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THE INTERFERON-INDUCIBLE AUTOANTIGEN, IFI 16: LOCALIZATION TO THE NUCLEOLUS AND IDENTIFICATION OF A DNA-BINDING DOMAIN

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The human *IFI 16* gene encodes a nuclear protein whose expression is associated with activation and differentiation of cells of the myeloid lineage, and which has recently been postulated to be a frequent target of human autoantibodies. We have used a unique monoclonal antibody in immunofluorescence studies to define the intranuclear distribution of IFI 16 antigen in primary human leukocyte sub-populations. We demonstrate that IFI 16 is expressed both within the nucleoli and nucleoplasm of mononuclear cells, but is not present in granulocytes. IFI 16 expression correlated with that of the known nucleolar antigen B23, suggesting that the function of IFI 16 is restricted to cells that have nucleoli and therefore are not terminally differentiated. These findings were supported by immunofluorescence studies in IFN-γ-stimulated HL-60 cells and by biochemical fractionation of Daudi nuclear extract. IFI 16 was extracted from whole nuclei at approximately 200-300mM NaCl, within the range described for known transcription factors. Together with our previous findings, these results support a possible role of IFI 16 in binding nucleic acid *in vivo*. Furthermore, a DNA-binding site was localized within a fusion protein containing the amino-terminal 159 amino acids. As the nucleic acid-binding domains of several proteins are targeted by autoantibodies in SLE, this region may be of significance in the pathogenesis of autoimmune disease.

Recently, a family of highly homologous interferon (IFN)-inducible proteins has been described in both mouse and humans, each possessing at least one copy of a 200 amino acid motif of unknown function. The family comprises the products of the mouse Gene 200 cluster, consisting of at least six closely-linked genes, and two human genes designated IFI 16 and the myeloid cell nuclear differentiation antigen (MNDA) (1-4). The majority of these genes have been mapped to a region of chromosome 1 which is syntenic in mouse and humans (5-7). In addition to IFN-inducibility, their expression is also regulated by agents which cause differentiation of myeloid cell lines (6,8). The members of this family which are best characterized at the protein level are IFI 16, MNDA, 202 and 204, all of which are nuclear proteins, though 202 is only localized to the nucleus during mitosis and after induction with IFNs (4, 8-10).

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Studies on the localization of the 204 protein revealed it to be present in both the nucleoplasm and nucleolus (9). This represented the first description of an IFN-induced protein localizing to the nucleolus. Recently, IFI 16 was also defined by Seelig and co-workers as a nucleolar antigen recognised by serum of patients with systemic lupus erythromatosus (SLE; Ref. 11). Both serum from SLE patients and a rabbit polyclonal antiserum raised against an IFI 16 fusion protein reacted with an antigen in the nucleoplasm and nucleolus in a range of cell lines. Autoantibodies to IFI 16 were present in approximately 30% of patients with SLE, whilst no activity was detected in serum from normal patients.

In order to examine the significance of these findings, IFI 16 expression was examined in primary, untransformed cells by using a monoclonal antibody (mAb) in immunofluorescence (IF) studies. The 1G7 mAb has previously been shown to specifcally recognize IFI 16, and does not cross react with the closely-related nuclear protein, MNDA (8). We demonstrate that not only was IFI 16 detected in the nucleoplasm and nucleoli of peripheral leukocytes, expression of the antigen was restricted to transcriptionally active cells which possess nucleoli i.e. cells that were not terminally differentiated. In addition, we have examined the DNA-binding characteristics of IFI 16 and defined a putative DNA-binding domain in the amino-terminal region of the molecule.

MATERIALS AND METHODS

Cell culture, reagents and antibodies.
The Burkitt's lymphoma cell line, Daudi, and the promyelocytic cell line HL-60 were maintained in RPMI medium supplemented with 10%(v/v) fetal calf serum (Flow Laboratories, Sydney, Australia), 2mM glutamine (Commonwealth Serum Laboratories (CSL), Melbourne, Australia), 100U/ml penicillin (CSL), and 100μg/ml streptomycin (Glaxo, Melbourne, Australia). For induction of IFI 16 protein, exponentially dividing cells were passaged into medium supplemented with recombinant human (r hu) IFN-α at 100U/ml (Dr Mark Smyth, Biological Response Modifiers Program, National Cancer Institute, Frederick, MD) for 7 days prior to harvest. Both the 1G7 monoclonal antibody (mAb) and a polyclonal antibody recognising the IFI 16 antigen were produced against a bacterial fusion protein containing the amino-terminal 159 amino acids of IFI 16 (8). The mAb directed against the nucleolar protein B23 (nucleophosmin) was a kind gift of Dr Pui K. Chan (Baylor College of Medicine, Houston, TX).

