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OTHER PROBLEMS

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Neutralizing Monoclonal Antibodies Cross-React with Fusion Proteins Encoded by *129L* of the Ectromelia Virus and *A30L* of the Variola Virus

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Abstract—PCR fragments containing the fusion protein genes *129L* of the ectromelia virus (EV) and *A30L* of the variola virus (VARV) were cloned in pQE32. The expression products, recombinant prA30L and pr129L, were isolated from *Escherichia coli* cell lysates by metal-chelate affinity chromatography. The recombinant proteins retained the capability of oligomerization, characteristic of their natural analogs. ELISA and immunoblotting were used to test 22 monoclonal antibodies (mAbs) to orthopoxviruses (19 mAbs to EV, 2 mAbs to the vaccinia virus (VACV), and 1 mAb to the cowpox virus (CPXV)) for interaction with prA30L, pr129L, and orthopoxviruses. Twelve species-specific epitopes were found in the EV fusion protein 129L and its recombinant analog. Ten cross-reacting epitopes were found in the EV, CPXV, and VACV fusion proteins. Of these, nine epitopes were present both in prA30L and in the VARV fusion protein. Five mAbs interacting with cross-reacting epitopes were capable of efficient neutralization of VACV; two of these mAbs neutralized VARV. It was demonstrated that there are species-specific epitopes in EV 129L and cross-reacting epitopes in the EV, VARV, CPXV, and VACV fusion proteins, including epitopes that induced synthesis of virus-neutralizing antibodies against VACV and VARV.

Key words: variola, ectromelia, virus, fusion protein, *A30L, 129L*, recombinant protein, monoclonal antibody, virus neutralization

INTRODUCTION

Poxviruses (the family Poxviridae) are large, structurally complex viruses whose genome is linear double-stranded DNA of a size varying from 130 to 230 kb within the family [1, 2]. Poxviruses affect many invertebrates and vertebrates, including humans. The human pathogens variola virus (VARV), monkeypox virus, vaccinia virus (VACV), and cowpox virus (CPXV) belong to the genus *Orthopoxvirus.* Orthopoxviruses are structurally similar and have cross-reacting antigens, which have made it possible to use VACV as a vaccine for eliminating variola from the global population [3]. Monkeypox is continuously recorded in the African population [4] and, in 2003, spread to the United States as a result of a contact with prairie dogs brought from Africa [5]. In view of these facts, it is necessary to develop modern techniques for preventing and treating orthopoxvirus infections. It should be noted, however, that only scarce data are available on the antigenic structure of orthopoxvirus proteins and their role in protective immunity. The genome of VACV, the best studied orthopoxvirus, codes for about 200 polypeptides, several dozens of which are present in virions [1]. Only six VACV proteins—the products of *H3L* [6], *A27L* [7], *B5R* [8], *D8L* [9], *L1R* [10, 11], and *A33R* [12]—are involved in the immune response and contain epitopes inducing synthesis of virus-neutralizing antibodies. The most detailed data have been obtained for the 14K protein, which is encoded by *A27L* and is capable of inducing virus-neutralizing antibodies and protecting the organism from orthopoxvirus infection [13, 14]. Experimental injection of the monoclonal antibody (mAb) C3 against the 14K protein suppresses VACV replication in animals. At the same time, there is almost no data on the 14K protein homologs encoded by *A30L* of VARV [15] and *129L* of the ectromelia virus (EV) [16].

The objective of this work was to study the properties and the antigenic structure of fusion proteins encoded by VARV *A30L* and EV *129L* with the use of their recombinant analogs and mAbs against EV, VACV, and CPXV.

EXPERIMENTAL

Viruses, their cultivation, and their purification. EV strain K-1, VACV strain LIVP, CPXV strain GRI-90, and VARV strain Ind-3a were obtained from the col-

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lection of the Vector State Research Center of Virology and Biotechnology. Viruses were propagated in Vero cells, which were cultured in DMEM supplemented with 2% fetal bovine serum, or on the chorioallantoid membrane of chicken embryos (Novosibirskii Poultry Farm, Kol'tsovo). Isolation and purification of viruses in sucrose density gradients were carried out according to published protocol [17]with some modification [18].

