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Heterogeneity of desmin, the muscle-type intermediate filament protein, in blood vessels and astrocytes

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Summary. Monoclonal antibodies were isolated from mice immunized with chicken gizzard desmin. Antibodies reacting with desmin on immunoblots and selectively decorating chicken and rat intestinal smooth muscle as well as the Z-line in striated muscle, were selected for this study. Based on their staining pattern on cryostat sections of chicken and rat cerebellum, spleen, kidney, aorta and femoral artery, monoclonal supernatants could be divided in three groups: (*i*) antibodies decorating astrocytes and vascular smooth muscle; (*ii*) antibodies decorating only vascular smooth muscle; (*iii*) antibodies decorating only astrocytes. Antibodies in group (*i*) and (*iii*) also stained GFA-negative Bergmann glia in chicken cerebellum. It is proposed that desmin may vary depending on the histological localization.

Introduction

In a study conducted with polyclonal antibodies we reported the immunohistological localization of desmin in astrocytes (Dahl and Bignami 1982). Desmin is the muscle-type intermediate filament protein (Lazarides and Hubbard 1976).

The localization of desmin in astrocytes was not confirmed in a subsequent study conducted with monoclonal antibodies (Debus et al. 1983a) thus suggesting that the astrocyte staining reported in our study could be due to crossreactivity with the two intermediate filament proteins in astroglia, that is GFA protein and vimentin. The similarity of intermediate filament proteins has been shown by amino acid sequence studies (reviewed by Weber and Geisler 1984).

The findings reported in this paper suggest another interpretation for the failure of desmin monoclonal antibodies to decorate astrocytes, i.e. desmin heterogeneity.

Materials and methods

For the purpose of immunization desmin was partially purified from chicken gizzard according to the procedure of Small and Sobieszek (1977). The preparation yielded a major band at 57 K daltons on SDS-PAGE. Antibody testing by the immunoblotting procedure was conducted with desmin further purified by preparative SDS-PAGE (Dahl and Bignami 1982). Ten-18% linear gradient electrophoresis was performed on a slab gel apparatus using the procedure described by Laemmli (1970). The electrophoretic transfer from gels to nitrocellulose paper followed by immunoperoxidase staining (Towbin et al. 1979) was performed as reported (Dahl 1983) using 1:10 dilutions of monoclonal supernatants. Indirect immunofluorescence was carried out on acetone-fixed cryostat sections of chicken and rat cerebellum, small intestine, spleen, kidney, aorta, femoral artery and skeletal myofibrils. Skeletal myofibrils were prepared from muscle of the thigh according to Lazarides and Hubbard (1976).

Production and screening of monoclonal antibodies. Ten BALB/c mice were injected with 0.5 mg of desmin. The injection was given weekly over a period of 5 weeks. About 100 μg of protein in complete Freund's adjuvant was injected subcutaneously in the first, third and fifth week, and intramuscularly in the second and fourth week. A few drops of blood were removed from the vein in the tail and the sera were tested by immunofluorescence on cryostat sections at 1:20 dilutions. The antisera of the six mice selected for the production of monoclonal antibodies stained vascular and intestinal smooth muscle as well as astrocytes. With three antisera staining of arteries was weak compared to astrocyte staining. Selected mice were boosted with 100 μg of antigen injected in the tail 3–5 days before fusion of spleen cells with NS-1 mouse myeloma cells. Fused cells were diluted in 30–46 wells (0.5 ml/well, 2×10^5 cells/well). Supernatants from wells showing clonal growth

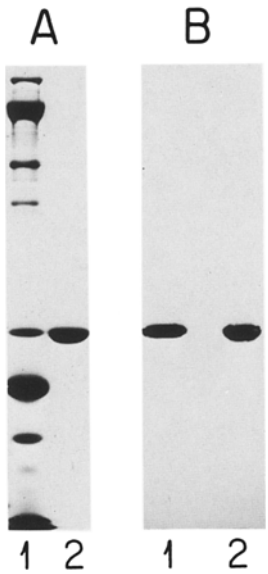

Fig. 1. Purification of desmin. Coomassie blue stained gels are shown in (**A**) and immunoblots in (**B**). **A** 1 chicken gizzard extract; 2, purified desmin. **B** 1 chicken gizzard extract; 2, purified desmin

