The EMBO Journal Vol.2 No.12 pp.2305-2312, 1983

Monoclonal antibodies to desmin, the muscle-specific intermediate filament protein

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Communicated by M. Osborn
Received on 19 August 1983; revised on 7 September 1983

A set of monoclonal antibodies to desmin has been isolated from a fusion of mouse myeloma cells with spleen cells from mice immunized with purified porcine desmin. Eleven group I antibodies recognized desmin in the immune blot, and using defined desmin fragments the epitope has been tentatively assigned as lying between residues 325 and 372. When cell lines were tested in immunofluorescence only the human line RD and hamster BHK-21 were positive. When tissue sections were used, skeletal, cardiac, visceral and some vascular smooth muscle cells were positive. Thus, the group I antibodies appear specific for desmin and do not recognize other intermediate filament proteins. Group II monoclonals recognized not only desmin in the immune blot but also other polypeptides. The epitope of this class is located between residues 70 and 280. In immunofluorescence on cell lines and tissues, the staining patterns of group II antibodies were more complicated and demonstrate that not only other intermediate filament proteins but also additional antigenic determinants are being recognized. The group I antibodies stain, as expected from their desmin specificity, rat and human rhabdomyosarcomas and thus appear to be useful reagents in pathology.

Key words: desmin/muscle/intermediate filaments/tumor diagnosis/rhabdomyosarcoma

Introduction

Intermediate filament (IF) expression is a cell- and tissue-specific event. Rabbit and guinea pig antibodies purified on their respective antigen have been instrumental in differentiating the five major subgroups of IF proteins: epithelial cytokeratins, neuronal neurofilaments, astrocyte glial acidic fibrillary protein (GFAP), myogenic desmin and mesenchymal vimentin (for review, see Holtzer *et al.*, 1982; Franke *et al.*, 1982; Osborn *et al.*, 1982). Gel electrophoretic results support these distinctions and have allowed a further dissection of the keratin subfamily into some 20 individual components. Some of the different keratin species have also been shown to be histologically interesting markers capable of distinguishing morphologically different epithelia (Fuchs and Green, 1978; Moll *et al.*, 1982) in line with refined immunological data (Lane, 1982; Debus *et al.*, 1982b, Tseng *et al.*, 1982). Amino acid sequence data derived from the purified proteins (Geisler and Weber, 1981, 1982; Geisler *et al.*, 1982a, 1982b), or their gene clones (Hanukoglu and Fuchs, 1982), have not only supported the distinctions made by immunology but have also stressed the common building principle of IFs. Related rod-like middle domains, some 310 residues in length, contain α-helices able to form coiled-coil elements and thus account for the X-ray diffraction pattern of IF. These rods are flanked by rather hypervariable terminal domains, which are not α-helical in nature but also contribute to filament organization. In addition, these studies have shown that along the rod domains the molecules are related proteins in which sequence identities between 30 and 70% can be observed. This finding further emphasizes the need for antibodies, which are not only well characterized but are known by various criteria to detect only one human IF polypeptide in complex mixtures.

IF antibodies have been clearly demonstrated to be of use in human surgical pathology where they can identify the major human tumor groups (for review, see Osborn and Weber, 1983). Thus, carcinomas are cytokeratin-positive, at least some tumors derived from the sympathetic nervous system are neurofilament-positive, gliomas are GFAP-positive, rhabdomyosarcomas are desmin-positive, while non-muscle sarcomas are vimentin-positive. The progress recently made in using such antibodies in pathological diagnosis as well as the hope of making such reagents available on a large scale and therefore readily exchangeable seemed to warrant resorting to the monoclonal antibody strategy invented by Kohler and Milstein (1975). Monoclonal antibodies specific for individual neurofilament polypeptides have been described (e.g., Debus *et al.*, 1982a; Anderton *et al.*, 1982) as have monoclonal antibodies which recognize either the whole cytokeratin family, certain subsets of cytokeratins or only the cytokeratin component 18 (Gigi *et al.*, 1982; Lane, 1982; Tseng *et al.*, 1982; Debus *et al.*, 1982b).

Here we describe a set of mouse monoclonal antibodies to desmin, the IF protein characteristic of myogenic cells. Such monoclonals should be useful not only in problems in pathology but should also allow certain currently controversial problems related to desmin expression to be resolved.

Results

Isolation of monoclonals to desmin

The assay procedure was designed to select clones that were not only positive for desmin by immunochemical tests but which reacted in fluorescence microscopy on specimens known from previous results with conventional antibodies to be desmin-positive. At day 14 after fusion, 600 out of 720 wells showed growth. Supernatants from 324 of the 600 (i.e., 61%) showed a positive reaction with porcine desmin in the enzyme-linked immunosorbent assay (ELISA) test. A further ELISA test performed in the presence of 500 mM NaCl (high salt ELISA) in the washing step served to identify 132 putative high affinity antibodies to desmin. Parallel to these tests, supernatants positive in the ELISA assay were tested by immunofluorescence microscopy using as a test object the human rhabdomyosarcoma line, RD. Experiments using conventional antibodies have shown that this cell line contains both desmin and vimentin (our unpublished results). RD was preferred to the hamster line BHK-21 which also contains desmin and vimentin (Tuszynski *et al.*, 1979), since RD cells are of human origin, and one of the aims was to obtain desmin monoclonals suitable for use in human pathology.