Enzymes. *Taq* DNA polymerase, the Klenow fragment of DNA polymerase I, T4 DNA ligase, *Sma*I, *Hae*II, and *Rsa*I were purchased from SibEnzyme (Russia).

Bacterial strains and viral DNAs. We used *Escherichia coli* strain XLBlue {recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac[F' proAB $lac^qZ\Delta M15$ $Tn10(Tet^r)$ Amy $Cam^r]^{c,d}$}, DNA of VARV strain Ind-3a (International Depositary, Vector State Research Center of Virology and Biotechnology), and DNA of EV strain MP-1n (kindly provided by H. Meyer, Institute of Microbiology, Munich, Germany).

Oligonucleotides. Oligonucleotide primers for PCR (5'-ATGGACGGAACTCTTTTCCCC and 5'-AAGAGTTAAGTTACTCATATGGA) were designed using the Oligo 3.3 program (Borland International, United States) on the basis of the VACV *A27L* nucleotide sequence (GenBank locus M35027).

Molecular cloning. PCR was carried out according to the following scheme: 95°C for 3 min; 18 cycles of 95°C for 45 s, 55°C for 45 s, and 72°C for 45 s; and 95°C for 5 min. Isolation of the amplified fragments, construction of recombinant DNAs, and restriction enzyme analysis followed standard protocols [19]. The structure of the cloned fragments was verified by sequencing the fragment insertion region by nascent strand termination in an ABM PRISM 310 automated sequencer (Perkin Elmer, United States), using standard primers.

Isolation of recombinant proteins. Individual colonies of Ap^r, Tc^r transformants of *E. coli* XLBlue (*QE32*/*129L*) and *E. coli* XLBlue (*QE32*/*A30L*) were grown in 100 ml of the LB medium at 37°C until $A_{550} = 0.6$. Expression of the target genes was induced by adding IPTG to the final concentration 1 mM. After 4 h, cells were collected by low-speed centrifugation; suspended in 5 ml of 100 mM Tris-HCl (pH 7.3), 10 mM EDTA, 2 mM PMSF, 10 mM imidazole; and disrupted by sonication. The soluble protein fraction was dialyzed against buffer A (20 mM Tris-HCl, pH 8.5, 20 mM imidazole, 5 mM 2-mercaptoethanol, 10% glycerol) supplemented with 500 mM KCl and applied onto a 5-ml column with Ni-chelate agarose equilibrated with the same buffer. The column was washed with 1 ml of buffer A containing 1 mM KCl. The target products were eluted with buffer B (20 mM Tris-HCl, pH 8.5, 100 mM KCl, 100 mM imidazole, 5 mM 2-mercaptoethanol, 10% glycerol, 2 mM PMSF). Protein was quantitated according to Bradford [20].

Hybridomas and monoclonal antibodies. To obtain mouse mAbs against VACV and CPXV, hybridomas were constructed by hybridizing spleen cells of immune BALB/c mice with NS-1 myeloma cells [21]. Positive hybridomas producing mAbs against orthopoxviruses were selected by ELISA with peroxidase-conjugated polyclonal antispecific antibodies [22]. As antigens, we used purified VACV and CPXV at 10 μg/ml. The antigens were adsorbed at 1 μg per well in 0.05 phosphate buffer (pH 7.4) overnight. For cryoconservation, hybridoma preparations in DMEM supplemented with 10% DMSO and 40% fetal bovine serum were frozen in liquid nitrogen. To obtain ascitic fluids containing mAbs, female BALB/c mice (Vector State Research Center of Virology and Biotechnology) weighing 20–22 g were injected intraperitoneally with pristane (Sigma, United States) and then with (10–20) × 10^6 hybridoma cells per mouse. Rat mAb to EV was obtained as described previously [23]. To purify mAb from ascitic fluids, nonspecific proteins were precipitated with caprylic acid and then immunoglobulins were precipitated with ammonium sulfate [24]. The mAb concentration was determined with a BioRad protein assay kit as recommended by the manufacturer, using purified rat IgG as a standard for calibration.