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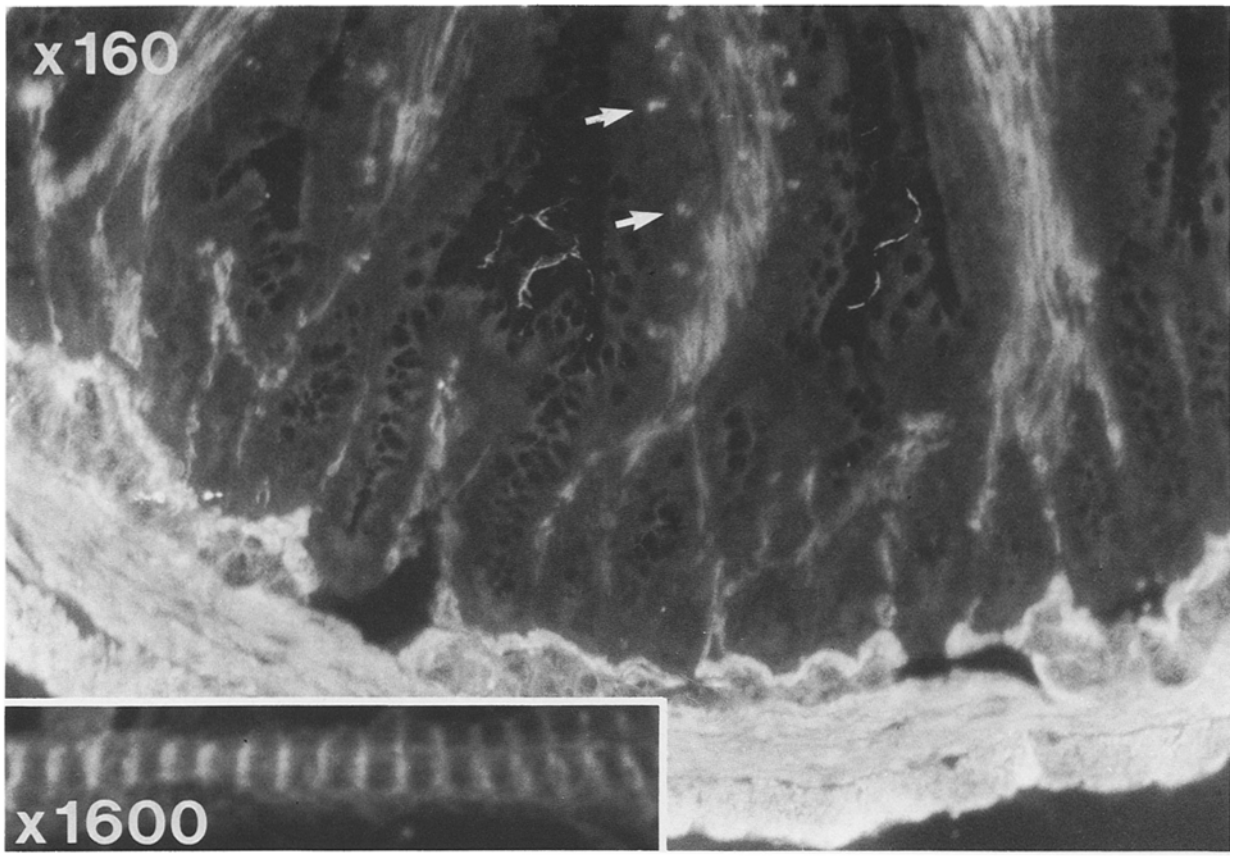

