Journal of Cellular Biochemistry 24:1-14 (1984)

A Monoclonal Antibody Recognizes an Epitope Common to an Avian-Specific Nuclear Antigen and to Cytokeratins

Richard M. Franklin, Lyman R. Emmons, Rebecca P. Emmons, Osamu Kai, Anna Oommen, J. Richard Pink, Anne-Marie Rijnbeek, Marianne Schnetzler, Leena Tuderman, and Eeva Vainio

Department of Structure Biology, Biocenter, University of Basel, CH-4056 Basel (R.M.F., L.R.E., R.P.E., O.K., A.O., M.S., L.T., E.V.) and Basel Institute for Immunology, CH-4005 Basel (J.R.P., A-M.R.), Switzerland

X3, a monoclonal antibody of unusual specificity, is described. This antibody reacts with one or more cytokeratin polypeptides and also reacts with an avian (chicken, quail) nuclear antigen that appears to be present in all cell types (chicken) tested, although with variable staining pattern and intensity. This antigen is distinct from the cytokeratins but does have an epitope in common with this class of proteins. It disappears from the nucleus during the early stages of cell division and reappears during anaphase as a granular cytoplasmic structure. In late telophase the antigen is relocated in the nucleus. This antigen, which we have designated as avian-specific nuclear antigen (AVNA), is not associated with chromatin or ribonucleoproteins. From immunoblotting experiments on chicken fibroblast nuclei, AVNA is probably a complex composed of one or several polypeptides, one of which has a molecular weight of approximately 60 kD. The proteins were identified as nuclear matrix proteins rather than pore complex-lamina proteins by immunoblotting experiments on the purified nuclear matrix of chicken erythrocytes. The major polypeptide had a molecular weight of 60 kD and the minor polypeptide a molecular weight of 69 kD.

Key words: species-specific nuclear matrix antigen, cytokeratins, monoclonal antibody

A monoclonal antibody of unusual specificity has enabled us to identify an avian-specific nuclear antigen (AVNA). This antigen is interesting because of the variation in nuclear distribution in different cell types and modulation in expression in different embryonic tissues. The purpose of this work is to characterize the specificities of the antibody and to provide an initial description of AVNA.

In the course of screening for monoclonal antibodies of a given type, one occasionally encounters some with anomalous specificities that may be very broad

Received April 12, 1983; accepted November 1, 1983.

due to a common epitope on several otherwise distinct antigens. This situation was found upon screening for monoclonal antibodies directed against surface determinants of the chicken thymocyte [1]. One anomalous antibody (X1) was directed against an epitope common to intermediate filament proteins, actin, and myosin [2]. A second anomalous antibody stained cells of many chick tissues, apparently in the nucleus. This antibody, called X3, is described here. X3 reacts with a cytokeratin epitope and therefore stains intermediate filaments of epithelial cells. Besides this, however, it stains a nuclear antigen in chicken and quail, but not mammalian, cells. We designate this antigen AVNA for avian nuclear antigen.

MATERIALS AND METHODS

Production and Screening of the Monoclonal Antibodies

Details of the preparation of monoclonal antibodies to chicken thymocytes have been described [1, 2]. Antibody activity against chicken thymus and bursa cells was checked by indirect immunofluorescence on frozen sections of the appropriate tissues.

Cell Cultures

Primary cultures of chick embryo cells (CEF) were prepared from 10-day-old embryos; of lung and kidney (mixed epithelial and fibroblastlike cells), from 14-day-old embryos; of forebrain, from 8-day-old embryos; and of tendon (fibroblasts), from 17-day-old embryos [3]. The lung, kidney, and forebrain cultures were used as primary cultures. The avian tendon cultures were used on the second to fourth passage. Some of the fourth passage tendon cells were infected either with the avian osteopetrosis virus MAV.2-O [4] or the Rous sarcoma virus (RSV) strain Prague B.

Primary cultures of Japanese quail cells (somites from 3-day-old embryo) were kindly provided by Dr A. Rapin, Department of Biochemistry, Biocenter, University of Basel; human amniotic fluid cells [5] and skin fibroblasts, by the Genetic Laboratory, Cantonal Children's Hospital, Basel; and the B16 melanoma, subline F1 [6], by Mr. K. Tullberg, Department of Biochemistry, Biocenter, University of Basel.

The following cell lines were obtained from the American Type Culture Collection: HeLa, Chang liver, and 3T3.

Immunofluorescence

Immunofluorescent labeling of cultured cells was done by the indirect method, as described in detail elsewhere [2]. Cells were grown on glass coverslips and routinely fixed in acetic ethanol (5% glacial acetic acid, 95% absolute ethanol) for 20 min at −20°C. Variations in the fixation procedure are mentioned below. Both the first antibody (mouse monoclonal X3) and the second antibody (fluorescein isothiocyanate [FITC] rabbit antimouse Ig, Nordic Immunological Laboratories, Tillburg, The Netherlands) were diluted in Weber-Osborn [7] phosphate buffered saline (PBS) containing 0.05% NP40. The nuclei were counterstained with bisbenzimide Hoechst 33258 and the slides were mounted in 50% PBS/50% fluorescent-free glycerol.

Embryonic peripheral blood was collected with micropipettes. The cells were fixed for 10 min in 3.5% paraformaldehyde in PBS, centrifuged, and taken up in small volumes of PBS. Smears were prepared and fixed in acetic ethanol (−20°C for 20 min). Immunofluorescent staining was carried out as described above.

Erythrocytes from adult chickens were also investigated. Smears of fresh peripheral blood from adult chickens were fixed for 5 sec in acetone at 20°C before immunofluorescent staining, which was carried out as described above except that NP40 was not included in the buffer.