Neutralization reaction with VACV and VARV were performed under maximal protection conditions (BSL-4), using positively pressured protective garments. Plaque formation was assayed in Vero cell cultures growing in 96-well plates [25]. As a positive control, we used mAb J2D5 [10].

Electrophoresis and immunoblotting. Proteins were separated by PAGE according to Laemmli [26] and transferred onto a nitrocellulose membrane (Schleicher & Schuell, United States) or a 0.45-μm nitrocellulose membrane (Millipore, United States) in a Bio-Rad electroblotting unit (United States). To block nonspecific binding, membranes were incubated in 25 mM Tris-HCl (pH 7.3), 137 mM NaCl, 3 mM KCl, 5% skimmed milk. Immune complexes of mAbs with recombinant fusion proteins were detected using horseradish peroxidase-conjugated goat antibodies against mouse IgG (Bio-Rad, 1:25,000), the chemiluminescent reagent ECL (Amersham Pharmacia Biotech, United States), and a CP-BU X-ray film (AGFA, Belgium). Alternatively, we used peroxidase-conjugated antispecific antibodies against mouse IgG (Bio-Rad) or rat IgG (Sigma) [23].

Table 1. Differences in the amino acid sequence between the fusion proteins of VACV, VARV, and EV

Amino acid position	Amino acid substitution:		
	A27L VACV	A30L VARV	129L EV
25	D (Asp)	A (Ala)	A (Ala)
30	A (Ala)	A (Ala)	D (Asp)
32	R (Arg)	R (Arg)	H (His)
35	I (Ile)	I (Ile)	T (Thr)
42	D (Asp)	N (Asn)	D (Asp)
77	E (Glu)	D (Asp)	E (Glu)

Note: Information about the amino acid sequences of the protein products of *A27L* of VACV strain Copenhagen, *A30L* of VARV strain India, and *129L* of EV strain Moscow is available at www.poxvirus.org.

RESULTS

Cloning and Expression in *E. coli* of EV *129L* and VARV *A30L*

VE *129L* and VARV *A30L* were PCR-amplified from the corresponding viral genomes and cloned into the *Sma*I site of pQE32 (Qiagen, Germany) to produce two recombinant plasmids, pQE32/129L and pQE32/A30L. Sequencing confirmed that the structure of the cloned genes was as theoretically expected from our protocol of plasmid construction: the fusion proteins had four species-specific amino acid substitutions clustered (residues 30–42) in the central region (Table 1) and additional N-terminal residues ($MRGSH_6GIRMRARTP$) encoded by the polylinker of the vector. Expression of *A30L* and *129L* in *E. coli* yielded two recombinant polypeptides, prA30L and pr129L. The apparent molecular weight of the recombinant proteins in PAGE assays was respectively 19 and 20 kDa (Fig. 1), somewhat higher than theoretically expected (≈16 kDa), even with correction for the additional N-terminal residues. It should also be noted that the recombinant proteins differed in electrophoretic mobility, which was lower in pr129L.