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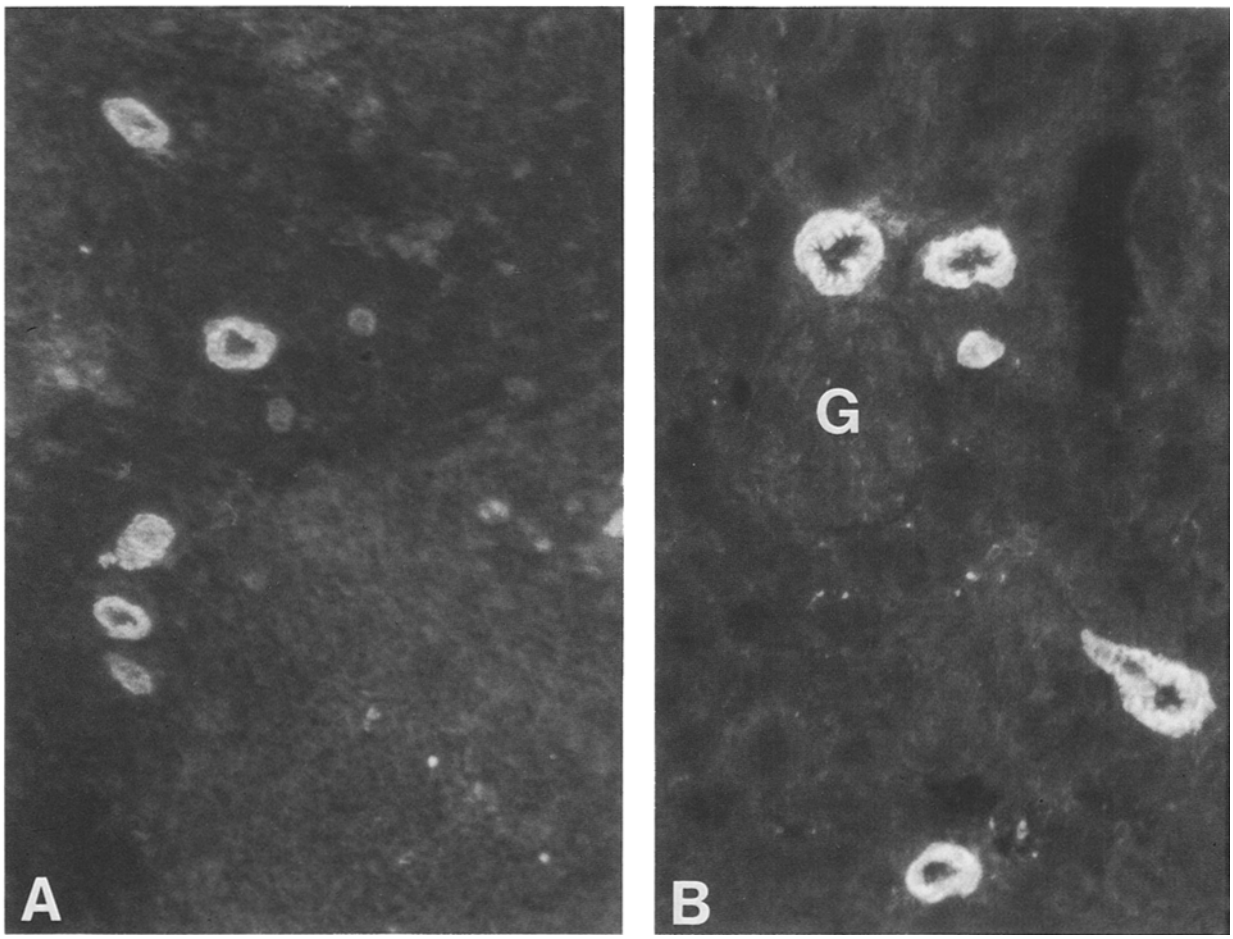