Immunohistochemistry.
Peripheral blood leukocytes from normal individuals were separated into mononuclear and polymorphonuclear cells for immunohistochemistry. Whole blood was mixed with 6% dextran in phosphate buffered saline (PBS) at a ratio of 9:1 to aggregate the red cells. Leukocytes were washed in PBS, layered on Isopaque ficoll (Pharmacia), and the cells separated by centrifugation. The upper "buoyant" fraction containing the mononuclear cells and the pelleted polymorphonuclear cells were each transferred to a fresh tube, pelleted and washed in PBS. Both the IFN-treated HL-60 cells and the fractionated peripheral blood leukocytes were resuspended in PBS supplemented with 5% fetal calf serum for cytopsin slide preparation. After air drying for one hour, slides were fixed in 2% paraformaldehyde, 0.01M sodium periodate, 0.075M L-lysine, 0.037M sodium phosphate buffer, according to (12). Samples were then incubated with the appropriate antisera diluted in 0.5% BSA/ DME at 4°C overnight, and bound antibody was detected with a fluorescein isothiocyanate-conjugated sheep anti-mouse Ig(Fab')$_2$ or swine anti-rabbit Ig(Fab')$_2$ (Silenus, Melbourne, Australia), as appropriate. Slides were washed in PBS and mounted with coverslips using a 2% N-propyl galate (Sigma)/ 87% glycerol solution, and examined for fluorescence using UV microscopy.

Isolation of nucleoli.
Nucleoli were prepared from Daudi cells using a modification of the method described in (13). Daudi cells (10^8) were washed twice in PBS and lysed in buffer containing 0.5% NP-40, 5mM $MgCl_2$, 25mM KCl, 10mM Tris HCl, pH8, 1mM dithiothreitol (DTT), 1mM iodoacetamide and 2mM phenoxymethylsulfonlyfluoride (PMSF). Nuclei were separated from cell debris by centrifugation, and washed in the same buffer. The nuclei were then washed twice in 0.88M sucrose/5mM $MgCl_2$ and sonicated in 10mls of 0.34M sucrose/0.5mM $MgCl_2$ (2 x 15 sec, output 7.0; Branson Sonifier, Moline, Illinois). Complete nuclear lysis was assessed by light microscopy. The sample was adjusted to 13mls in volume and layered over 25ml 0.88M sucrose/5mM $MgCl_2$. Nucleoli were pelleted by centifugation at 4,000g in a swinging-bucket rotor for 20 min at room temperature, and washed twice with 0.88M sucrose/5mM $MgCl_2$ before anaylsis by western blotting.

Western blotting.
Protein preparations were electrophoresed on 10 or 12.5% (w/v) SDS-polyacrylamide gels (SDS-PAGE) and electrophoretically transferred onto Immobilon P membranes (Millipore, Bedford, MA). Non-specific binding of antibodies was blocked with 3% casein in PBS. Membranes were then probed with the 1G7 mAb, and bound Ig detected by horse radish peroxidase-conjugated rabbit anti-mouse Ig (DAKO, Santa Barbara, CA) and visualized using the ECL detection system (Amersham, Sydney, Australia).

Salt extraction of proteins from whole nuclei.
Nuclei were isolated from Daudi cells as described above and incubated in extraction buffer (0.1% NP-40, 10mM Tris pH 8.0 and 5mM ethylenediaminetetraacetic acid, 1mM DTT, 1mM iodoacetamide, 2mM PMSF and 1mM aprotinin) containing 100, 200, 300 or 500mM NaCl. The soluble protein lysate was separated from the insoluble nuclear debris by ultracentrifugation at 4°C for 20min (100K; TLA 100 rotor, Beckman model TL-100). The resultant chromatin pellet was boiled in sample buffer and the solubilized proteins analyzed by western blotting.

DNA-cellulose affinity chromatography.
The glutathione S-transferase (GST)-IFI 16 fusion protein (5μg) in buffer B (10mM potassium phosphate, pH 6.8, 1mM $MgCl_2$, 0.5% NP-40, 1mM DTT, 10% glycerol) was incubated with double-stranded calf thymus DNA covalently coupled to cellulose beads (Sigma), preswollen in buffer B. The column was washed thoroughly with buffer B and bound proteins eluted with incremental increases in NaCl concentration diluted in Buffer B (three elutions at each concentration). Eluted fractions were analyzed by Western blotting.