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Table I.

	Iso type	ELISA against desmin	Western blot analysis				Characterization on cell lines				Characterization on tissue sections					
			Desmin (kd)	Cys part	Rod piece (kd)	RD cell extract	RD[c]	Glioma[a] HeLa IMR-90	BHK-21[c]	CV-1 PtK2 3T3[b]	Human		Rat			Toad skeletal muscle
											Skeletal muscle[c]	Rhabdomyosarcoma	Tongue muscle	Tongue other cell types	Rhabdomyosarcoma	
I. Monoclonals recognizing 18-K rod piece																
1. DE-A-7	IgG1	+	53	CII	18	53	+		+		+	+	+	–	+	+
2. DE-B-5	IgG1	+	53	CII	18	53	+	–	+	–	+	0	+	–	+	+
3. DE-C-3	IgG1	+	53	CII	18	53	+	–	+	–	+	+	+	–	+	+
4. DE-D-1	IgG1	+	53	CII	18	53	+		+		+	0	+	–	0	+
5. DE-E-1	IgG1	+	53	CII	18	53	+	–	+	–	+	+	+	–	+	+
6. DE-H-1	IgG1	+	53	CII	18	53	+		+		+	0	+	–	0	+
7. DE-I-1	IgG1	+	53	CII	18	53	+		+	–	+	+	+	–	+	+
8. DE-L-3	IgG1	+	53	CII	18	53	+	–	+		+	+	+	–	+	+
9. DE-R-11	IgG1	+	53	CII	18	53	+	–	+	–	+	0	+	–	0	0
10. DE-S-8	IgG1	+	53	CII	18	53	+	–	+	–	+	0	+	–	0	0
11. DE-U-10	IgG1	+	53	CII	18	53	+	–	+	–	+	0	+	–	+	+
II. Monoclonals recognizing 21K rod piece																
DE-J-4	IgM	+	53	CI	21	Multiple bands	See text for staining patterns									
III. Other monoclonals																
G-2	IgG1	-	-	-	-	-		Stress fibers								
IV. Conventional desmin antibody (H87)																
		+	53		18,21		+		+	–	+	+	+	–	+	+

+ Gives typical desmin pattern on cell line or tissue in question, e.g. stains Z-lines in skeletal muscle but not cells lying between the muscle fibers, in tongue, stains tongue muscle but not other cell types, in rhabdomyosarcoma stains tumor cells, in RD cells stains 80 – 90% interphase cells, in BHK-21 cells most but not all cells are stained. In both these cases typical IF profiles are seen. – Staining not observed. 0 not tested.

[a] In these cases DE-A-7, DE-C-3 and DE-H-1 staining of ~2 – 5% of cells of the glioma line was observed.

[b] With DE-C-3 cytoplasmic dots were observed and with DE-H-1 nucleoli were stained.

[c] In these instances cell lines or tissue sections were tested after formaldehyde treatment. After formaldehyde treatment of human muscle sections staining of the Z-lines was still seen with several of the group I monoclonals, e.g., Figure 3h. After formaldehyde treatment of RD or of BHK-21 cells the percentage of cells showing strong staining was very much reduced.

Screening of the supernatants of the 324 wells that were positive in the first ELISA test showed that 170 decorated RD cells when assayed by immunofluorescence microscopy. 124 of these supernatants resulted in staining of filamentous arrays, i.e., patterns compatible with the staining of IFs in these cells seen with polyclonal antibodies. Variations in the intensity of staining as well as in the percentage of desmin-postive and -negative cells were frequently seen. Presumably these variations reflect the fact that not all cells of the RD line are stained by normal desmin antibodies although all cells are stained by the vimentin antibodies. Other staining patterns detected by the immunofluorescence tests included the occasional clone that stained either stress fibers, or dotted structures in the cytoplasm or lamin-like staining of the nuclear membrane. 70 clones that were strongly positive in the high salt ELISA assay and in fluorescence microscopy on RD cells were selected for further characterization, and stocks of these were frozen. Here we describe results with a representative 13 clones that were independently isolated and which have been made monoclonal by limiting dilution or by agar cloning (see Table I).

Identification of desmin as the target: approximate location of the epitope

The Western blot technique (Towbin *et al.*, 1979) was used to test the monoclonal antibodies on both porcine and chicken gizzard demin and on different defined fragments of desmin from chicken gizzard (Table I). The fragments used were CI (residues 1 – 323) and CII (residues 324 – 463) obtained by cleavage at the single cysteine residue using 2-nitro-5-thiocyanobenzoic acid (Geisler and Weber, 1981, 1982), the rod domain (residues 70 – 415), as well as the amino-terminal 21-K fragment and the carboxy-terminal 18-K fragment derived from this domain by prolonged chymotryptic cleavage (Geisler *et al.*, 1982a). The precise cleavage point separating the two sub-domains of the rod is not yet known. All antibodies in groups I and II reacted with the full desmin molecule and its rod in the ELISA assay and on blots (e.g., Figure 1A). The two groups could be distingiushed, however, when a mixture of the 18-K and 21-K fragments was used (Figure 1B). Eleven of the antibodies reacted only with the 18-K fragment (Figure 1B, slots 2, 3), whereas one, which was