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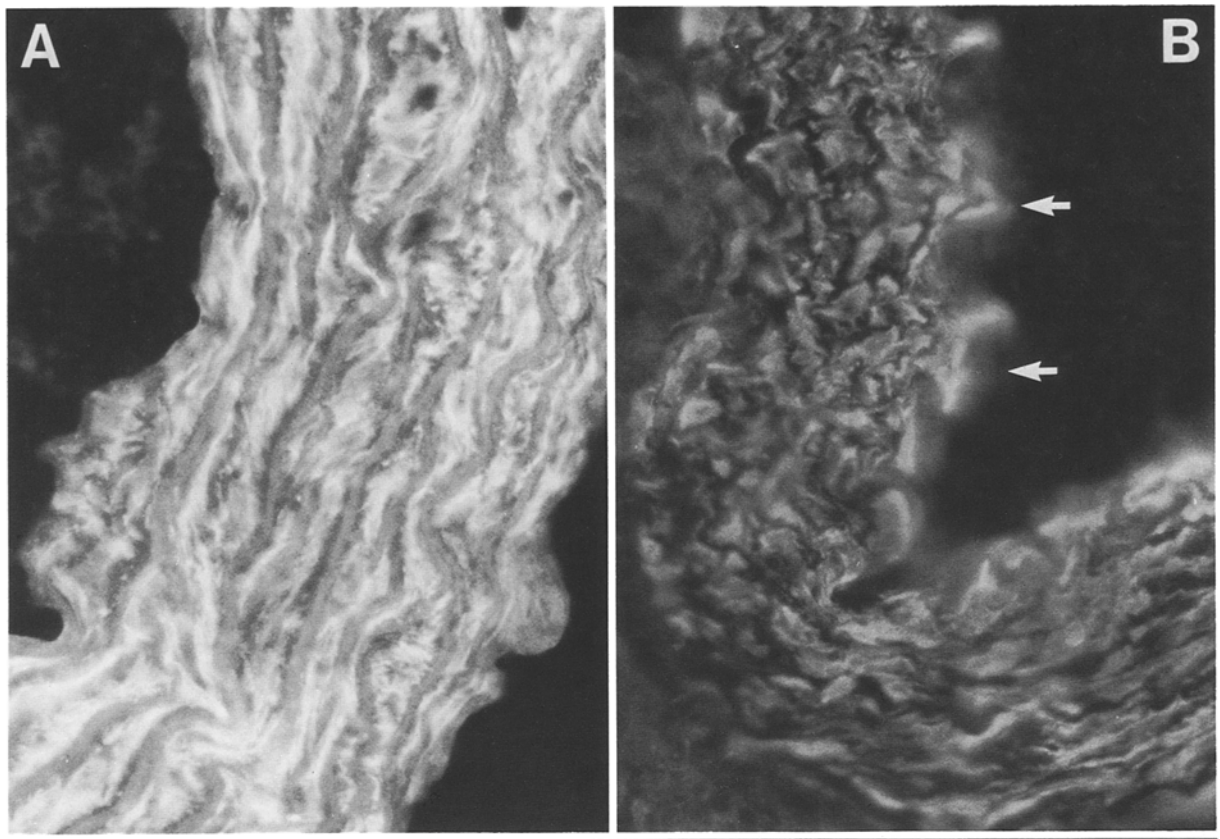
Fig. 2. Immunofluorescent staining with desmin monoclonal antibodies of smooth muscle in a cryostat section of rat small intestine and of the Z-bands in rat skeletal myofibrils (*insert*). Arrows point to autofluorescent lipid in the gut. Compare with Fig. 7 and 21 in the original paper reporting the muscle-specificity of desmin (Lazarides and Hubbard 1976). All monoclonals reported in this study showed this staining pattern. × 160 and × 1600 (*insert*)

Fig. 3. Immunofluorescence staining with desmin monoclonal antibodies of small arteries in a Malpighian corpuscle of the spleen (**A**) and of cortical arteries in the kidney (**B**). *G*, glomerulus. Four out of 6 monoclonals reported in this study showed this pattern. × 160