Besides the frozen sections of chicken tissues, mentioned above, imprints of tissues were used for immunohistochemistry. These were prepared by pressing the cut surfaces of fresh organs briefly against glass microscope slides. Material transferred to slides by this procedure was fixed in acetone and stained as above.

Immunochemistry

Proteins and DNA used as antigens. Brain intermediate filament proteins were extracted from rat brain stem [8] and consisted of a mixture of neurofilament proteins and glial fibrillary acidic proteins (GFAP), keratins were isolated from bovine hoof [9], vimentin from porcine lens [10; cf 2], actin and desmin from chicken gizzard [11; cf 2]. Single-stranded DNA [12] was heat-denatured calf thymus DNA (Serva Feinbiochemica, Heidelberg, FRG), and double-stranded DNA was purified from bacteriophage PM2 [13]. The total histone fraction from calf thymus was a commercial preparation (Sigma Chemical Co, St Louis MO).

Enzyme-linked immunosorbent assay (ELISA) tests. The ELISA plates were coated as follows: brain intermediate filament proteins, 10 μg/ml; keratins, 25 μg/ml; vimentin, 50 μg/ml; desmin, 15 μg/ml; actin, 8 μg/ml; histones, 1 μg/ml; single-stranded (ss)DNA, 1 μg/ml; double-stranded (ds) DNA, 1 μg/ml. Binding of dsDNA was facilitated by prior coating of the plates with poly-L-lysine at 0.1 μg/ml [14]. Blocking of nonspecific binding was achieved using 1% normal goat serum in PBS (NGS-PBS), and the antibodies were detected using horseradish peroxidase labeled goat antimouse IgM (Nordic Immunological Laboratories, Tillburg, The Netherlands). This reagent was used because the monoclonal antibody in question was of the IgM class [1]. Standard automated techniques were used for reading the ELISA optical-density values [cf 2].

Immunodot [15] and immunoblot [16] tests were made on purified proteins or cell and nuclear extracts initially separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). A buffer described by Zeller and co-workers [17] was used to block nonspecific binding sites on the filters. The specific antibody reaction was visualized using horseradish-peroxidase-labeled rabbit antimouse immunoglobulins (DAKO-immunoglobulin a/s, Copenhagen, Denmark), or in some cases ^{125}I-labeled protein A (20 nCi/ml) and fluorography.

Immune precipitation was used to test possible reactions of the antibody with ribonucleoprotein (RNP) from HeLa and chick embryo fibroblast cells [18]. The procedure was as described [18] except that rabbit antimouse IgM was used as the intermediate.

For total cellular protein extracts the cell monolayers were scraped into PBS, washed several times, and then extracted with a buffer containing 2% SDS, 1% glycerol, 1 mM phenylmethyl sulfonyl fluoride (PMSF), 25 mM ethylenediamine tetraacetic acid (EDTA), 5% 2-mercaptoethanol, and 50 mM Tris-HCl (pH 6.8), immediately followed by boiling for 5 min.

For nuclear extracts the monolayers of cells were trypsinized, washed once with 10% foetal calf serum (FCS; Gibco Bio-Cult, Ltd, Paisely, Scotland) in PBS, twice with PBS, and then suspended in PBS containing 25 mM EDTA, 1 mM PMSF, and

1 mM N-ethylmaleimide. NP40 was then added to a final concentration of 1%; the cells were gently shaken for 1–2 min, and the resulting nuclear fraction was collected by centrifugation at 3,500 rpm for 10 min. The pellets were washed once with PBS containing the proteinase inhibitors and 0.1% NP40, transferred to extraction buffer, and treated as the whole cell extracts were treated. In both cases the DNA, which made the extracts very viscous, was sheared by forcing the extracts through an 18-g needle several times. Then the extracts were run on a 10% SDS-PAGE under standard conditions [19] and electrotransferred (1 A, 75 min) to a nitrocellulose filter for immunoblotting.

The above procedure was also carried out on chicken erythrocytes and on various stages of purification of the nuclear matrix of chicken erythrocytes. The nuclear matrix was purified by a modification of published procedures [20]. The complete procedure is described here and variations will be mentioned under Results. Heparinized or citrate-treated chicken blood was centrifuged and the red blood cell (RBC) pellet was washed twice in RBCW (0.145 M NaCl, 5 mM KCl, 1.5 mM $MgSO_4$, 1 mM phenylmethylsulfonylfluoride, 0.01 M Tris, pH 7.4). After lysis in RSB (10 mM NaCl, 1.5 mM $MgSO_4$, 1 mM PMSF, 0.01 M Tris, pH 7.4, 1% NP40) the nuclei were washed once and resuspended in HRSB (0.11 M NaCl, 10 mM $MgSO_4$, 1 mM PMSF, 0.01 M Tris, pH 7.4) and incubated with DNAase I (Serva Feinbiochemica GMBH, D-6900 Heidelberg, FRG; 1× crystallized, 0.3 mg/ml) and RNAase (Worthington Biochemical Corp, Freehold, NJ, 100 μg/ml) for 20 min at 20°C. The mixture was layered over 1 M sucrose in HSRB and the nuclei pelleted, washed once in 0.4 M ammonium sulfate containing 10 mM $MgSO_4$, and 0.01 M Tris (pH 7.4), followed by washing once in RSB. Immunoblotting analysis was carried out as described above.