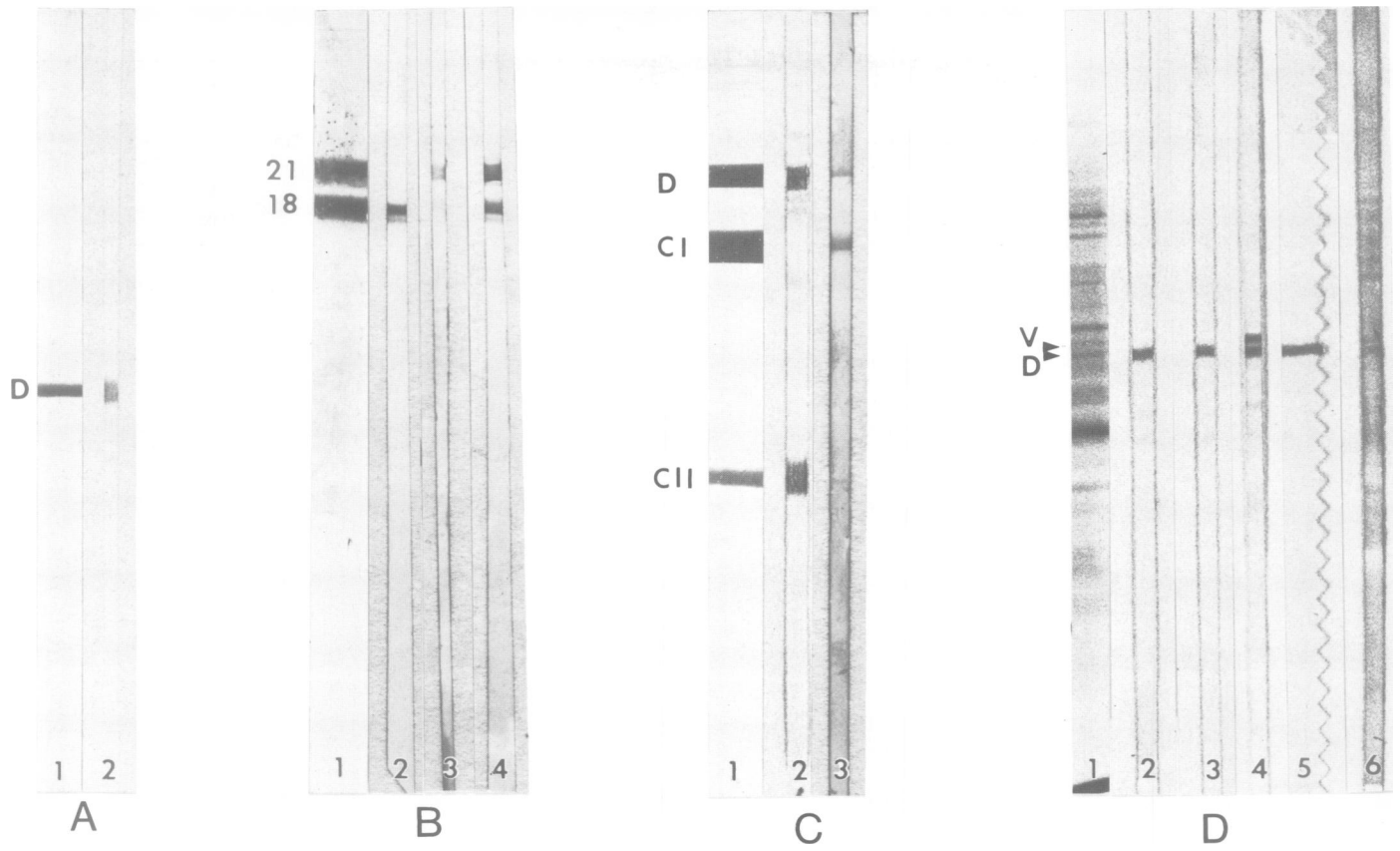


Fig. 1. Western blots of monoclonal antibodies against **(A)** desmin, **(B, C)** defined fragments of desmin and **(D)** an RD cell extract. **(A) Slot 1** Coomassie-stained gel porcine desmin, **slot 2** Western blot with DE-R-11, **(B) Slot 1** Coomassie-stained gel showing 21-K and 18-K fragments of chicken desmin, **slots 2–4** Western blots with **slot 2** DE-B-5, **slot 3** DE-J-4, **slot 4** conventional rabbit desmin antibody. **(C) Slot 1** Coomassie-stained gel showing chicken desmin D and cys I and II fragments, **slots 2** and **3** Western blots with **slot 2** DE-B-5, **slot 3** DE-J-4. **(D) Slot 1** Coomassie Blue-stained gel of RD cell extract, **slots 2–5** Western blot with **slot 2** DE-A-7, **slot 3** DE-C-3, **slot 4** DE-B-5 together with a vimentin monoclonal, **slot 5** DE-E-1 and **slot 6** DE-J-4. Note that the group I desmin antibodies recognize desmin, the 18-K fragment and the CII fragment. Group II monoclonals recognize desmin, the 21-K fragment and the CI fragment.

