detection of myocardial cell death. Radiolabeled anti-myosin antibodies can bind to insoluble myosin after the loss of myocyte membrane integrity, therefore allowing the scintigraphic detection of myocardial necrosis [31]. Anti-myosin imaging may be helpful as a non-invasive method in identifying cardiac rejection and its progression in heart transplants [2], suspected myocarditis [5], muscular tumors [32] and cardiac side-effects of anthracycline treatment [6,33]. Moreover, B7 is of human origin, and human antibodies are known to reduce or eliminate immune reactions frequently elicited in patients upon administration of a murine antibody [34].

However, human monoclonal IgM antibodies may not have an optimal imaging capability due to their large size [35]. It is generally assumed that due to steric encumbrance, IgM have a slow tissue penetration rate [36]. The recent development of genetically engineered scFv, offer the potential to alleviate this problem [7]. We have constructed a gene encoding for the B7 antibody scFv fragment into the pHOG21 vector and the scFv were shown to be expressed in a soluble form in *E. coli*. Nevertheless, our recent results of scFv purification (manuscript in preparation) showed that the level of B7 scFv expression in the periplasmic space combined to culture medium is around 0.1 mg/l. It is well known that scFv from different antibodies can show very different yields of secretion within *E. coli*, as their unique amino acid sequences can affect their expression [37]. For example, in identical *E. coli* host/vector systems, 1–5 mg/l of anti-glycophorin A 1C3 scFv were found in the culture medium [38] compared to 0.1–0.5 mg/l of anti-DNP U7.6 scFv and no anti-transferrin receptor OKT9 scFv [39]. The low yield of B7 scFv production can be improved by using fermentation protocols as established by Schiweck et al. [40]. Here, we have proved that the B7-related scFv fragments have conserved their specificity for the myosin of human heart. However, diagnostic applications of scFv fragments often require large amounts of protein. Thus, prior to clinical application, we will compare B7 scFv production in *E. coli*, *Pichia Pastoris* [41] and in the baculovirus-insect cells expression system [42]. Both eukaryotic expression systems have been reported to reach yields in the range of several hundred milligram per liter. By virtue of their unique myosin specificity and human origin, B7 scFv targeting to damaged myocytes would be a potential important tool in the imaging of myocardial necrosis for a number of myocardial disorders, as a non-invasive method.

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