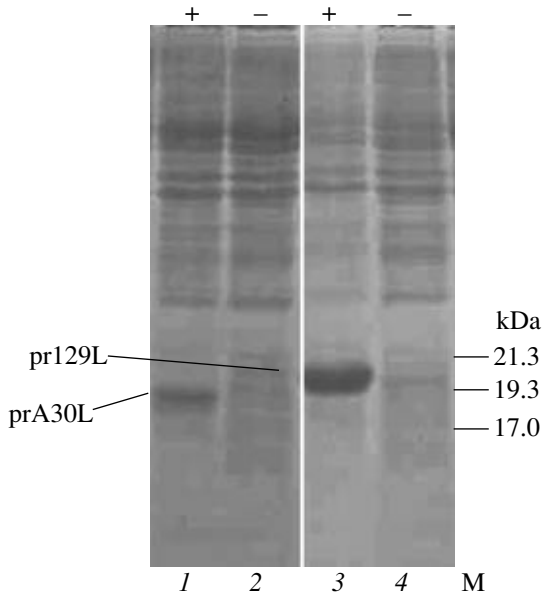

Fig. 1. Expression of EV *129L* and VARV *A30L* in *E. coli* XLBlue. Total cell lysates were obtained from transformants carrying (*1, 2*) pQE32/A30L or (*3, 4*) pQE32/129L before (–) or after (+) induction with IPTG and separated by PAGE in 15% gel. M, marker proteins of 21.3, 19.3, and 17.0 kDa from kits of prestained SDS-PAGE standards, Kaleidoscope prestained standards, and gel filtration standards (Bio Rad). Gel was stained with Coomassie R-250.

Both prA30L and pr129L were isolated from bacterial cell lysates by affinity chromatography on Ni-NTA. Electrophoretic analysis of the preparations obtained during prA30L isolation from a bacterial cell lysate (Fig. 2, lane *1*) showed that prA30L was synthesized to a high level and that our procedure was efficient and yielded high-purity (~90%) prA30L (lanes *7*, *8*). The method allowed us to obtain the recombinant proteins in considerable amounts and to study their properties.

To check whether the recombinant analogs preserve the capability of natural proteins to form homologous oligomers despite of the additional amino acid residues, the affinity-purified proteins were resolved by PAGE in 12% gel in the presence (denaturing conditions) or absence of 2-mercaptoethanol (Fig. 3a), transferred onto a nitrocellulose membrane, and analyzed by immunoblotting (Fig. 3b). In the absence of 2-mercaptoethanol, oligomers (trimers) were observed for pr129L (lanes *4, 8*) and, to a lesser extent, for prA20L (lanes *2, 6*). In the presence of 2-mercaptoethanol, pr129L occurred in two, major and minor (having a higher electrophoretic mobility), forms (lane *3*). A possible explanation of such occurrence is that pr129L is less tolerant of bacterial proteases than prA30L.

Interaction of mAbs with Recombinant Fusion Proteins

We have previously obtained a collection of hybridomas producing 125 mAbs to EV and have selected 19 mAbs reacting with the 14-kDa protein of EV [23]. This mAb panel was supplemented with three additional mAbs: 1E1 against CPXV and 8B11 and 11G7 against VACV, which were obtained in this work and reacted with the recombinant proteins. The interaction of the mAbs with affinity-purified pr129L and prA30L is characterized in Table 2. Both in ELISA and in immunoblotting, 21 out of the 22 mAbs interacted with purified pr129L. The only exception was mAb 112H12 (against EV), which efficiently interacted with the fusion protein contained in EV particles [23] and failed to recognize its recombinant analog pr129L, though reacting with prA30L in ELISA. Nine mAbs cross-reacted with prA30L in ELISA. The