RESULTS

Immunofluorescent Staining Patterns Given by Monoclonal Antibody X3

Clone X3, producing antibody X3, was isolated as part of a collection of hybridoma clones producing antibodies to surface determinants of chicken thymocytes [1]. This particular antibody, however, did not react with a surface component of thymocytes or any other chicken lymphocyte, a property it shared in common with anitbody X1 [2]. A survey was made of the staining pattern of antibody X3 with frozen sections of chicken tissues. Many tissues were stained with a discrete V- or Y-shaped pattern in the individual cells and the stain appeared to be localized in the nucleus (not shown). This observation prompted us to look at the specificity of antibody X3, first by immunofluorescent techniques using cultured cells and then by immunochemical methods.

HeLa cells (Fig. 1) were stained in a manner characteristic of the cytokeratin intermediate filaments [21], but not all cells were stained with equal intensity. Similar results have been reported for HeLa cells stained with monoclonal antibodies to cytokeratins [22]. Primary human epithelial cells from amniotic fluid, on the other hand, all stained strongly with a typical intermediate filament pattern (Fig. 2a) with no nuclear staining (compare Fig. 2a and b). A primary culture of human fibroblasts derived from normal skin (Table I) did not stain at all. This information suffices to make an initial prediction with respect to the specificity of antibody X3. Since the staining pattern in HeLa cells corresponds to that of cytokeratin and not vimentin [21]

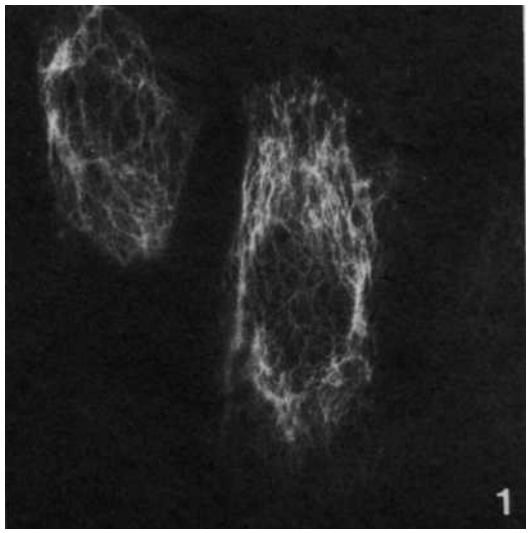
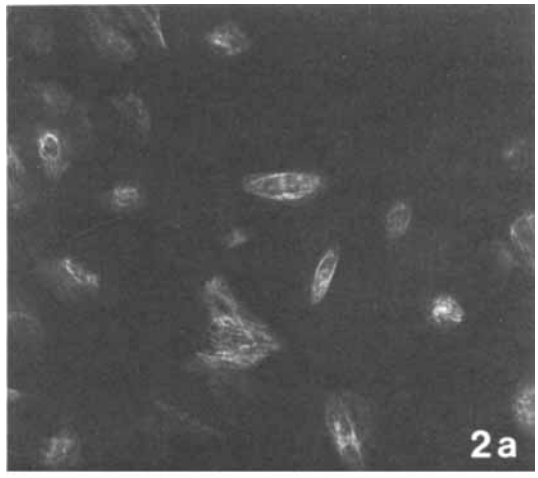


Fig. 1. HeLa cells, immunofluorescent staining with antibody X3 ×1,060.



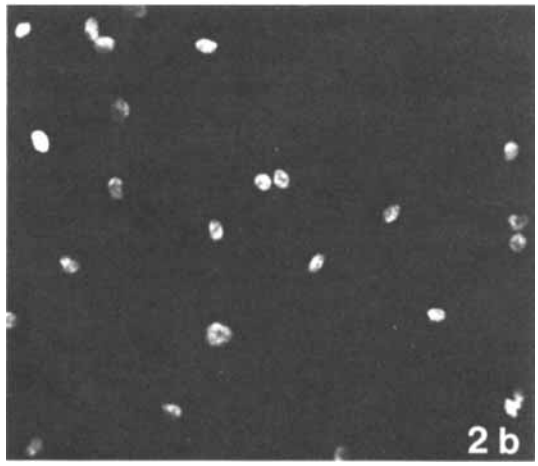


Fig. 2. Primary culture of cells from human amniotic fluid ×450. a. Immunofluorescent staining with antibody X3. b. Fluorescent stain of the nuclei with Hoechst 33258.

TABLE I. Pattern of Immunofluorescent Labeling of Cultured Cells With Monoclonal Antibody X3

Species	Source of cell	Cell type	Cytokeratin	Nuclear antigen
Chicken	Tendon (16-day-embryo)	Fibroblast	–	+
	Lung (14-day-embryo)	Epithelial	+	+
	Kidney (14-day-embryo)	Epithelial	+	+
	Forebrain (8-day-embryo)	Glial	–	+
	MAV. 2-0-infected CEF[a]	Fibroblast	–	+
	RSV-infected CEF[b]	Transformed fibroblast	–	+
Quail	Neural crest	Fibroblast	–	+
Human	Skin	Fibroblast	–	–
	Amniotic fluid	Epithelial	+	–
	Cervical carcinoma, HeLa	Epithelial	+	–
	Non-malignant liver, Chang	Epithelial	+	–
Hamster	Baby hamster kidney, BHK-21	Fibroblast	–	–
Mouse	Swiss albino embryo, 3T3	Fibroblast	–	–
	B16 melanoma, subline Fl[c]	Melanoma	–	–

[a]Tertiary CEF culture infected with the B-subgroup avian osteopetrosis virus MAV.2-0 [cf 4].
[b]Tertiary CEF culture infected with the Prague B strain of Rous sarcoma virus.
[c][cf 6.]

and since fibroblasts, which are not stained, have only vimentin intermediate filament protein [cf 23], we may conclude that X3 is an anticytokeratin. There are three cytokeratin polypeptides that are shared between HeLa cells and human amniotic epithelial cells—those of molecular weights 52.5, 45, and 46 kD [24]. X3 might, therefore, be specific for an epitope on one or all of these polypeptides. We cannot say whether it reacts with other cytokeratin polypeptides.