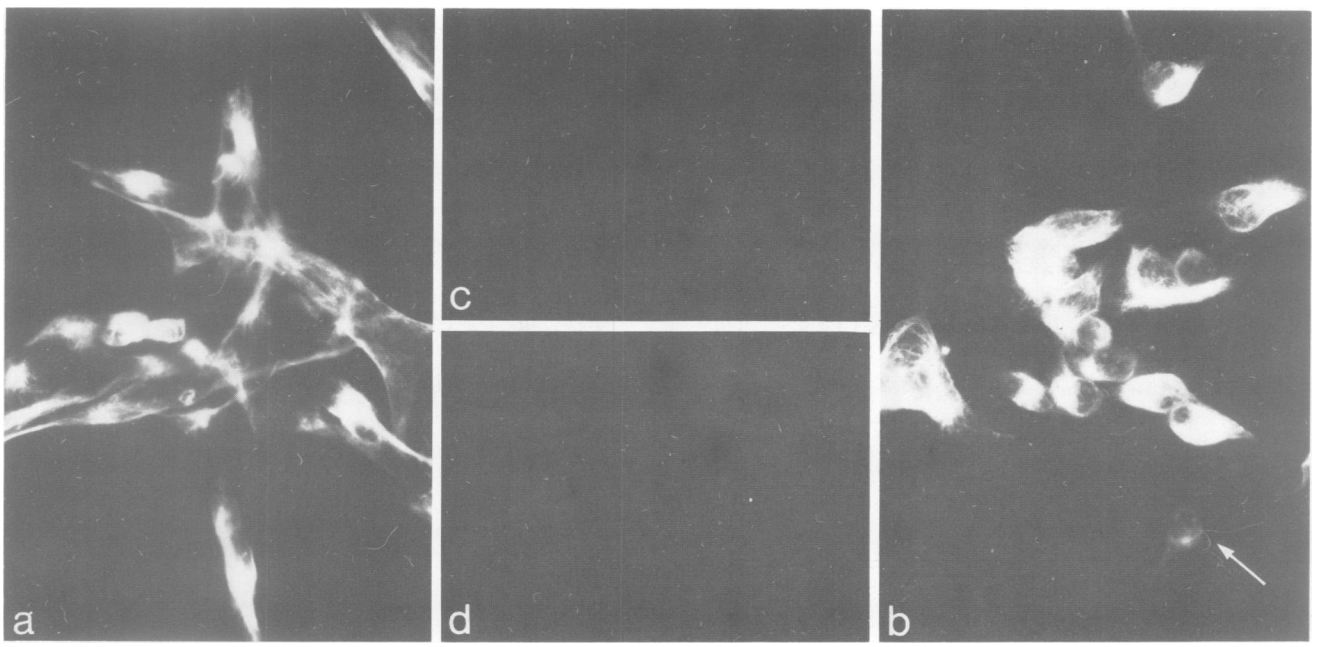
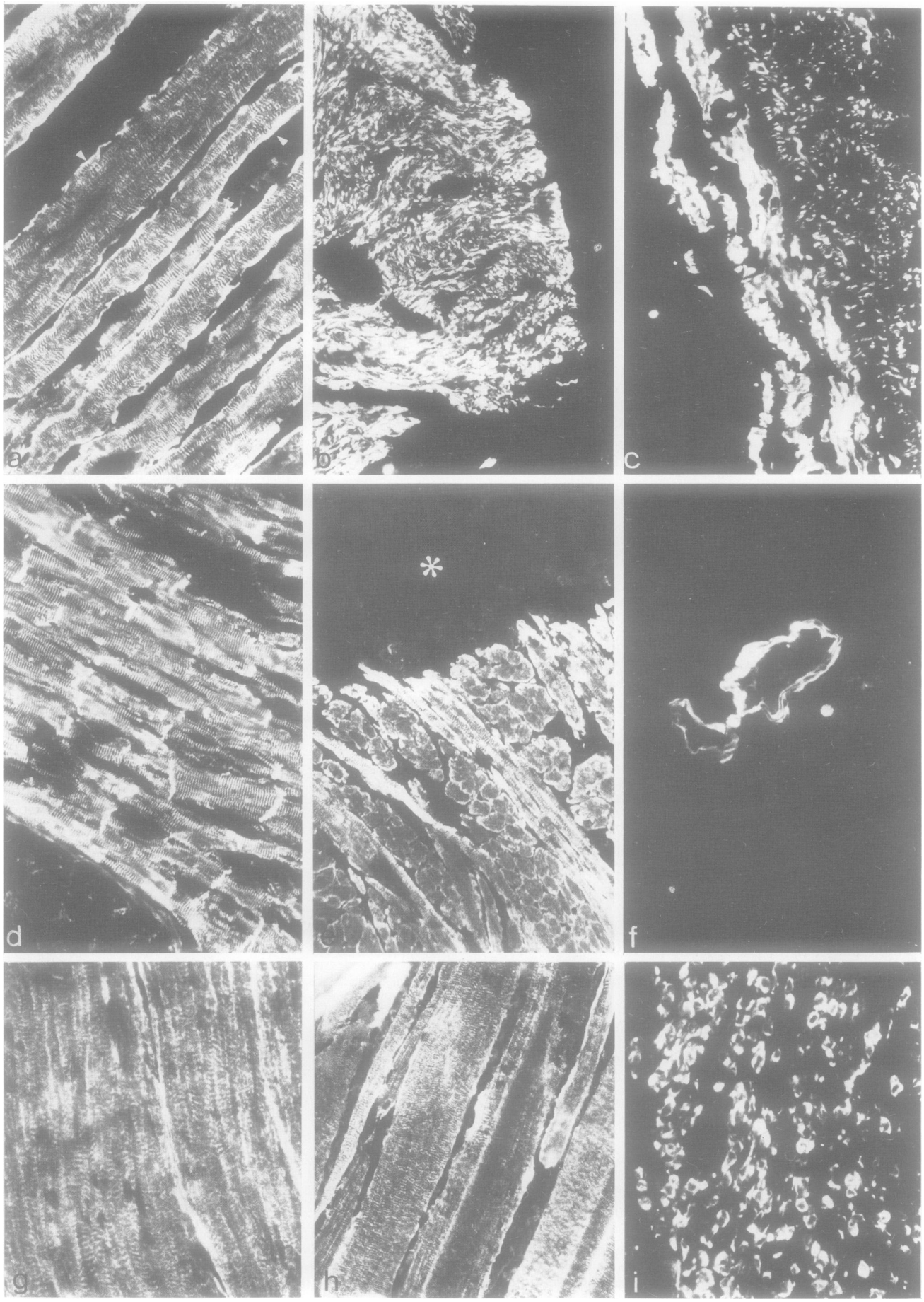