RESULTS

Intranuclear distribution of IFI 16.
Previously we have used the mAb 1G7 in immunoperoxidase studies to show that IFI 16 was localized to the nucleus. The nuclear staining observed in HL-60 cells induced to differentiate with retinoic acid and dimethylsulfoxide was punctate and patchy in appearance (8). Subsequently we have employed a more sensitive technique, IF, to examine the intranuclear localisation of the IFI 16 protein. Indirect IF staining using the 1G7 mAb on cytospin preparations of untreated (Figure 1A) and IFN-α-stimulated HL-60 cells (Figure 1C) revealed bright nucleolar staining only in the IFN-α treated cells. The nucleolar staining was accompanied by a more diffuse reticular pattern of staining in the nucleoplasm, similar to that observed previously (11). Very weak nucleoplasmic staining was detected in only a few (10%) of the untreated HL-60 cells (Figure 1A), a similar finding to that observed previously with immunoperoxidase staining. This population of cells may represent the estimated 5-15% of HL-60 cells in normal culture shown to have a more mature phenotype (14). Cells

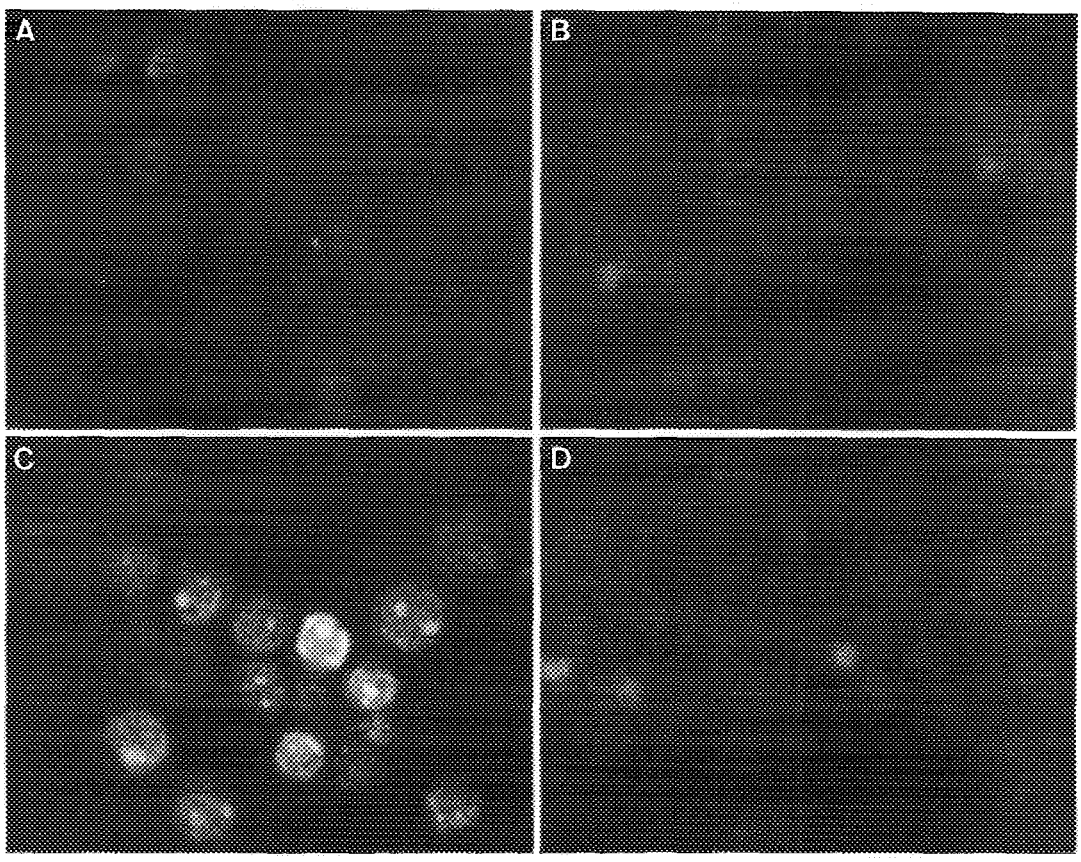


Figure 1. Indirect IF microscopy of unstimulated HL-60 (A) and IFN-α-treated HL-60 cells (C) stained with the mAb 1G7 (A and C,respectively) or an irrelevant isotype-matched antibody (B and D, respectively). Antibody binding was visualized with a fluorescein-conjugated secondary antibody (see *Materials and Methods*). Magnification=500X.

incubated with an irrelevant isotype-control antibody showed no staining in the presence (Figure 1D) or absence (Figure 1B) of IFN-α.