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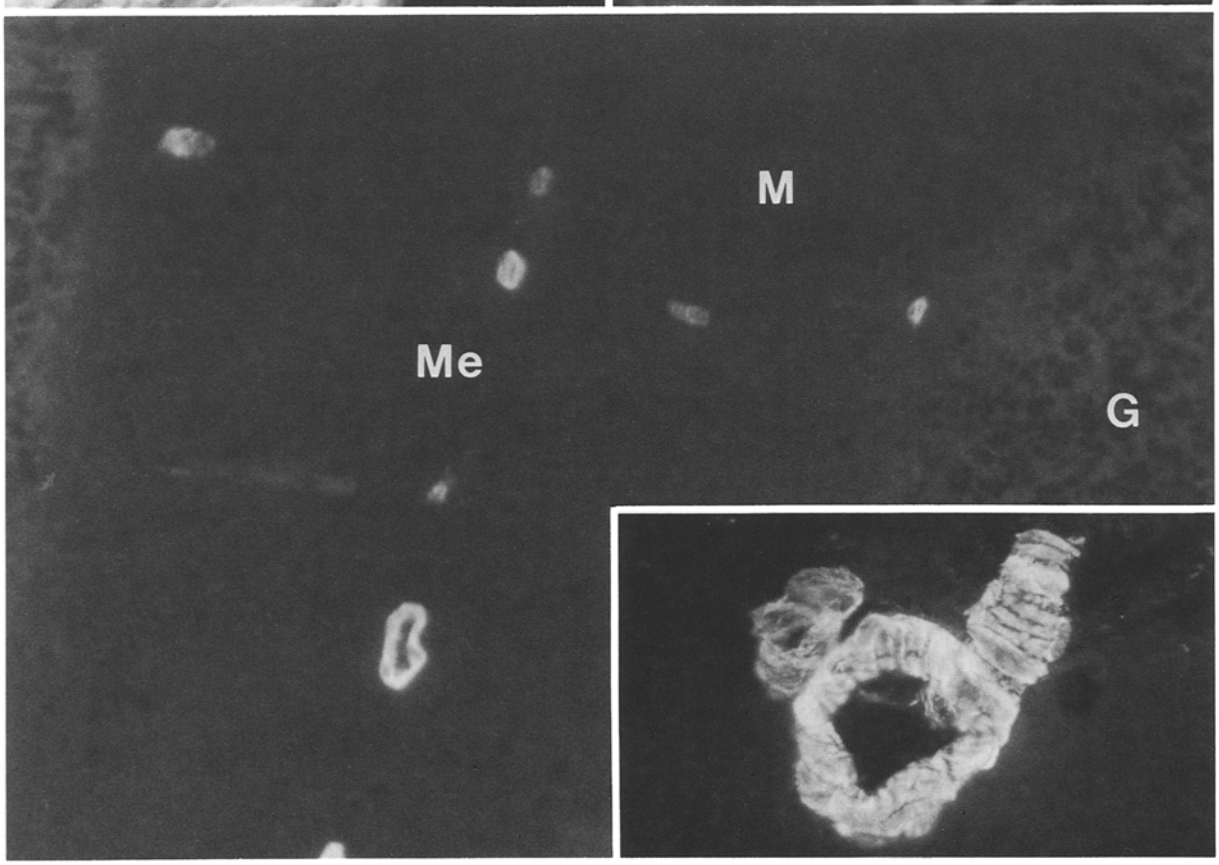

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Fig. 4. Immunofluorescence staining of rat aorta (**A**) and femoral artery (**B**) with desmin monoclonal antibodies decorating vascular smooth muscle. Note in (**B**) the discontinuous autofluorescence of the *elastica interna* (*arrows*). ×160

Fig. 5. Selective immunofluorescence staining of small arteries in a cryostat section of rat cerebellum. Only one desmin monoclonal out of six showed this pattern. *Me*, meninges. *M*, molecular layer. *G*, granular layer. ×160. *Insert:* Small artery in the leptomeninges at higher magnification. ×400