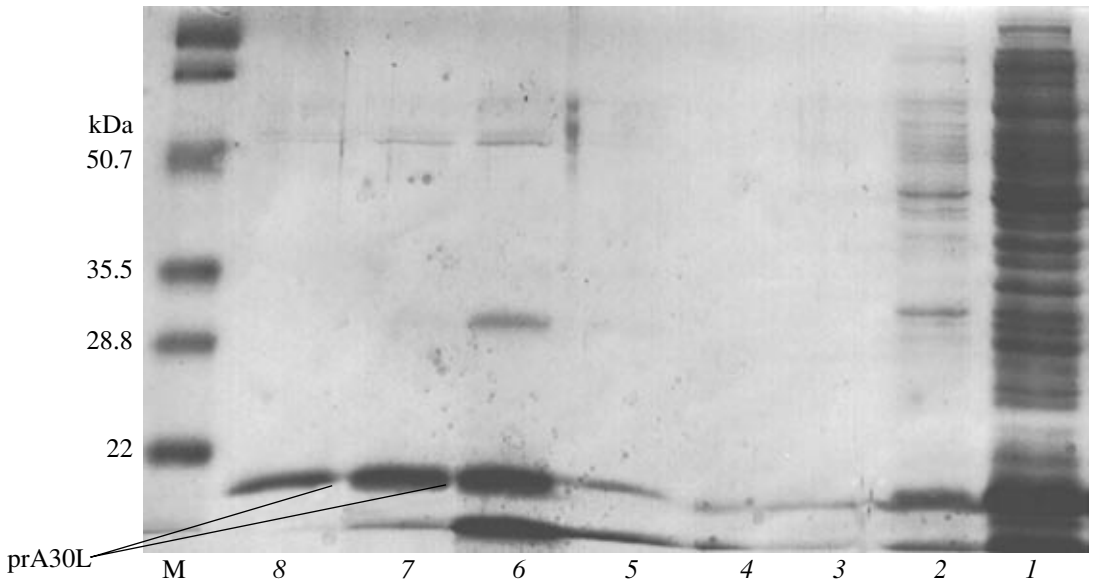

Fig. 2. Electrophoresis in 12% PAG of preparations obtained during isolation of prA30L from *E. coli* XLBlue (pQE32/A30L): (*1*) a clarified lysate of cells disrupted by sonication; (*2*) protein nonbound on Ni-NTA agarose; (*3, 4*) washing with buffer A; and (*5–8*) fractions 1–4, respectively. Elution of prA30L was performed with buffer B as recommended by Qiagen. Bands of prA30L are indicated with arrows. M, prestained SDS-PAGE standards (114.0, 88.0, 50.7, 35.5, 28.8, and 22 kDa; Bio Rad). Gel was stained with Coomassie R-250.

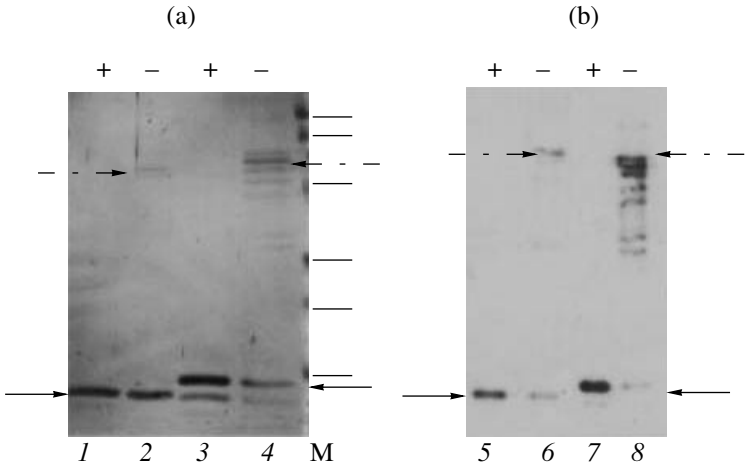

Fig. 3. Electrophoresis (a) and immunoblotting (b) of purified (*1, 2, 5, 6*) prA30L (0.85 μg per lane) and (*3, 4, 7, 8*) pr129L (0.93 μg per lane). PAGE in 12% gel was performed in the presence (+) or absence (–) of 2-mercaptoethanol in the sample application buffer. Immunoblotting was performed with a mixture of mAbs 111A3, 111C4, 113D5, 113F8, 117A3, and 122C10 (Table 2) at 1:500 dilution. The monomeric (solid arrows) and trimeric (dot-dashed arrows) forms of the recombinant proteins are indicated. M, prestained SDS-PAGE standards (114.0, 88.0, 50.7, 35.5, 28.8, and 22 kDa; Bio Rad, United States); the marker positions are shown with horizontal lines.

cross-reaction was confirmed by immunoblotting for eight mAbs (Table 2), suggesting common antigenic determinants for the fusion proteins of EV and VARV, as well as for their recombinant analogs. However, 13 mAbs did not react with prA30L, which can be explained by the difference in antigenic structure between the recombinant product and the VARV fusion protein. To obtain more detailed data, we studied the cross-reactivity of the mAbs from our panel with the four orthopoxviruses (EV, VACV, CPXV, and VARV) by ELISA and the neutralization reaction.