Fig. 2. Representative staining patterns of group I monoclonals on cell lines. Both the human RD line **(a)** and the hamster BHK-21 line **(b)** which are cell lines known to contain desmin are strongly stained by the group I desmin monoclonals. Not all interphase cells are equally strongly stained (e.g., arrow in **d**). Cell lines which do not contain desmin such as the human fibroblast line IMR-90 **(c)** and rat kangaroo PtK2 cells **(d)** are not stained. The desmin monoclonals shown are **(a)** DE-L-3, **(b–d)** DE-E-1. Magnification x 430.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selected as a representative of a second group, reacted with the 21-K fragment. A conventional rabbit desmin antibody (Osborn *et al.*, 1982) reacted with both 18-K and 21-K fragments (Figure 1B, slot 4). A more precise location of the epitope of group I antibodies recognizing the 18-K fragment was possible by the use of the C-fragments (Figure 1C). All group I antibodies recognized CII but not CI (Figure 1C, slot 2), whereas the group II 21-K fragment-specific antibody recognized CI but not CII (Figure 1C, slot 3). Use of the full amino acid sequence of chicken desmin (Geisler and Weber, 1982) allows assignment of the epitopes of group I antibodies to residues 324–415 and of group II antibodies to residues 70–280. Arguments which further restrict the epitope location are given in the Discussion.

In agreement with the finding that the group I monoclonal antibodies recognize purified desmin and its fragment on a Western blot, a positive result was also obtained when these monoclonals were tested by the Western blot method on human RD cells which contain desmin and vimentin. A single band of mol. wt. 53 kd was decorated with the group I antibodies (e.g., Figure 1D). When a vimentin antibody was included with the group I desmin monoclonals two bands were detected (Figure 1D, slot 4) and, as expected from the known mol. wts. of desmin and vimentin, the vimentin band was of higher mol. wt. (~57 kd).

The DE-J-4 monoclonal antibody gave a different result. Although it clearly stained a 53-kd band, a band corresponding to vimentin as well as other additional bands were also visible in the Western blot (Figure 1D, slot 6).

Characterization of desmin monoclonals on cell lines

The IF content of several hundred cell lines is now known and it is therefore possible to select representative lines of known IF content as prototypes. Thus each monoclonal antibody was tested on the following human cell lines: rhabdomyosarcoma RD (desmin-positive, vimentin-positive), the glioma line U333/343MG (GFAP-positive, vimentin-positive). HeLa (cytokeratin- and vimentin-positive), and the lung fibroblast IMR-90 (vimentin-positive). Only the RD cells gave strong positive staining consistent with the fact that the other human cell lines do not contain desmin when tested either in immunofluorescence microscopy using conventional desmin antibodies or on gels. The group I desmin monoclonals stained 80–90% of interphase RD cells (Figure 2a). Thus, not all interphase RD cells are stained, a result also found with a conventional desmin antibody. In contrast, all interphase RD cells are stained to an approximately equivalent amount by a conventional vimentin antibody. RD cells analyzed by gel electrophoresis reveal vimentin and desmin in an approximate ratio of 3 to 2 (data not shown: for nitrocellulose transferred material see Figure 1D). Presumably these results show that individual RD cells may vary in their desmin content. The human cell lines HeLa (cytokeratin-positive, vimentin-positive), IMR-90 (vimentin-positive) and glioma line (GFAP-positive, vimentin-positive) did not show IF staining (Figure 2c, d). Likewise, when animal cell lines were used, only baby hamster kidney, BHK-21 cells, which contain desmin and vimentin (Tuszynski *et al.*, 1979), stained positively with the group I monoclonals (Figure 2d). Monkey CV-1 and rat kangaroo PtK2 cells (both are cytokeratin- and vimentin-positive) as well as mouse 3T3 cells (vimentin-positive) did not show IF staining.

The DE-J-4 antibody in group II, in contrast, stained IFs not only in RD cells, but also in other cell types such as RMCD and HeLa. If cells such as RD were first fixed with formaldehyde and then examined, the staining patterns with DE-J-4 became even more complex. Not only IFs but also sub-membraneous staining was then visible (data not shown).