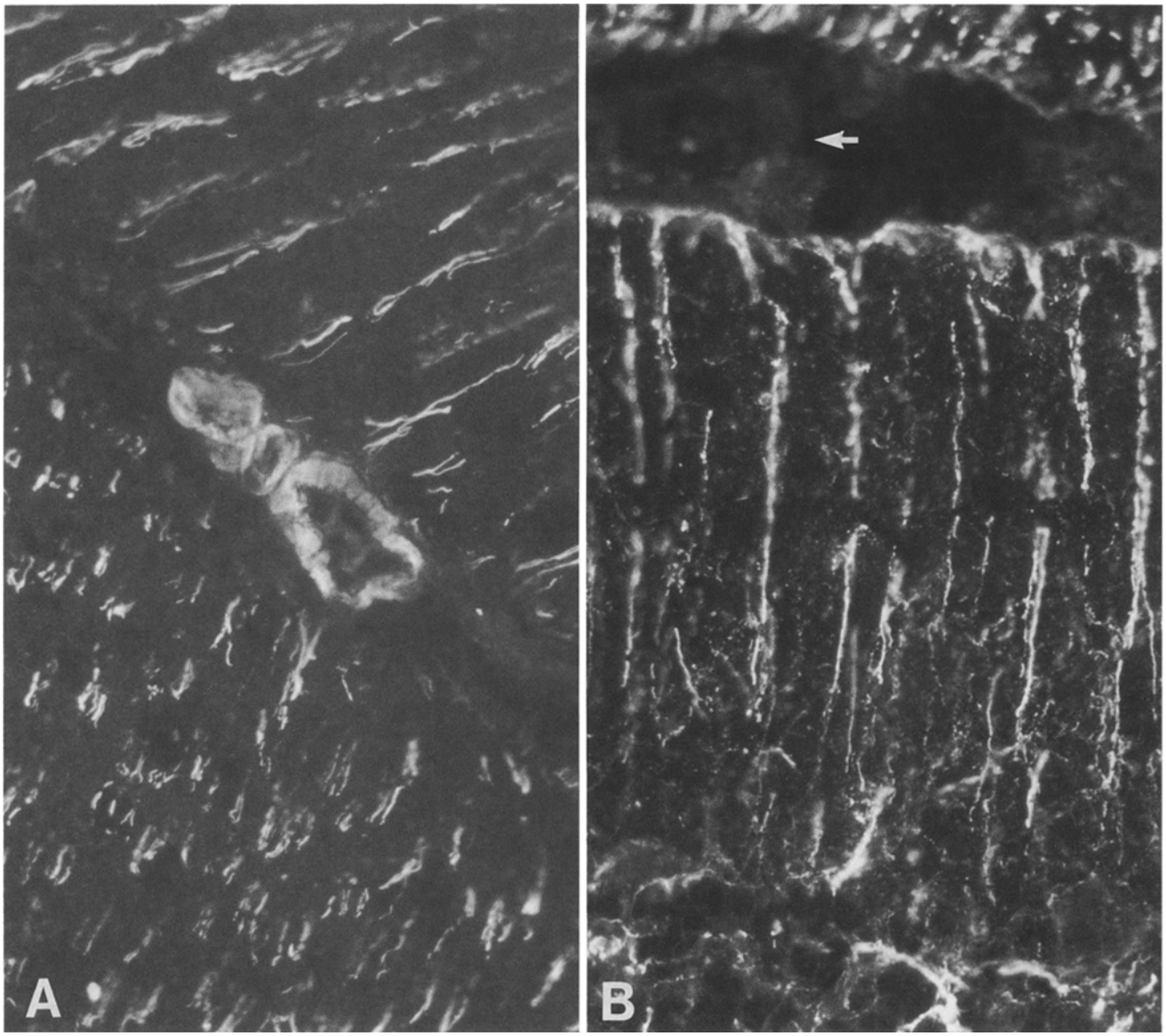

Fig. 6. Immunofluorescent staining of rat cerebellum with desmin monoclonal antibodies staining astrocytes and small arteries (**A** 3 monoclonals out of 6 reported) and with desmin monoclonal antibodies staining only astrocytes (**B** 2 monoclonals out of six reported). *Arrow* in (**B**) points to unstained small artery in the leptomeninges. × 400

were tested by immunofluorescence on cryostat sections and by the immunoblotting procedure on chicken gizzard extracts and purified desmin. For subcloning cells were distributed in 96 well microplates at the density of 0.3 cell/well. Supernatants from both original clones and subclones displayed the same activity by immunofluorescence and immunoblotting.

Results

Six out of 19 monoclonal antibodies were selected for this study on the basis of immunoblotting (Fig. 1) and immunofluorescence findings, i.e. staining of intestinal smooth muscle (Fig. 2) and of the Z-band in skeletal muscle (Fig. 2, insert) in both chicken and rat. Monoclonals reacting with desmin on immunoblots but negative on tissue sections (Dahl et al. 1984), as well as monoclonals only staining chicken tissues by immunofluorescence, were not entered in this study. Monoclonals displaying staining patterns incompatible with the known distribution of desmin, e.g. decoration of meninges, connective tissues and capillaries, were also discarded.

The reactivity of the six selected monoclonals with astrocytes and arterial smooth muscle was studied in the cerebellum, spleen, kidney, aorta and femoral artery. One monoclonal only stained arteries. Three monoclonals stained astrocytes and arteries. Two monoclonals stained only astrocytes including GFA-negative Bergmann glia in chicken. Figures 3–7 illustrate the findings.