IFI 16 expression in peripheral blood leukocytes.

As IFI 16 has been shown to be a potential target of human autoantibodies *in vivo*, it was important to examine the intranuclear localization of the IFI 16 antigen in primary, untransformed human cells. Accordingly, the expression of IFI 16 and the nucleolar antigen B23 (15) was examined in peripheral blood leukocytes. In mature cells such as peripheral polymorphonuclear cells, the state of terminal differentiation is associated with a loss of nucleoli. Therefore the mononuclear and polymorphonuclear cells of a normal donor were examined for IFI 16 antigen expression by IF (Figure 2). In the mononuclear cell preparation, which contained lymphocytes and monocytic cells, strong staining was observed with the 1G7 mAb in the nucleoplasm and nucleoli (Figure 2A). The nucleolar staining was not as distinct as that observed in IFN-treated HL-60 cells, which may reflect the relative proliferative states of the two cell types. Accordingly, IF staining of Daudi cells using the anti-B23 antibody

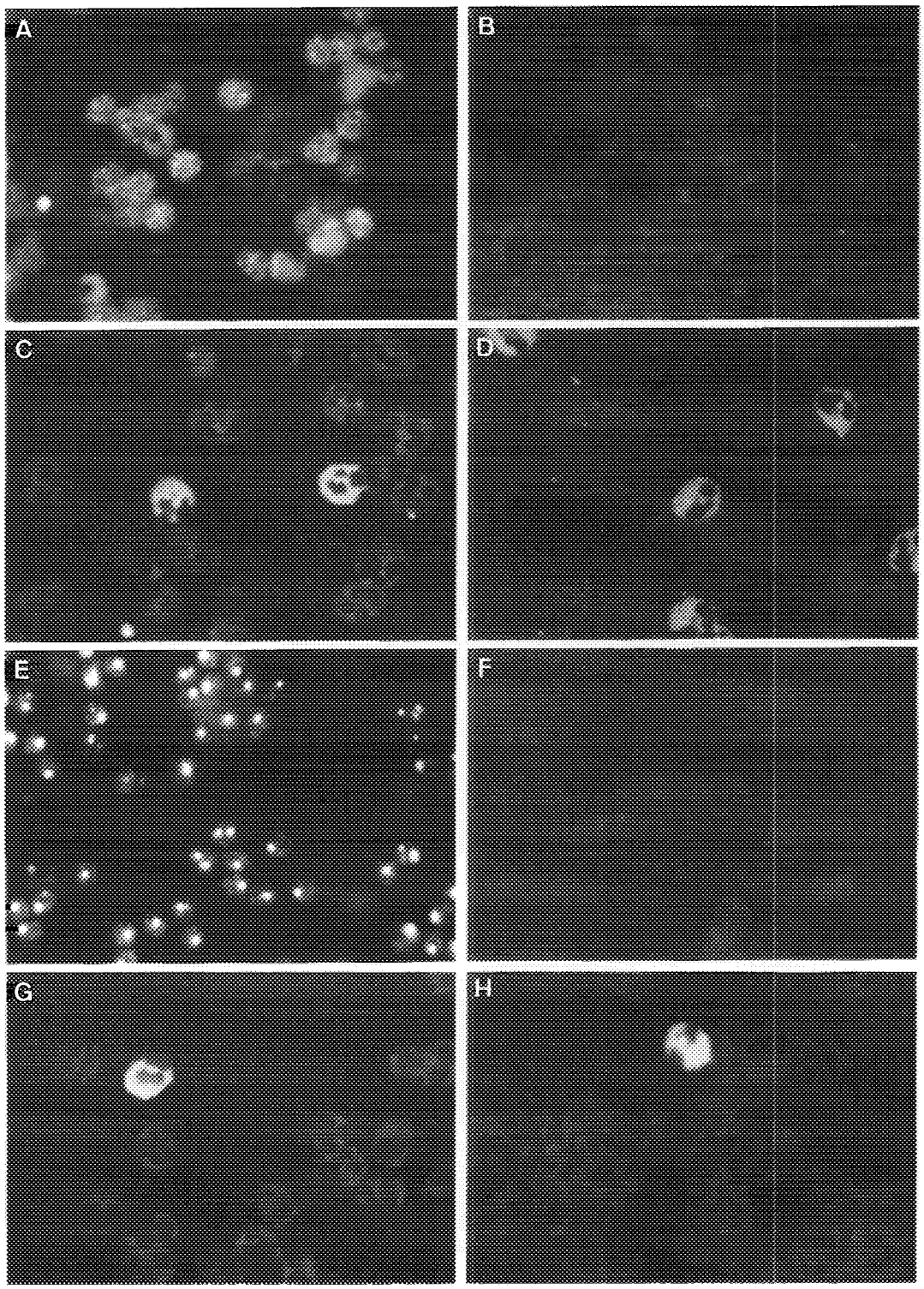


Figure 2. Indirect IF microscopy of peripheral blood leukocytes stained with antibodies directed against IFI 16 and the nucleolar protein B23. PBMC were stained with 1G7 mAb (A) and anti-B23 mAb (C) or irrelevant isotype-matched antibodies (B and D, respectively). Polymorphonuclear cells were stained with 1G7 mAb (E) and B23 (G)

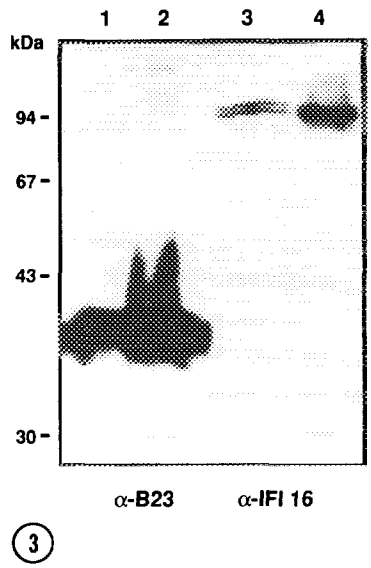
revealed three to seven large nucleoli (data not shown), while no more than three were observed in freshly isolated peripheral mononuclear cells (Figure 2E). This observation was compounded by the greater relative abundance of IFI 16 in the nucleoplasm than in the nucleolus, whilst the lack of nucleoplasmic staining for B23 made its nucleolar staining more obvious. Minimal background staining was observed with irrelevant isotype control antibodies for IFI 16 and B23 