Characterization of desmin monoclonals on tissue sections

When human skeletal muscle sections were examined, positive staining of the Z-lines was seen with each of the group I monoclonals (Figure 3a). Increased staining around the periphery of the muscle cell, underlying the membrane, was often observed (arrowhead, Figure 3a). Cells lying between the muscle fibers were not stained, in agreement with results using conventional desmin antibodies. Other human muscle tissues that were strongly stained included smooth muscle (e.g., human uterus, Figure 3b) and certain vascular smooth mucle cells (see, e.g., artery shown in Figure 3c). Note, however, that also in these cases only the cells of muscle origin are stained. Sections of rat cardiac muscle, and of rat tongue were also examined. The Z-lines as well as the intercalated discs of the cardiac muscle are strongly positive (Figure 3d). In tongue the muscle is strongly positive but cells in the epithelium, in the *lamina propria* and in the nerve bundles of the tongue are not stained (Figure 3e). The group I monoclonals were also tested on sections of rat cerebellum where, except for vascular smooth muscle cells present in blood vessels, no positive staining was seen (Figure 3f). Finally, since sections of skeletal muscle from the toad *Bufo marinus* showed strong staining of Z-lines (Figure 3g), the cross-species reactivity of the group I monoclonal antibodies as well as the conventional H87 desmin antibody extends at least from man to toad. Formaldehyde fixation of human skeletal muscle sections clearly decreased the intensity of the Z-line staining but Z-lines were clearly visible after such treatments (e.g., Figure 3h). Finally, we had available sections from a single human rhabdomyosarcoma (Altmannsberger *et al.*, 1982) and from a single experimentally induced rat rhabdomyosarcoma. As can be seen from Figure 3i and from Table I those group I monoclonals that were tested on these rhabdomyosarcomas stained the tumor cells positively.

DE-J-4 did not give a typical desmin pattern on sections of human skeletal muscle and also stained cells lying between the muscle fibers. On rat tongue almost all cell types present in this tissue appeared to be stained, although again the profiles were not typical of IFs.

During the course of this study two other interesting monoclonals were isolated. G-2, which does not recognize desmin in the Western blot, was isolated by chance during subcloning. This clone stains regularly spaced bands in human skeletal muscle which appeared thicker than the Z-lines stained by the desmin antibody. On the human line IMR-90 this

Fig. 3. Staining patterns of group I desmin monoclonals on different tissues. **(a)** Human skeletal muscle DE-I-1, **(b)** human uterus DE-B-5, **(c)** human arteria iliac externa DE-I-1, **(d)** rat cardiac muscle DE-I-1, **(e)** rat tongue DE-R-11, **(f)** rat cerebellum DE-A-7, **(g)** toad skeletal muscle DE-E-1, **(h)** formaldehyde-fixed human skeletal muscle DE-E-1, **(i)** human rhabdomyosarcoma DE-L-3. Note the strong positive staining of Z-lines in striated muscle **(a,d,e,g,h)** and of the intercalated discs in cardiac muscle **(d)**, the staining of visceral smooth muscle **(b)** and of vascular smooth muscle **(c,f)**. Note also the strong staining of the tumor cells in **(i)**. Note that other cell types of non-muscle origin are not stained by the group I monoclonals (e.g., cells in tongue epithelium (*) and in lamina propria in **e**, astrocytes in **f**). The staining patterns seen with the desmin monoclonals are therefore equivalent to those obtained with conventional rabbit desmin antibodies. Magnificiation **(a,d,f,h,i)** x 260, **(b,c,e)** x 170, **(g)** x 430.

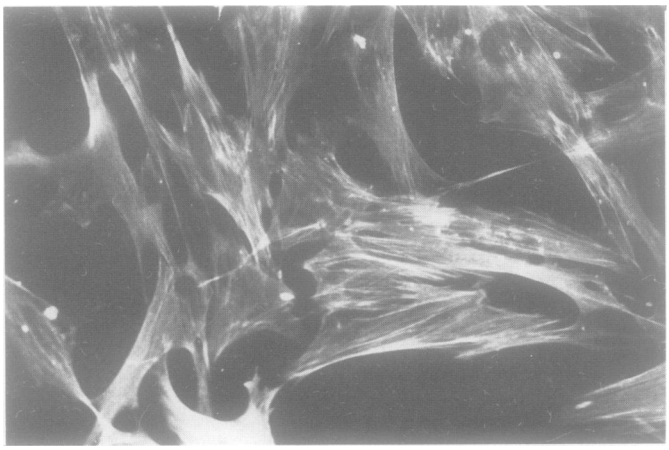

Fig. 4. Staining of the IMR-90 human fibroblast line by the DE-J-4 antibody, which does not recognize desmin in the immune blot. Note the positive staining of stress fibers. Magnification x 260.

clone gave a typical stress fiber pattern (Figure 4). Thus G-2 appears to recognize a protein that forms part of the microfilament bundles. A further clone, F-6, also gave an interesting pattern in that it gave positive fluorescence in human skeletal muscle only in a region coincident with or just underneath the membranes surrounding the muscle fibers, and also stained cells lying between the muscle fibers in a similar manner. Further work is required to identify more fully the target molecules recognized by these two monoclonal antibodies.

Discussion

The immunochemical and immunocytological data accumulated above argue strongly that the 11 group I monoclonal antibodies recognize desmin and not any of the other major IF proteins. Thus we have shown decoration both of purified chicken and porcine desmin (53 kd) and of a 53-kd polypeptide in a human rhabdomyosarcoma line. The finding that the group I monoclonals recognize only desmin is also consistent with the demonstration that, in tissues, only cells of muscle origin are stained, and that of the cell lines tested only those that contain desmin, but not those that contain cytokeratin, vimentin, or GFAP, are positive with the group I monoclonals.