Discussion

The findings reported in this paper, i.e. desmin monoclonal antibodies only reacting with intestinal smooth muscle and desmin monoclonal antibodies reacting with both intestinal and vascular smooth muscle, strongly suggest desmin heterogeneity. Desmin heterogeneity probably explains the conflicting results in the literature as to the immunohistological localization of the muscle-type intermediate filament pro-

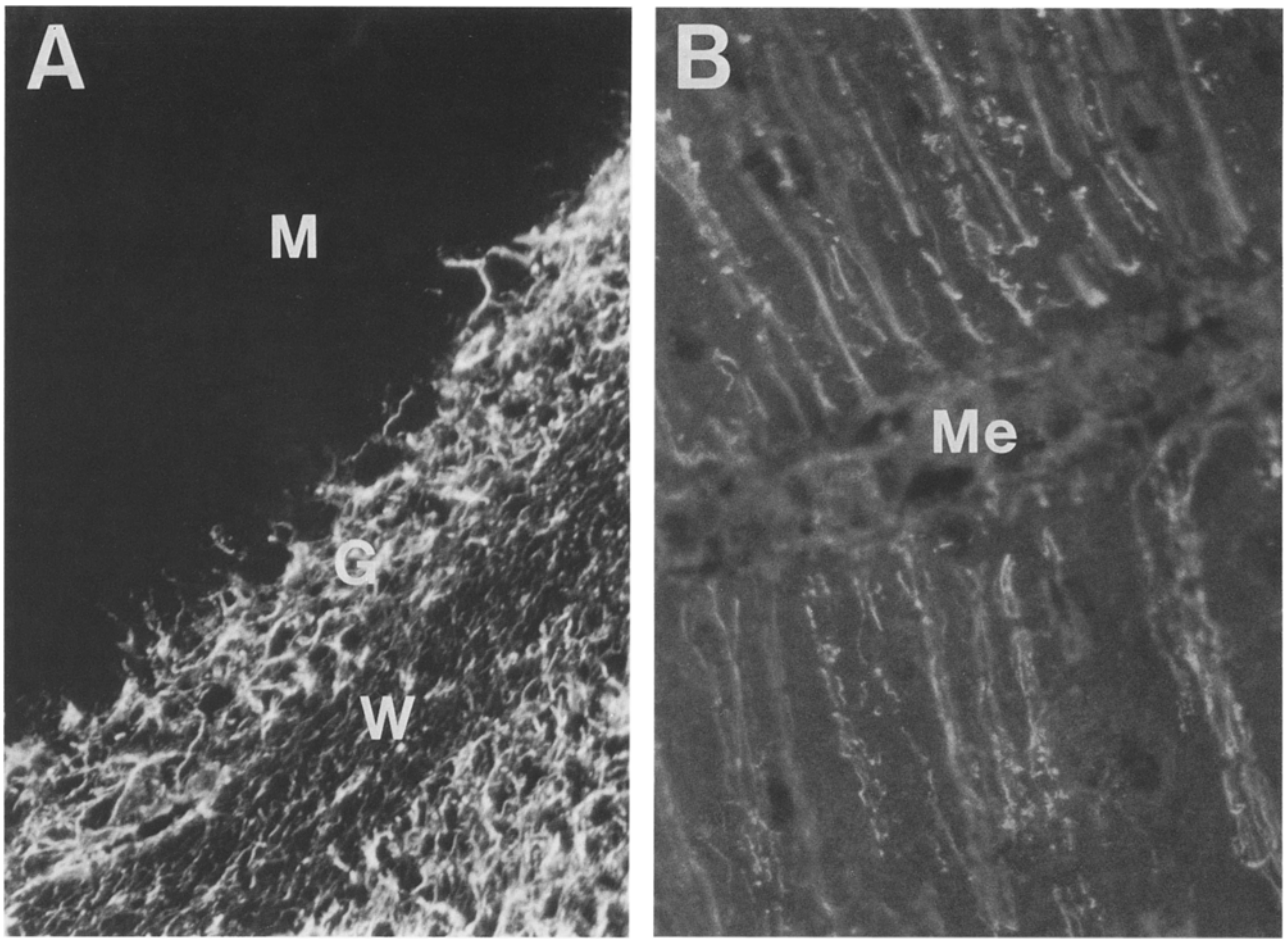

Fig. 7. **A** In chicken cerebellum Bergmann glia in the molecular layer (*M*) was GFA negative with both polyclonal and monoclonal antibodies. Note the GFA immunoreactivity in the granular layer (*G*) and white matter (*W*). **B** Staining of Bergmann glia in the molecular layer of chicken cerebellum with desmin monoclonal antibodies decorating astrocytes. *Me*, meninges. × 400