Cross-Reacting Neutralizing Epitopes of the Fusion Proteins of Orthopoxviruses (EV, VARV, CPXV, and VACV)

The interaction with the recombinant and viral proteins was studied for the 22 mAbs against the ortho-

Table 2. Interaction of mAbs with purified recombinant pr129L of EV and prA30L of VARV

No.	mAb	Immunogenic virus	Interaction with recombinant polypeptide			
			pr129L		prA30L	
			mAb titer in ELISA*	immunoblotting**	mAb titer in ELISA*	immunoblotting**
1	111A3	EV	3.65×10^5	+	2.2×10^5	+
2	111C4	EV	1.29×10^5	+	<100	–
3	112H12	EV	<100	–	4.05×10^4	–
4	113D5	EV	4.5×10^4	+	<100	–
5	113F8	EV	3.65×10^5	+	2.2×10^5	+
6	115F11	EV	4.05×10^4	+	4.05×10^4	+
7	117A3	EV	4.05×10^4	+	<100	–
8	122C10	EV	1.29×10^5	+	<100	–
9	122E9	EV	3.65×10^5	+	<100	–
10	122G4	EV	3.65×10^5	+	<100	–
11	122H9	EV	3.65×10^5	+	3.65×10^5	+
12	123C3	EV	1.29×10^5	+	<100	–
13	124B6	EV	3.65×10^5	+	<100	–
14	124H9	EV	3.65×10^5	+	<100	–
15	124H2	EV	1.29×10^5	+	<100	–
16	125G9	EV	1.29×10^5	+	1.29×10^5	+
17	126C9	EV	1.29×10^5	+	<100	–
18	126C3	EV	1.29×10^5	+	<100	–
19	127F2	EV	4.05×10^4	+	<100	–
20	1E1	CPXV	2.43×10^4	+	2.43×10^4	+
21	8B11	VACV	2.43×10^4	+	2.43×10^4	+
22	11G7	VACV	4.4×10^4	+	4.4×10^4	+

Notes: * The reverse of the mAB titers estimated in ELISA is shown.

** The specific interaction of mAbs with the recombinant peptides (+) was or (–) was not detected.

poxvirus fusion proteins, including 19 mAbs against EV, 2 mAbs against VACV, and 1 mAb against CPXV (Tables 2, 3). Considerable similarity of the antigenic structure was observed for the fusion protein contained in EV virions and its recombinant analog, pr129L. Of the 19 mAbs against EV, 12 reacted both with species-specific epitopes of the EV protein (Table 3) and with epitopes of pr129L (Table 2). Seven mAbs interacted with cross-reacting orthopoxvirus epitopes, which are also present in the EV fusion protein. Six of these mAbs (with the exception of mAb 112H12) recognized these epitopes in pr129L (Tables 2, 3). Only ten mAbs of our panel (seven against EV, two against VACV, and one against CPXV) cross-reacted with EV, VACV, and CPXV (Table 3). Of these, nine mAbs (six against EV, two against VACV, and one against CPXV) interacted with prA30L (Table 2) and cross-reacted with VARV (Table 3), which confirmed the similarity of the VARV fusion protein and its recombinant analog. Moreover, our findings suggest common cross-reacting epitopes for the fusion proteins of EV, VACV, CPXV, and VARV.

The virus-neutralizing activity of the 22 mAbs recognizing the orthopoxvirus fusion proteins was studied with cultured Vero cells. Five cross-reacting mAbs against EV (112H12, 113D5, 113F8, 122H9, and 125G9) displayed the virus-neutralizing activity with respect to VACV (Table 3). Two mAbs, 122H9 and 125G9, showed the highest VACV-neutralizing activity and were capable of neutralizing VARV. Our results allowed us to conclude that the fusion proteins of EV, VACV, CPXV, and VARV have cross-reacting epitopes capable of inducing synthesis of virus-neutralizing antibodies against VARV in B cells and that the recombinant proteins pr129L and prA30L retain the structure of these epitopes.