We then turned to cultured chicken cells, a system we were interested in because of the extensive staining of chicken tissues with antibody X3. The nuclei of chick embryo fibroblasts were stained in a discrete fashion. The granular distribution of the antigen was distinct from that of the DNA, as revealed by staining with Hoechst 33258 (Fig. 3a,b). Fibroblasts derived from the total chick embryo, from embryonic tendon, or in mixed cultures derived from embryonic brain, kidney, or lung all had a nuclear staining pattern with no cytoplasmic staining. Fibroblastlike cells infected with the avian osteopetrosis virus MAV.2-O or transformed by RSV also had a similar pattern (Table I). Epithelial cells, derived from kidney (Fig. 3a,b) or lung (Table I) had a nuclear pattern similar to that found in fibroblasts but also a cytoplasmic pattern characteristic of intermediate filaments, as would be expected because of the suggested specificity of antibody X3 toward cytokeratins. Cultured nonneuronal brain cells resembling primary culture glial cells were only labeled in the nucleus but with less intensity and with a different staining pattern than fibroblasts, the antigen being located centrally in the nucleus (Fig. 4a,b), often as a V-shaped structure. These cells grew *above* a fibroblast layer. In Figure 4 the focus is on the nonneuronal brain cells, the fibroblast layer is out of focus. The absence of cytoplasmic fluorescence in these cells (Fig. 4a) suggests that X3 does not react with GFAP [cf 23]. This was confirmed using immunochemical methods (see below).

These findings prompted a survey of staining patterns in cells from other species (Table I). Of the species we surveyed (chicken, quail, human, hamster, and mouse),

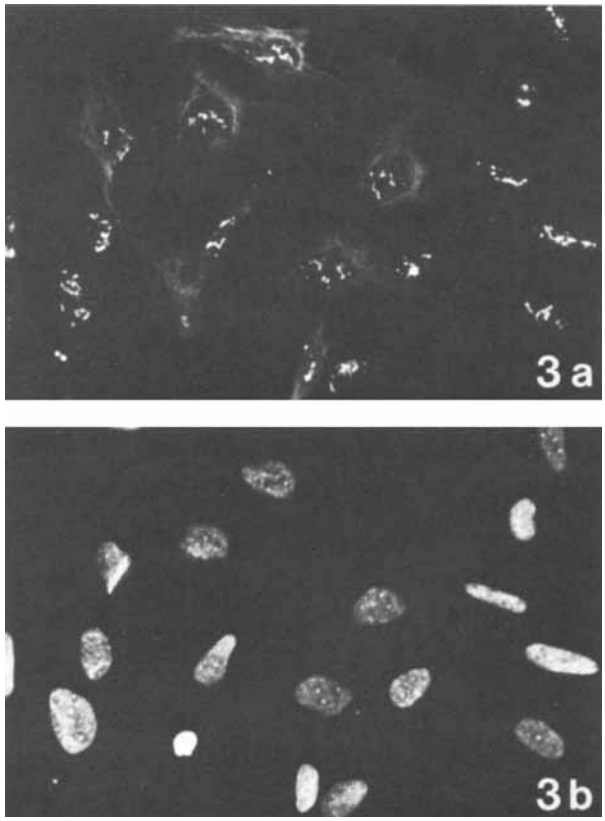


Fig. 3. Primary culture of 14-day-old chick embryo kidney, a mixed culture of epithelial cells and fibroblasts. The epithelial cells are clustered in the center and are decorated with X3 in both the nucleus and cytoplasm ×450. a. Immunofluroescent staining with antibody X3 showing nuclear staining of fibroblasts and nuclear plus intermediate filament staining of epithelial cells. b. Fluorescent stain of the nuclei with Hoechst 33258.

only chicken and quail cells were labeled in the nucleus. The pattern in the quail fibroblast nucleus was different from that in the chick fibroblast nucleus; nucleoli in the quail nucleus being very clearly delineated but not stained. We imagine that cells expressing the appropriate cytokeratin polypeptide(s) would be stained cytoplasmically, regardless of species. The nuclear antigen, however, is species specific, and has only been demonstrated in chicken and quail so far. We refer to this antigen as avian nuclear antigen (AVNA) and suggest that X3 cross-reacts with an epitope common to one or several cytokeratin polypeptides and AVNA. In view of the dual specificity of X3, we subcloned the parent hybridoma line. Three out of five subclones produced antibody in sufficient concentration to give a good immunofluorescent pattern, which was the same for HeLa cells and CEF as the pattern found for X3 itself.