Immune blots of desmin and its defined fragments clearly separate group I and group II antibodies as far as epitope location is concerned. Group I antibodies recognize an epitope between residues 324 and 415 (see Results). Since these antibodies are specific for desmin and do not recognize either vimentin or the neurofilament 68-K protein, we can use the sequence data available for the three proteins in this region (for a summary see Weber *et al.*, 1983). Thus, we tentatively assign the epitope to residues 325 – 372, because of extensive sequence identities in the subsequent array (residues 372 – 407). In addition, residues 407 – 415 are an unlikely target since, in this region, porcine and chicken desmin show several amino acid exchanges (Geisler and Weber, 1981). Use of even shorter fragments, such as CNBr fragments and tryptic peptides, may further refine the location of the epitope. In contrast, group II antibodies must recognize an epitope located between residues 70 and 280 and this must involve a site common to several distinct IF proteins. Here again, use of smaller fragments together with the available sequence data may further delineate this more general epitope. It is interesting to note that, contrary to a proposal made without experimental data (Steinert *et al.*, 1983), all the desmin-specific monoclonal antibodies (group I) recognize their epitope not as predicted in the two terminal and hypervariable domains of the IF molecule but rather within a defined portion of the rod domain. In contrast, the group II antibody recognizes not only desmin but also other polypeptides in RD cells. From the staining pattern on tissues and cells it clearly recognizes not only other IF proteins but also additional antigenic determinants.

Desmin was one of the first IF proteins to be purified in biochemically useful amounts (Small and Sobieszek, 1977; Lazarides and Hubbard, 1976; Huiatt *et al.*, 1980) and is still the only non-epithelial IF protein for which the complete amino acid sequence is known (Geisler and Weber, 1982). In spite of these studies and the elegant work locating desmin to the sarcomeric Z-line (Granger and Lazarides, 1979; Richardson *et al.*, 1981; Tokuyasu, 1983), arguments as to whether desmin is restricted to myogenic cells have continued. Although different laboratories have agreed that skeletal, cardiac, visceral and many but not all vascular smooth muscle cells are characterized by desmin (for reviews, see Holtzer *et al.*, 1982; Lazarides *et al.*, 1982; Osborn *et al.*, 1982), expression of this protein in cultured non-myogenic lines has remained controversial. Two approaches were taken. We and others have argued that desmin expression is restricted to myogenic differentiation (Bennett *et al.*, 1982; Osborn *et al.*, 1982) and that its occurrence in a very few lines such as BHK-21 is due to a derivation from embryonic kidney vascular smooth muscle or mesangial cells (Frank and Warren, 1981; Travo *et al.*, 1982). Alternatively Lazarides (1981) has proposed that 'filaments containing vimentin, desmin or synemin or combinations of all three proteins exist in virtually all cell types'. Although this argument was discontinued in a subsequent review (Lazarides, 1982) there has been the occasional immunofluorescence microscopic study reporting some filament staining of non-myogenic cells by conventional sera against desmin (e.g., Campbell *et al.*, 1979). Our results with conventional desmin antibodies and now with a series of desmin monoclonal antibodies which do not stain a variety of vertebrate non-muscle tissues, or cell lines other than RD and BHK-21, show that desmin expression is restricted to myogenic cells. Thus, it seems that claims as to desmin staining in fibroblasts and related cells should be interpreted as either: (a) due to a weak cross-reaction between desmin and vimentin when certain polyclonal antisera, which have not been further antigen affinity-purified, are used, because the two proteins reveal 70% sequence identity along their rod sequences (Quax-Jeuken *et al.*, 1983; Weber *et al.*, 1983), or (b) due to some cross-reaction between desmin and an IF-associated protein. The latter possibility would be consistent with the hypothesis that those IF-associated proteins which are directly incorporated into the filament backbone could share sequence homology with the major filament protein along the rod portion of the molecule (Geisler *et al.*, 1983). Also, we cannot readily explain the recent report by Ip *et al.* (1983) who have used a conventional anti-desmin antibody in immunoelectron microscopy on 'fibroblasts' present in a culture of embryonic chicken cardiac myocytes. They detected a very light labelling which they related to a small amount of desmin present in addition to vimentin. Since it is unclear if

such cells reflect normal fibroblasts, we feel that a generalization is not possible. Alternatively, some antibodies against one of the specific IF proteins can also react with a minor 66-kd protein possibly associated with the filaments (Pruss *et al.*, 1981; W.W. Franke, personal communication). An abstract on monoclonal desmin antibodies (Danto and Fischman, 1981) has been followed by some immunofluorescence microscopic data (Lazarides *et al.*, 1982), which raises the question as to whether some desmin epitopes may change or become obscured during certain stages of myotube differentiation. Although we have not yet performed a myogenesis study with our group I desmin antibodies, all are positive on muscle and those which were tested reacted positively on rhabdomyosarcomas.