DISCUSSION

The VACV A27L protein, which is responsible for the fusion of viral and cell membranes, induces synthesis of VACV-neutralizing antibodies in B cells [27, 28]. In addition, A27L binds with heparin, forms a stable

Table 3. Virus-neutralizing activity and cross-reaction of mAbs with orthopoxviruses

mAb	mAb titer (reverse) in ELISA			ELISA of mAb with VARV*	Neutralization reaction**	
	EV	VACV	CPXV		VACV	VARV
111A3	3.65×10^5	1.09×10^6	1.2×10^5	+	–	nd
111C4	3.65×10^5	<100	<100	–	–	nd
112H12	1.09×10^6	3.65×10^5	1.09×10^6	+	80	nd
113D5	1.2×10^5	8.1×10^3	8.1×10^3	–	200	nd
113F8	1.09×10^6	3.65×10^5	1.2×10^5	+	200	nd
115F11	1.09×10^6	1.09×10^6	3.65×10^5	+	–	nd
117A3	1.2×10^5	<100	<100	–	–	nd
122C10	1.2×10^5	<100	<100	–	–	nd
122E9	1.2×10^5	<100	<100	–	–	nd
122G4	4.1×10^4	<100	<100	–	–	nd
122H9	2.2×10^6	2.2×10^6	2.2×10^6	+	12000	500
123C3	1.4×10^4	<100	<100	–	–	nd
124B6	3.65×10^5	<100	<100	–	–	nd
124H9	3.65×10^5	<100	<100	–	–	nd
124H2	3.65×10^5	<100	<100	–	–	nd
125G9	1.09×10^6	2.2×10^6	1.5×10^6	+	8000	500
126C9	4.1×10^4	<100	<100	–	–	nd
126C3	4.1×10^4	<100	<100	–	–	nd
127F2	1.2×10^5	<100	<100	–	–	nd
1E1	2.4×10^4	1×10^3	2.4×10^4	+	–	nd
8B11	<300	2.2×10^5	1×10^3	+	–	nd
11G7	6.6×10^6	6.6×10^6	2.4×10^5	+	–	nd

Notes: * VARV was treated as in [12], and the resulting lysate was used as an antigen in ELISA; (+), A495 ≤ 0.02; (–), A495 ≥ 1.0. Ascitic fluids were used as a source of mAbs at 1:500 dilution.

** Indicated is the reverse of the ascitic fluid dilution inhibiting more than 50% of plaque-forming units (PFU) after infection with 1000 PFU/well. The neutralizing effect was (–) not observed or (dn) not determined.

complex with A17L and A14L of VACV, and is necessary for the formation of extracellular VACV virions [10, 29]. VACV A27L consists of 110 amino acid residues, which are highly conserved. The EV fusion protein 129L has four amino acid substitutions as compared with VACV A27L; three to five substitutions are characteristic of the fusion proteins of various VARV strains (Table 1). The VACV fusion protein has the apparent molecular weight of about 14 kDa according to denaturing electrophoresis and occurs as a trimer in virions [30]. The N-terminal region of the VACV fusion protein harbors the heparin-binding domain (residues 21–33) and the fusion peptide (residues 29–37) [31, 32]. The first 29 residues of the protein are involved in the formation of extracellular VACV virions. Starting from residue 37, the protein has the α-helical structure; region 37–49 is responsible for the formation of a stem of three α-helices in the trimer. It is of interest to note that most species-specific amino acid substitutions characteristic of this protein are in the region of the fusion peptide (Table 1).

Another important function of the VACV fusion peptide is based on its marked antigenic character and the capability of inducing antiviral immunity. It is known that A27L induces synthesis of virus-neutralizing antibodies and activates the cell immune response in infected organisms [10, 25, 27–29]. The progress of orthopoxvirus infection can be prevented using virus-neutralizing antibodies against the 14 kDa product of *A27L*. This fact demonstrates the important role of the fusion protein in antiviral immunity.