in the mononuclear cells (Figures 2B and 2F respectively). Alternatively, only non-specific cytoplasmic staining was observed when 1G7 mAb was used to stain polymorphonuclear cells, which consisted mainly of mature granulocytes (Figure 2C). This observation differs from our previous finding using immunoperoxidase, where weaker staining was observed in the polymorhonuclear cells in comparison to the PBMC (8). Bright cytoplasmic staining observed in a few cells was probably due to non-specific binding of Ig through Fc receptors, as the same pattern of staining was apparent with the isotype control antibody (Figure 2D). B23 was also not expressed in these cells (Figures 2G), confirming that nucleoli were indeed absent. Thus, IFI 16 antigen expression in normal peripheral blood leukocytes was restricted to those cell populations which were transcriptionally active and therefore possessed nucleoli.

In order to confirm this result, nuclei isolated from Daudi cells were further fractionated and analyzed by western blot. Two identical filters containing unfractionated nuclear extract or the nucleolar extract for the same number of nuclei, were probed with the 1G7 mAb or B23 mAb . 1G7 detected three bands ranging from 90-95 kDa in the whole nuclear lysate (Figure 3, lane 3), although the lowest band was barely discernible. As noted previously, the smallest species was the least abundant, and was difficult to visualize in some experiments (ref. 8; see also Figure 4). IFI 16 was also detected in the nucleolar lysate, and was significantly enriched during the purification process (Figure 3, lane 4). A similar pattern was noted for the B23 antigen, which migrated as a 37 kDa protein in the whole nuclear sample (Figure 3, lane 1). As with IFI 16, there was a significant increase in the amount of protein detected in the fractionated nucleoli (Figure 3, lane 2).

Mapping of a DNA binding domain within the IFI 16 protein.