Our interest in AVNA was stimulated by its behaviour during cell division, as observed with cultured tendon or whole chick embryo fibroblasts (Fig. 5a–f). During

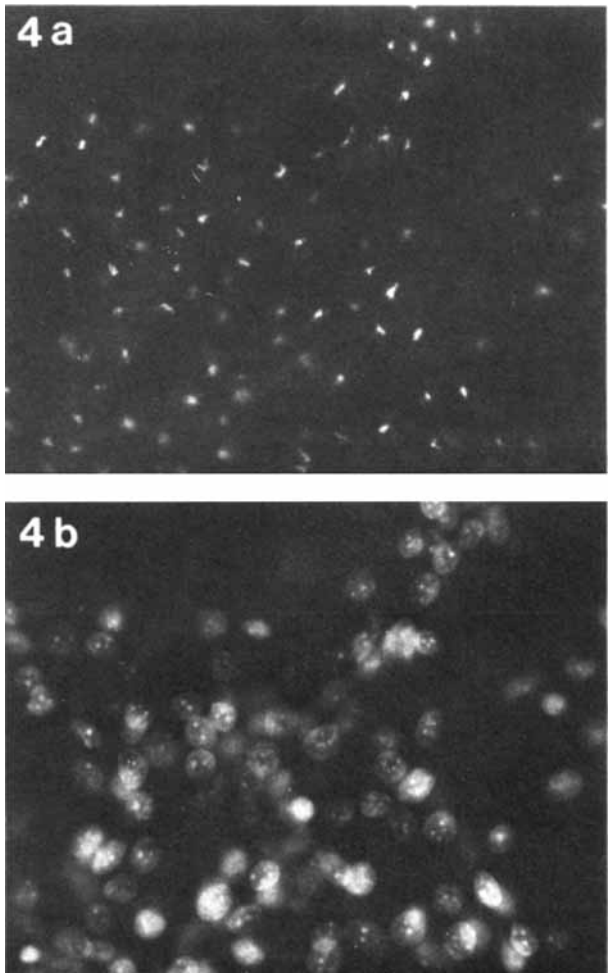


Fig. 4. Cultured cells from the brain (hemisphere) of an 8-day-old chick embryo, a mixed culture of fibroblasts as the adhering layer and nonneuronal brain cells resembling primary culture glial cells above the fibroblasts. The focus is on the glial cells. ×580. a. Immunofluorescent staining with antibody X3. b. Fluorescent stain of the nuclei with Hoechst 33258.

prometaphase or metaphase there is no detectable staining (Fig. 5a,b) but as soon as the chromosomes separate the antigen reappears as distinct spheroid bodies in the cytoplasm (Fig. 5c,d). In late telophase or early interphase there are still some cytoplasmic AVNA bodies, but there is now nuclear staining as well (Fig. 5e,f).

Immunofluorescent Pattern of Chicken Tissues Stained With Antibody X3

A preliminary survey has been made of the staining pattern of chicken cells and tissues. Peripheral blood cells from 3 to 15-day-old embryos had the nuclear antigen, but the staining intensity was weak and not every cell was stained (not shown). The red cells from 3 to 5-day-old embryos represent erythrocytes of the primitive series,