To study in greater detail the properties of the EV and VARV fusion proteins, homologs of the VACV fusion protein, we cloned EV *129L* and VARV *A30L* in pQE32 and obtained affinity-purified recombinant pr129L and prA30L in amounts sufficient for such a study. An important structural feature of the VACV fusion protein is the capability of forming trimers, because it is in the trimeric form that the protein

occurs in VACV virions and on the membrane of infected cells [30, 33]. Electrophoresis and immunoblotting showed that pr129L and prA30L are similar to the natural proteins in forming trimers.

Using a large collection of hybridomas to poxviruses, we constructed a panel of 22 mAbs specific for the orthopoxvirus fusion proteins. It is important to note that live viruses were used for immunizing animals in order to obtain hybridomas; i.e., the resulting mAbs are against live viruses. Almost all mAbs efficiently interacted with EV pr129L in ELISA. The only exception was mAb 112H12, which did not react with EV pr129L but did react with VARV prA30L. Hence, the 112H12 epitope was changed in pr129L compared with its natural analog, while a similar epitope was formed in VARV prA30L. We think that 12 mAbs are species-specific, reacting with the EV fusion protein and pr129L but not with prA30L nor any other orthopoxvirus examined. Of the five amino acids differing between pr129L and prA30L (Table 1), four are in region 30–42 residues of the EV fusion protein. Since this region corresponds to the fusion peptide, the above 12 mAbs can be assumed to recognize the specific sequence of the EV fusion peptide, which thereby differs from its counterparts of the other orthopoxviruses. Structural analysis has shown that the VACV fusion protein lacks globular domains [32]. Hence, we think that the antigenic determinants of this protein are linear. Indirect evidence for this assumption was provided by the results of immunoblotting of the recombinant proteins with the mAb panel after denaturing electrophoresis. Taken together, the above data suggest that the amino acid substitutions in the fusion peptide region are responsible for the species-specificity of mAb interactions with monomeric and trimeric (natural) forms of the EV and VARV fusion proteins.

Nine mAbs efficiently reacted with prA30L; native VACV, CPXV, and EV; and inactivated VARV in ELISA. This finding suggests that the fusion proteins of different orthopoxviruses have cross-reacting or genus-specific epitopes and that the recombinant proteins fully retain or mimic the epitope structures. Five mAbs cross-reacting with the orthopoxviruses were active in the neutralization reaction against VACV strain LIVP. In addition, mAbs 122H9 and 125G9 neutralized VARV strain Ind-3a. Thus, we were the first to demonstrate that VARV A30L has an epitope(s) directly involved in virus neutralization. Region 26–40 resudues of VACV A27L has been shown to induce synthesis of virus-neutralizing antibodies against VACV [31]. It is possible to assume that the same region of the fusion protein is recognized by VARV-neutralizing mAbs 122H9 and 125G9. However, the presence of amino acid substitutions in this region of the VARV protein compared with its VACV counterpart makes this assumption questionable and requires further investigation and more exact mapping of virus-neutralizing epitopes of the VARV fusion protein.

Thus, detailed analysis of the interaction of the mAb panel against orthopoxviruses with the orthopoxvirus fusion proteins and their recombinant analogs, pr129L and prA30L, suggests at least three different groups of epitopes for the fusion protein (Table 3). Group 1 epitopes are species-specific because 12 mAbs to these epitopes interact only with EV. Group 2 and 3 epitopes are genus-specific. Ten mAbs to these epitopes cross-react with EV, VACV, CPXV, and VARV. In addition, group 3 epitopes, which are recognized by five mAbs, are involved in the induction of virus-neutralizing antibodies.

We think that our hybridoma collection and the recombinant proteins pr129L and prA30L can be used to further study the role of the fusion proteins and its particular regions both in poxvirus reproduction and in immunity against pathogenic orthopoxviruses. Our results support previously recorded data which demonstrated that, owing to the presence of cross-reacting virus-neutralizing epitopes in the fusion protein, its recombinant analogs can be used to design vaccines against orthopoxviruses pathogenic for humans. It is also possible to develop new preventive and therapeutic preparations on the basis of native or recombinant virus-neutralizing antibodies against the fusion protein.

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