The previous finding that native IFI 16 binds to DNA *in vitro*, and the nucleolar localisation demonstrated here suggests that the protein may bind to DNA *in vivo*. Most DNA-binding proteins are basic, and characteristically elute from DNA in a range of NaCl concentrations from 200-400 mM. To determine the ionic strength of the interaction of IFI 16 with its putative binding site(s) within the nucleus, whole nuclei were treated with buffer containing concentrations of NaCl ranging from 100mM to 500mM. The eluted proteins were then separated from the residual nuclear fraction by ultracentifugation, and both samples were analyzed by western blotting using the 1G7 mAb (Figure 4). IFI 16 was first detected in the buffer containing in 200mM NaCl, with the maximum amount of eluted antigen obtained in the

or irrelevant isotype-matched antibodies (F and H, respectively). The antibodies were detected with a fluorescein-conjugated secondary antibody (see *Materials and Methods*). Magnification= 500X.



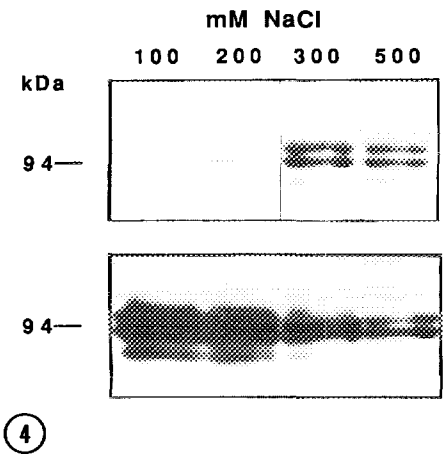


Figure 3. Western blot analysis of fractionated nuclei from Daudi cells. Whole nuclear lysate (lanes 1 and 3) and the fractionated nucleolar lysate (lanes 2 and 4) were probed with anti-B23 mAb or anti-IFI 16 mAb as indicated. Bound antibodies were visualized using a chemiluminescence detection system (see *Materials and Methods*). Molecular weight markers in kDa are shown at left.

Figure 4. Western blot analysis of proteins extracted from whole nuclei and the corresponding residual nuclear pellet. Whole nuclei from Daudi cells was incubated with extraction buffer containing the salt concentration indicated, and the insoluble nuclear material was removed by centrifugation. The eluted proteins and the proteins remaining in the pellet were analyzed by Western blotting with 1G7 mAb. Molecular weight markers are shown at left.

300mM NaCl extract (Figure 4, upper panel). Correspondingly, the amount of IFI 16 in the residual nuclear fraction began to decline at 200mM NaCl, and the minimum level of residual protein was reached at 300mM NaCl (Figure 4, lower panel). Approximately 80% of the IFI 16 protein appeared to be eluted from the nucleus with salt concentrations of 300mM or greater, though the remaining protein could not be removed from the residual nuclear pellet even after treatment with 2M NaCl (data not shown). Similar observations were also noted for MNDA, where approximately 20% of the total protein has been shown to resist extraction with 0.35M NaCl (16). The nature of the unextractable protein persisting in the nucleus remains unresolved. The elution of IFI 16 between 200 and 300mM NaCl concurs with the previous finding, where IFI 16 was shown to elute from DNA-cellulose at 250mM NaCl (8). Therefore, approximately the same concentration of salt is required to disrupt the interaction of native IFI 16 with its natural binding site(s) within the nucleus, and with DNA *in vitro*.

The possibility that IFI 16 binds to DNA was supported further by the identification of a DNA-binding region within IFI 16. As native IFI 16 binds to DNA *in vitro*, this interaction was examined further by investigating the binding of GST-IFI 16 to DNA-cellulose. This fusion protein contains the amino-terminal 159 amino acids of IFI 16, which is a highly basic portion of the protein and contains 26 lysine residues. The fusion protein was incubated with DNA-

cellulose, bound protein was eluted with incremental increases in salt concentrations, and the resultant eluates were analyzed by western blotting. Most of the GST-IFI 16 protein eluted from DNA at 250 mM NaCl, with the remaining protein eluted by the third elution with 300mM NaCl (Figure 5, indicated by arrow). None of the protein remained bound to the DNA-cellulose beads (data not shown). This region of IFI 16 exhibits the same propensity to bind DNA as the whole IFI 16 molecule, and therefore encodes a potential DNA-binding domain.