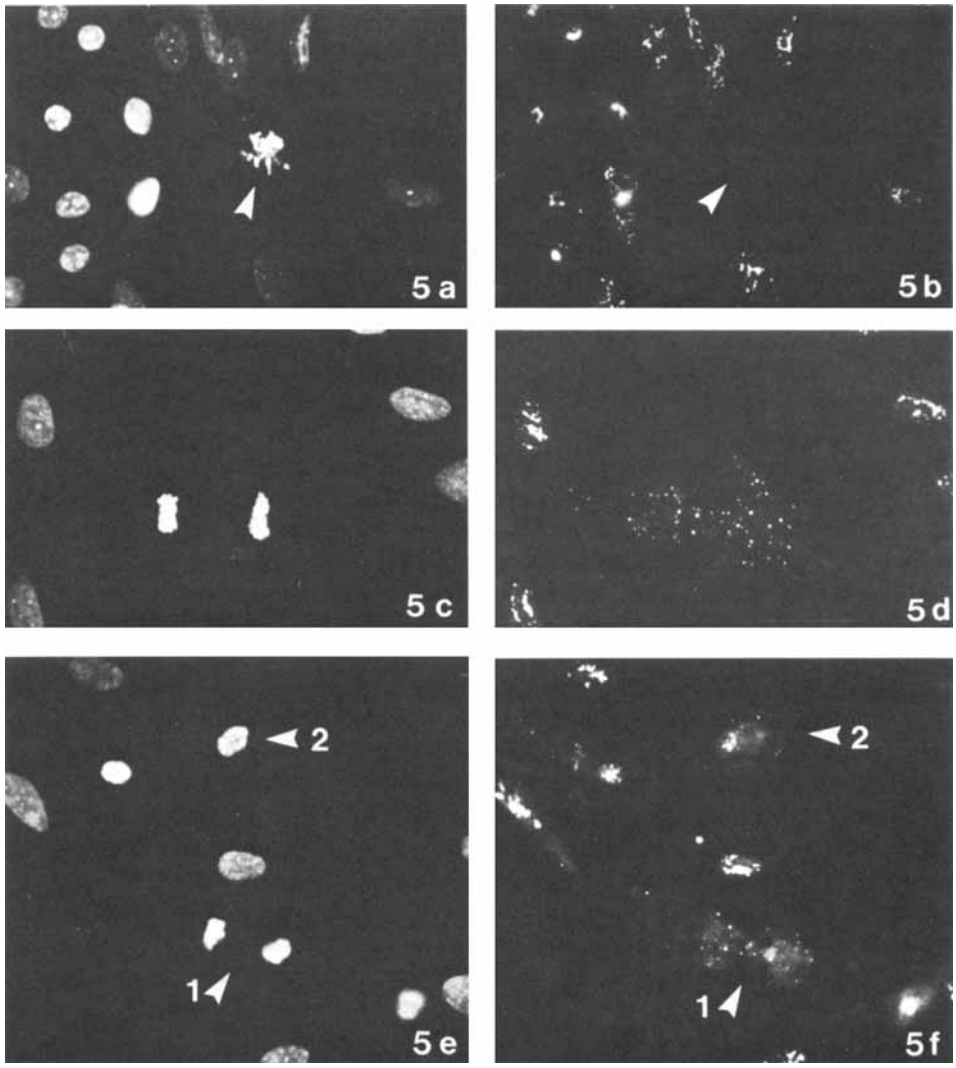


Fig. 5. Distribution of AVNA in CEF during cell division. a. Prometaphase or metaphase (arrow), fluorescent stain with Hoechst 33258. ×630. b. Same field as a, immunofluoresence with antibody X3. c. Telophase, fluorescent stain with Hoechst 33258. ×680. d. Same field as c, immunofluorescence with antibody X3; AVNA is localized in the cytoplasm. e. Two pairs of cells (arrows 1, 2) in late telophase or early interphase, fluorescent stain with Hoechst 33258. ×670. f. Same field as e, immunofluorescence with antibody X3; AVNA is localized in the cytoplasm but some staining is also found in the nucleus, particularly in pair 2.

whereas those from 15-day-old embryos are already of the definitive series [25]. Adult RBC were also stained (not shown). Chicken thymocytes and bursal lymphocytes contained AVNA, as demonstrated by immunofluorescent staining of imprints (not shown). Dividing cells were also seen in such imprints and the distribution of AVNA in such pairs was similar to that found in dividing pairs in culture. Furthermore, cryosections of 5- or 8-day-old chick embryos, as well as semithin sections of

hydroxypropyl methacrylate-embedded embryos, showed a modulated AVNA staining pattern. Some tissues such as mesenchyme stained very intensely, others were stained more weakly.

Immunochemical Analysis of X3 Specificity

ELISA tests were done using monoclonal antibodies X14 and X15, which only reacted with surface markers of the chicken thymocyte [1] to establish background levels. Only if the reaction with X3 was at least twice this background level was the reaction considered to be positive. The following proteins were tested—desmin, vimentin, bovine hoof keratins, brain intermediate filament proteins, actin, and histones. Of these there was only a positive reaction with the keratins. Furthermore ELISA reactions with single-stranded and double-stranded DNA were negative and there was no reaction with RNP as tested by immunoprecipitation of P^{32}-labeled CEF and HeLa cell extracts.

Dot tests were made with purified bovine hoof keratins, desmin, vimentin, brain intermediate filament proteins, and actin as the antigens. Whereas a 1:32 dilution of a X3 culture supernate gave a strong positive reaction with 0.5 μg of keratins, a 1:8 dilution was negative with the other proteins at 2 μg per dot.

Immunoblots (not shown) were made from purified keratins and vimentin, from partially purified desmin and actin, and from the brain intermediate filament protein preparation. A reaction could only be demonstrated with the keratins.

When immunoblots were made from extracts of whole cells or nuclei, a relatively large number of polypeptide bands were labeled (Fig. 6). If antibody X3 was omitted from the reaction, there were no labeled bands; if a polyclonal antiactin or antidesmin was used as the first antibody, only single bands were labeled in the whole cell extracts [cf 2]. Immunoblots of whole cell extracts gave the same pattern for X3 and for the subclones mentioned above. Of particular interest was the band of approxiamtely 60 kD found in nuclear extracts of CEF but not HeLa cells. In order to examine the origin of this 60-kD protein more carefully, nuclear matrix was isolated from chick erythrocyte nuclei. In immunoblots of this material X3 stained the 60-kD band strongly and to a lesser extend a 69-kD protein (Fig. 7). The antigenic bands were not affected by treatment of the nuclear preparation with DNAase, RNAase, or by ammonium sulfate precipitation (Fig. 7). All of these procedures are used in preparation of the nuclear matrix [20].

DISCUSSION

Monoclonal antibody X3, derived from mice immunized with chicken thymocytes, reacts with cytokeratins and a species-specific nuclear antigen. Since thymocytes have not been reported to contain cytokeratins and since X3 reacts only with a nuclear component of chicken thymocytes, we speculate that the antibody was raised against the nuclear component of disrupted thymocytes. That the nuclear antigen, which we have called AVNA, has an epitope in common with one or several cytokeratin polypeptides appears to be fortuitous, since no cytokeratins or any other intermediate filaments, for that matter, are found within the nucleus [cf 26; 27 for example]. In addition, cytokeratins are not found in fibroblasts [cf. 23; 28], a cell type which was used for many of our studies on AVNA. The nuclear antigen is found in RSV-transformed cells as well as those infected by a nondefective virus of the