If one accepts recent classification schemes that have used IF typing to classify cells in normal and abnormal tissues according to their IF content, antibodies to IFs are obviously useful reagents in pathology (for review, see Osborn and Weber, 1983). Although such classifications are generally accepted, and can be obtained in a variety of laboratories using different antibodies (Gabbiani *et al.*, 1981; Altmannsberger *et al.*, 1981, 1982; Miettinen *et al.*, 1982), reports of exceptions that do not seem to fit the general classification scheme are worrying and have to be followed up, especially since some IF antibodies are increasingly being used in human tumor diagnosis. One such exception is the report by Dahl and Bignami (1982) that astrocytes in rat brain contain both GFAP and desmin. In terms of the view that expression of cytokeratins, GFAP, desmin and neurofilaments indicate mutually exclusive pathways of differentiation, the idea that a cell expresses GFAP and desmin is difficult to understand. It is thus reassuring that in the current study the group I desmin monoclonals did not stain astrocytes in rat cerebellum. One of the desmin monoclonals (DE-I-1) has also been used on a section of human astrocytoma in which the tumor cells were strongly GFAP-positive. Again no staining was seen by this desmin monoclonal antibody.

The isolation of the group I desmin antibodies adds to the increasing list of monoclonal antibodies against IFs which may be expected to be of use in human pathology. Aberrant arrangements of desmin have been noted in several human skeletal and cardiac myopathies using conventionally prepared antibodies (e.g., Edström *et al.*, 1980; Stoeckel *et al.*, 1981; Fidzianska *et al.*, 1983). Likewise, in our hands, conventionally prepared desmin antibodies appear to be an excellent marker for rhabdomyosarcoma and can distinguish these tumors from other small cell childhood tumors which are treated by different clinical regimens (Altmannsberger *et al.*, 1982). Although this has been confirmed by another study (Miettinen *et al.*, 1982), other laboratories using different desmin antisera have not been able to reliably identify rhabdomyosarcomas using desmin antibodies. In one case only the larger rhabdomyoblasts which can already be diagnosed by routine histological assays were reported as desmin-positive (Gabbiani *et al.*, 1981); in a second study which is probably attributable to the different methods of fixation used, only 50% of the rhabdomyosarcomas were desmin-positive (Kahn *et al.*, 1982). The availability of monoclonal desmin antibodies which appear to stain tumor cells strongly in rhabdomyosarcomas of human and rat origin should allow these differences to be resolved, and thus provide a further reagent useful in human tumor diagnosis.

Materials and methods

Preparation of desmin and its fragments

Desmin was purified from porcine stomach (Geisler and Weber, 1981) and was the kind gift of Dr. N. Geisler. The protein purified in the presence of urea was dialyzed against phosphate buffered saline (PBS) before being used as antigen. For preparation of the desmin fragments see Geisler *et al.* (1982a).

Immunization

Four female BALB/c mice 6–8 weeks in age were immunized with purified desmin (50–100 μg protein/injection) using Freund's complete adjuvant for the first injection and incomplete adjuvant for subsequent injections. After three injections, sera were tested in the peroxidase spot test against the original antigen and in immunofluorescence microscopy on sections of human uterus. All mice reacted at least at 1:1000 in the spot test, and at least at 1:50 on uterus smooth muscle.

Fusion

Spleen cells from the mouse giving the strongest reaction were fused with cells from the mouse myeloma line PAI (Stocker *et al.*, 1980) at an approximate ratio of 1:1 using the general procedure described by Fazekas de St. Groth and Scheidegger (1980). After fusion, cells were aliquoted into thirty 24 well plates in HAT medium. Medium was changed twice a week and macrosopic colonies were visible by day 7.

Assays

Antibody type was determined by immunodiffusion tests using sera specific for each of the distinct immunoglobulin classes (Litton Bionetics, Kensington, MD).

Electrophoretic transfer onto nitrocellulose and immunoperoxidase detection was as in Towbin *et al.* (1979) and Debus *et al.* (1982a).

ELISA assays used porcine desmin as antigen.

Human cell lines used were RD (ATCC CCL136) (rhabdomyosarcoma), HeLa (epitheloid carcinoma of the cervix), IMR 90 (lung fibroblast) and the U333 CG/343 MG (glioma). Non-human cell lines including hamster BHK-21 cells (kidney), monkey CV-1 cells (kidney), mouse 3T3 cells and rat kangaroo PtK2 cells. Cells were fixed either with methanol alone or on occasion with 3.7% formaldehyde in PBS for 10 min followed by methanol.

Human, rat and toad tissue sections were from material frozen in isopentane at −140°C and stored at −70°C until use. Material to be fixed in formaldehyde was incubated in 3.7% formaldehyde in PBS for 10 min. Sections of the human rhabdomyosarcoma were from material fixed in alcohol and embedded in paraffin.

Standard procedures for immunofluorescence were used with both cells and sections. FITC-labelled goat anti-mouse (IgG + IgA + IgM) (Cappell Laboratories, Cochranville, PA) was used as the second antibody.

Acknowledgements

We thank Sabine Schiller, Susanne Isenberg and Wolfgang Koch for skilled technical assistance, Drs. J. Stocker and T. Staehelin for discussion concerning the monoclonal technique, and Dr. M. Altmannsberger for providing some of the human material used in these studies.

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Note added in proof

Isolation of monoclonal antibodies specific for either glial fibrillary acidic protein (GFA) or for each individual neurofilament triplet protein (i.e., 68 K or 160 K or 200 K) is described elsewhere (Debus,E., Weber,K. and Osborn,M. 1983 *Differentiation*, **25**, in press).