DISCUSSION

This study demonstrates for the first time that the nuclear localizaiton of IFI 16 is dependent upon the presence of nucleoli within a cell. Using specific mAbs in IF, both IFI 16 and the nucleolar antigen B23 were detected in the nucleus of unstimulated peripheral blood mononuclear cells, whereas polymorphonuclear cells did not express either antigen. Thus, only transcriptionally active cells expressed IFI 16, while those cells which have undergone terminal differentiation do not. IFI 16 is also capable of binding to DNA, and a putative DNA-binding domain was identified in the amino-terminal region. Taken with previous findings on the association of IFI 16 mRNA and protein expression with differentiation (8), this data supports the hypothesis that IFI 16 may be involved in transcriptional regulation associated with the state of differentiation/activation of haematopoietic cells.

Recently, Seelig and co-workers have used antisera from patients with SLE to localize the IFI 16 antigen to the nucleoplasm and nucleolus of a number of cell lines by IF, thus demonstrating IFI 16 to be a frequent target of autoantibodies (11). Anti-nuclear antibody-

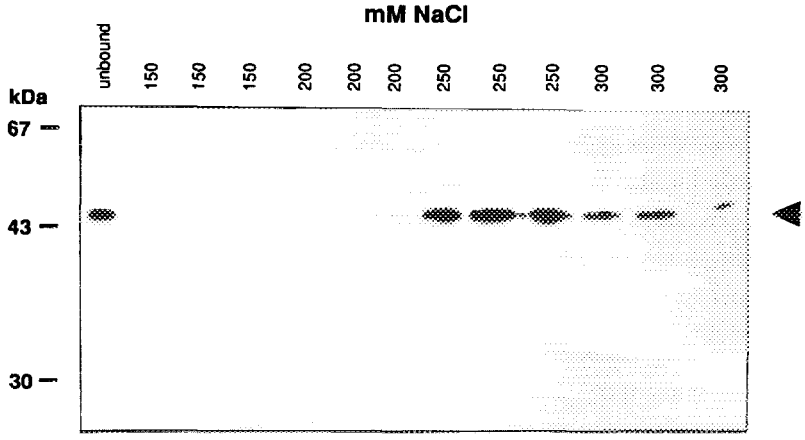


Figure 5. Binding of GST-IFI 16 and its elution from DNA-cellulose. GST-IFI 16 (10μg) was equilibrated in Buffer B containing 100mM NaCl and passed through a DNA-cellulose column. Bound proteins were sequentially eluted with Buffer B containing the NaCl concentration indicated (see *Materials and Methods*). "Unbound" indicates the column effluent. Eluted proteins were analyzed by Western blotting with 1G7 mAb. The 45 kDa GST-IFI 16 protein is indicated by an arrow on the right and molecular weight markers shown in kDa on the left.

positive antisera from SLE patients were found to exhibit a similar nuclear staining pattern as antiserum raised against IFI 16. The findings in our study extend this observation by defining the intranuclear distribution in subset of peripheral blood leukocytes. However, there were significant differences between our findings and those of Seelig et al., which were obtained using polyclonal and affinity-purified antiserum. In particular, these investigators report that the IFI 16 antigen was detected in approximately 50% of unstimulated HL-60 cells, whilst only 5-10% of HL-60 stain with 1G7. This difference could result from cross-reactivity of the polyclonal serum with the closely-related MNDA molecule. Experiments designed to demonstrate the specificity of the affinity-purified polyclonal antiserum utilized the HEp-2 hepatoma cell line, in which the expression of MNDA has not been examined. MNDA is constitutively expressed at low levels in HL-60 cells, but is absent from K562 and HeLa (17), whilst IFI 16 can be detected in nuclear lysates from HeLa cells (our unpublished data). Thus, in every instance where an antigen was detected with the polyclonal antiserum, IFI 16 and/or MNDA could have potentially been detected. Immunoprecipitation of HL-60 nuclear lysate using the purified polyclonal Ig would resolve the question of specificity.

In IF studies utilizing a mAb detecting IFI 16, the intranuclear staining of IFN-treated HL-60 cell line differed only quantitatively from that observed for cells of peripheral blood. The nucleolar staining in the immortalized cells was somewhat more distinct than in PBMC, in which only one or two small nucleoli were discernible amongst generally stronger nucleoplasmic staining. Continuously proliferating cell lines require constant rRNA production, whereas the majority of PBMC are "resting" and require added stimuli for activation/proliferation. This is reflected in the smaller size and lower number of nucleoli present in PBMC. However, the nature of the relationship between nucleolar and nucleoplasmic localization remains to be defined. It seems unlikely that IFI 16 has two different intranuclear targets, as IFI 16 was not detected in the nucleoplasm in the absence of nucleoli. It remains to be determined whether alternative binding sites exist within the nucleoplasm, or whether nucleoplasmic IFI 16 acts as an intranuclear pool for the protein localizing within nucleoli. The finding that a proportion of IFI 16 resisted salt extraction from whole nuclei was similar to that observed for MNDA, where the amount remaining in the nuclei was proportional to the extent of damage to the DNA caused by DNase I digestion (16). As the nuclei exposed to DNase I retained more MNDA, so the amount of salt-extractible protein decreased, suggesting that the salt-extractible MNDA might act as a pool to replenish the bound protein.