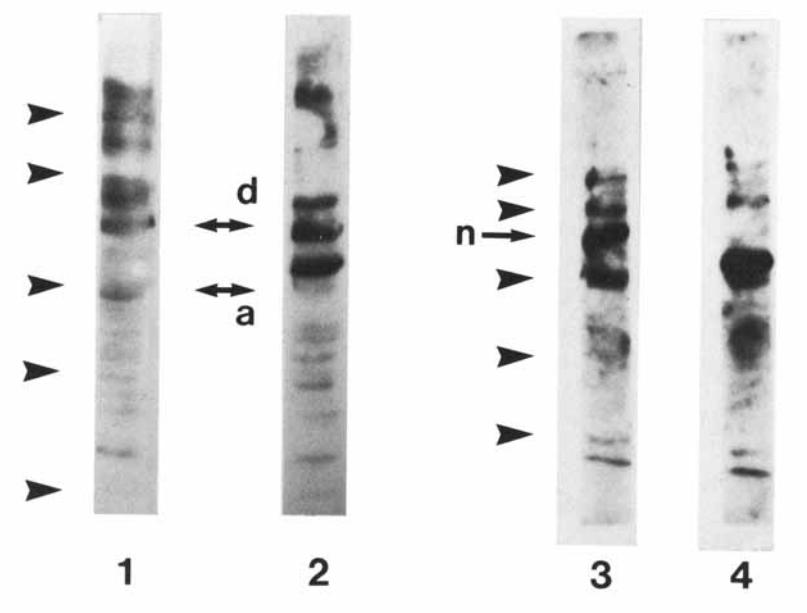


Fig. 6. Immunoblots of total cell extracts and nuclear extracts stained with antibody X3. The positions of the molecular weight standards are indicated by arrowheads. These standards, from top to bottom, are phosphorylase b, 94 kD; bovine serum albumin, 67 kD; ovalbumin, 43 kD; carbonic anhydrase, 30 kD; and α-lactalbumin, 14 kD. Track 1, CEF total cell extract; track 2, HeLa total cell extract; Track 3, CEF nuclear extract; track 4, HeLa nuclear extract. The arrows between tracks 1 and 2 indicate the positions of the bands identified as desmin (d) and actin (a) by polyclonal antibodies to desmin and actin, respectively [cf 2]. In track 2 the broad band just above a may represent a cytokeratin of 45 or 46 kD. The arrow beside track 3 (n) indicates the polypeptide which is labeled in the CEF but not the HeLa cell nuclear extract.

lymphoid leukosis class. In view of the universal occurrence of AVNA in chicken cells, the antigen must be of fundamental importance for the integrity and/or function of the nucleus. It is quite possible that similar antigens exist in other species.

By comparing the distribution of AVNA in resting and dividing cells with that of other nuclear antigens that have been studied, we can distinguish several classes of proteins from AVNA. Since AVNA disappears from the nucleus and reappears in the cytoplasm during cell division we can eliminate centromere antigen, histones, and any other proteins associated with chromatin from consideration. Comparison of the distribution of AVNA with the distribution of DNA in the resting nucleus, as well as with the distribution of nucleoli (not shown), indicates that AVNA is associated neither with DNA nor with the nucleolus in the resting cell. Furthermore, the staining pattern in both the resting nucleus and in the cytoplasm of the dividing cell is very much different from that found for the pore complex-lamina fraction [29]. A nuclear matrix protein [cf 30] is the most likely candidate for the 60-kD protein we have identified as a component of AVNA. Indeed in biochemical studies we have been able to identify AVNA in a nuclear matrix preparation from chicken erythrocytes. In this case immunoblotting of the nuclear matrix has revealed only one major band (60 kD) and a minor band (69 kD).

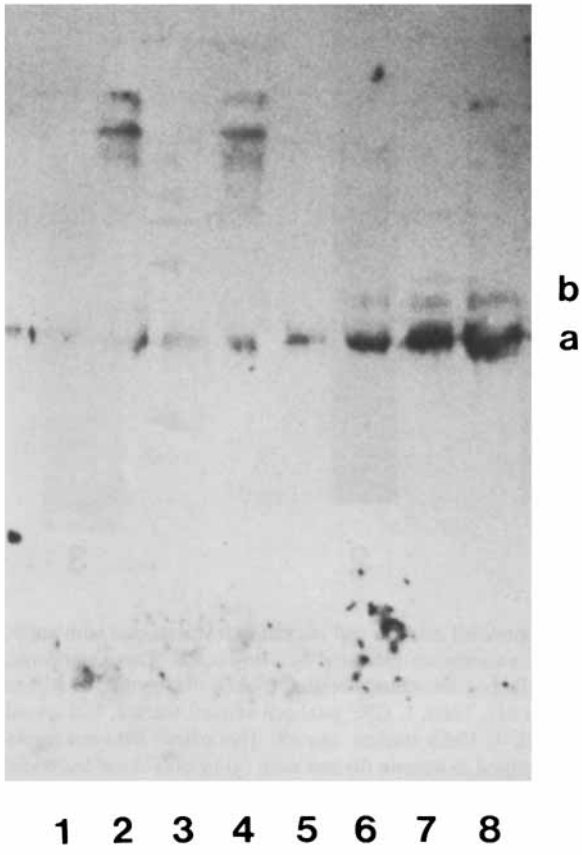


Fig. 7. Immunoblot of chick erythrocyte nuclear extracts stained with X3. The extracts were prepared as described in Materials and Methods and immunoblotted with X3 and ^{125}I-labeled protein A. From the relative mobilities of the two proteins the molecular weights are 60 kD and 69 kD. Tracks 1, 2: Nuclear extracts treated with DNAase alone. Tracks 3, 4: Nuclear extracts treated with DNAase plus RNAase. Tracks 5, 6: Ammonium sulfate precipitate of 1, 2. Tracks 7, 9: Ammonium sulfate precipitate of 3, 4. Tracks 1, 3, 5, 7 have 30 μl of extract; tracks 2, 4, 6, 8 have 100 μl of extract.

Whatever the nature of AVNA, it has several interesting properties which make its study worthwhile. The most important of these properties is the apparent modulation of this antigen as observed in sections of the chick embryo and in cultured cells. In some tissues the antigen is stained very strongly, whereas in others it is very weakly stained. There are also changes in the staining pattern of AVNA as seen, for example, when brain cells are compared with fibroblasts. We do not yet have enough information on this modulation to offer an explanation, but this may well be associated with cellular and molecular controls of differentiation. The second interesting property is the behaviour during cell division. With the dissassembly of the nuclear envelope at the end of prophase [29], AVNA becomes undetectable by immunofluorescent staining, possibly because it is diffuse within the cytoplasm. The antigen reforms into cytoplasmic granular structures during the later phases of cell division.