tein in vascular smooth muscle. According to Gabbiani et al. (1981), and Frank and Warren (1981), smooth muscle cells of blood vessels stained with antivimentin but not with anti-desmin. Interestingly, vascular smooth muscle cells differed from other smooth muscle cells in another respect, i.e. they contained a specific α-type actin (Gabbiani et al. 1981). These studies suggested that vascular smooth muscle cells were comparable to myofibroblasts, that is fibroblasts acquiring the morphological and functional characteristics of smooth cells when stimulated to move and contract under conditions of tissue remodeling. Vimentin is the fibroblast-type intermediate filament protein. It was also noted (Gabbiani et al. 1981) that this situation was reminiscent of that in myoepithelial cells, which are contractile and contain large amounts of intermediate filaments of the prekeratin type but no detectable desmin (Franke et al. 1980).

Conversely, other studies have convincingly demonstrated the presence of desmin in vascular smooth muscle, both by immunostaining and two-dimensional gel electrophoresis (Osborn et al. 1981; Schmid et al. 1982). It was also shown that vascular cells in primary culture could be divided into three groups on the basis of filament-typing: desmin-positive cells, vimentin-positive cells and cells that stained with both desmin and vimentin antisera in double-labeling experiments (Travo et al. 1982).

Molecular heterogeneity depending on the histological location has been also reported for GFA protein, the astrocyte-type intermediate filament protein. GFA antisera decorate a subpopulation of glia in the peripheral nervous system, i.e. enteric glia (Jessen and Mirsky 1980, 1983), non-myelinating Schwann cells in peripheral nerve (Yen and Fields 1981; Dahl et al. 1982) satellite cells in spinal ganglia (Jessen et al. 1984). Conversely, GFA monoclonal antibodies recently reported in the literature only stained astrocytes and enteric glia (Jessen et al. 1984; Dahl et al. 1985).

In view of a recent paper reporting a single gene for desmin in the hamster genome (Quax et al. 1984) it is tempting to speculate that desmin heterogeneity is due to post-translational modification. Monoclonal antibodies reacting with phosphorylated epitopes of the 200 kDa and 150 kDa neurofilament proteins have been recently reported (Sternberger and Sternberger 1983; Carden et al. 1985; Dahl and Bignami 1986).

Desmin heterogeneity may also explains conflicting results concerning the presence of this protein in astrocytes. As we said before, our previous report on the localization of desmin in astrocytes (Dahl and Bignami 1982) was not confirmed in a subsequent study conducted with monoclonal antibodies (Debus et al. 1983a). In accordance with this study, we found desmin monoclonal antibodies that did not stain astrocytes. However, other monoclonal antibodies decorated both astrocytes and blood vessels as previously

reported with desmin antisera (Dahl and Bignami 1982) or alternatively, only astrocytes.

Astrocyte IFs are heteropolymers of GFA protein and vimentin (Quinlan and Franke 1983), the astrocyte-specific IF protein (GFA) being the major subunit in the mature astrocyte, and the mesenchymal-type IF protein the major subunit in immature glia (Dahl and Bignami 1985). Our findings suggest that astrocytes also contain desmin, the muscle-type IF protein.

As pointed out by Debus et al. (1983a), the expression by the same cells of three intermediate filament proteins is exceptional, and immunological cross-reactivity could be responsible for the finding. However, the decoration of astrocytes with desmin monoclonal antibodies was not easily explained by cross-reactivity with vimentin and GFA protein. In brain sections cross-reactivity with vimentin could be excluded by the lack of reactivity with capillaries and meninges (see Figs. 3 and 4 in Dahl et al. 1981). Cross-reactivity with GFA protein was not compatible with the staining of Bergmann glia in chicken. Bergmann glia in chicken are exceptional in that intermediate filaments in this location are of the vimentin type and fail to stain with monoclonal and polyclonal GFA antibodies (Debus et al. 1983b; Shaw and Weber 1984; Dahl et al. 1985). However the possibility could still be considered that staining of astrocytes with anti-desmin could be due to cross-reactivity with an hitherto not identified astrocyte protein.

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