Whether these structures move into the reassembling nucleus as such or whether they disassemble prior to final assembly in the resting nucleus is unclear at this time. Certainly very large protein complexes can migrate from cytoplasm to nucleus provided they are nuclear-specific proteins [31].

Finally, a word concerning the keratin specificity of X3. We have tested the specificity of X3 with a limited number of purified antigens using immunochemical methods. All of the tests fully support the immunofluorescent studies, which show that cytoplasmic labeling is confined to a well-defined intermediate filament network in epithelial cells. When we look at the reaction of X3 with the polypeptides of whole cell extracts from both HeLa and CEF cells, however, we find many polypeptides labeled. This is also true if we look at crude nuclear extracts of these cell types. We have *purposely* used whole cell or crude nuclear extracts in these experiments in order to expose antibody X3 to all possible polypeptides of the cells in question. As with other monoclonal antibodies of broad specificity, we imagine that X3 interacts with a very short polypeptide sequence, which may be common to many polypeptides [cf 32]. Whereas the epitope in question may be hidden in most polypeptides as they exist within cells during an immunofluorescent staining reaction, they may be exposed in the denatured state of the polypeptide as found in the immunoblotting method. This could explain the discrepancy in apparent specificity. These considerations only emphasize the necessity of investigating the specificity of monoclonal antibodies by a large number of methods. Antibody X3 may, however, be a very useful reagent in studying the nuclear matrix, but only by virtue of a detailed understanding of its specificities.

ACKNOWLEDGMENTS

We hereby acknowledge the technical assistance of Mr R. Longato, Miss U. Schaub, Miss D. Lenig, and Mrs M.T. Martin. We gratefully acknowledge the assistance of Professor E. De Robertis and Mrs. R.F. Parisot with the immune precipitation test for antibodies to RNP and to Professor G. Schatz and Mr. K. Suda for kindly providing ^{125}I-labeled protein A. Dr A. Matus, Friedrich-Miescher-Institut, Basel, helped us with the preparation of the intermediate filament proteins from rat brain. We are grateful to Dr P. Liesi, Department of Virology, University of Helsinki for helpful evaluation of the brain cell cultures. This work was supported in part by grants (3.392.78, 3.311.81) from the Swiss National Fund and the Sandoz Fund. The Basel Institute for Immunology was founded and is supported by Hoffmann-LaRoche Ltd., Basel, Switzerland.

REFERENCES

1. Pink JR, Rijnbeek AM: Hybridoma 2:287, 1983.
2. Franklin RM, Emmons LR, Emmons RP, Oommen A, Pink JR, Rijnbeek AM, Schnetzler M, Tuderman L, Vainio E: Hybridoma 2:275, 1983.
3. Schwarz RI, Farson DA, Soo W-J, Bissell MJ: J Cell Biol 79:672, 1978.
4. Banes AJ, Smith RE: Infect Immun 16:876, 1977.
5. Valle M, Pentinnen K: Ann Med Exp Fenn 40:342, 1962.
6. Poste G, Fidler IJ: Nature 283:139, 1980.
7. Weber K, Bibring T, Osborn M: Exp Cell Res 95:111, 1975.
8. Matus A, Pehling G, Ackerman M, Maeder J: J Cell Biol 87:346, 1980.

9. Steinert PM, Idler WW, Zimmerman SB: J Mol Biol 108:547, 1976.
10. Geisler N, Weber K: FEBS Lett 125:253, 1981.
11. Geisler N, Weber K: Eur J Biochem 111:425, 1980.
12. Gripenberg M, Linder E, Kurki P, Engvall E: Scand J Immunol 7:151, 1978.
13. Brack C, Bickel TA, Yuan R: J Mol Biol 96:693, 1975.
14. Kaci AA, Monier JC: Pathol Biol 30:73, 1982.
15. Hawkes R, Niday E, Gordon J: Anal Biochem 119:142, 1982.
16. Towbin H, Staehelin T, Gordon J: Proc Natl Acad Sci USA 76:4350, 1979.
17. Zeller R, Nyffenegger T, De Robertis EM: Cell 32:425, 1983.
18. De Robertis EM, Lienhard S, Parisot RF: Nature 295:572, 1982.
19. King J, Laemmli UK: J Mol Biol 61:465, 1971.
20. Van Ekelen CAG, Salden MHL, Habets WJA, Van de Putte LBA, Van Venrooij WJ: Exp Cell Res 141:181, 1982.
21. Franke WW, Schmid E, Weber K, Osborn M: Exp Cell Res 118:95, 1979.
22. Debus E, Weber K, Osborn M: EMBO 1:1641, 1982.
23. Lazarides E: Annu Rev Biochem 51:219, 1980.
24. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R: Cell 31:11, 1982.
25. Bruns GAP, Ingram WM: Proc R Soc Lond [Biol] 266B:225, 1973.
26. Blose SH: Proc Natl Acad Sci USA 76:3372, 1979.
27. Blose SH, Bushnell A: Exp Cell Res 142:57, 1982.
28. Osborn M, Weber K: Cell 31:303, 1982.
29. Gerace L, Blum A, Blobel G: J Cell Biol 79:546, 1978.
30. Agutter PS, Richardson JCW: J Cell Sci 44:395, 1980.
31. De Robertis EM, Longthorne RF, Gurdon JB: Nature 272:254, 1978.
32. Nigg EA, Walter G, Singer SJ: Proc Natl Acad Sci USA 79:5939, 1982.