The function of this family of proteins is as yet undefined. IFNs are thought to affect cells by modulating gene transcription, and therefore this family of IFN-inducible nuclear proteins may play a role in transcriptional regulation. Recently, the IFN-responsiveness of MNDA was shown to be restricted specifically to cells which were capable of differentiating along the monocytic pathway (6). Expression of the MNDA mRNA was also shown to decline upon terminal differentiation of HL-60 initiated by PMA. This effect was not due to cessation of proliferation, and would therefore appear to be associated with the process of differentiation. Our finding that IFI 16 is absent from terminally differentiated granulocytes is reminiscent of

the observations with MNDA. Thus, at least for the two human members of this family, induction of expression in late stage myelopoiesis is followed by a decline in expression at terminal differentiation. Further investigation into the nature of the interaction(s) of these proteins within the nucleus will give an insight into mechanisms of the IFN response in haematopoietic cells.

It is also unclear whether IFI 16 becomes the target of human autoantibodies as an initiating event or as a consequence of autoimmune disease. As IFI 16 is inducible with IFNs, it has been suggested that perhaps IFN-γ may cause the induction of the autoantigen following autoimmune inflammation (11). However, our demonstration of the constitutive expression of IFI 16 in PBMC would indicate otherwise. Our findings are consistent with the observation that DNA-binding proteins are often the target of autoantibodies, and are usually bound to double-stranded DNA, another major autoantigen. Interestingly, the nucleic acid binding domains of some nuclear proteins have been shown to be the target of a subset of auto-antibodies found in SLE patients. However, the final definition of IFI 16 as the target of the SLE antisera requires formal confirmation by preclearing or blocking studies with recombinant IFI 16 antigen.

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REFERENCES

1. Choubey D., Snoddy J., Chaturvedi V., Toniato E., Opdenakker G., Thakur A., Samanta H., Engel D.A. and Lengyel P. (1989) J. Biol. Chem. 264, 17182-17189.

2. Tasnnenbaum C.S., Major J., Ohmori Y. and Hamilton T.A. (1993) J. Leuk. Biol. 53, 563-568.

3. Trapani J.A., Browne K.A., Dawson M.J., Ramsay R.G., Eddy R.L., Shows T.B., White P. C. and Dupont B. (1992) Immunogenetics 36, 369-376.

4. Briggs J.A., Burrus G.R., Stickney B.D. and Briggs R.C. (1992) J. Cell. Biochem. 49, 82-92.

5. Opdenakker G., Snoddy J., Choubey D., Toniato E., Pravtcheva D.D., Seldin M.F., Ruddle F.H., Lengyel P. (1989)Virology 171, 568-578.

6. Briggs R., Dworkin l., Briggs J., Dessypris E., Stein J., Stein G. and Lian J. (1994) J. Cell. Biochem. 54, 198-206.

7. Trapani J.A., Dawson M.J., Apostolidis V.A. and Browne K.A. (1994) Immunogenetics 40, 415-424.

8. Dawson M.J. and Trapani J.A. (1995) J. Cell. Biochem. 57, 39-51.

9. Choubey D. and Lengyel P. (1992) J. Cell Biol. 116, 1333-1341.

10. Choubey D. and Lengyel P. (1993) J. Interferon Res. 13, 43-52.

11. Seelig H.P., Ehrfeld H. and Renz M. (1994) Athritis and Rheumatism 37, 1672-1683.

12. McLean I.W. and Nakane P.K. (1974) J Histochem. Cytochem. 22, 1077-1083.

13. Yung B.Y-M., Busch H. and Chan P-K. (1990). Biochem. Biophys. Acta 826, 167-173.

14. Collins S.J. (1987) Blood 70, 1233-1244.

15. Ochs R., Lischwe M., O'Leary P. and Busch H. (1983) Exp. Cell Res. 146, 139-149.

16. Duhl D.M., Gaczynski M., Olinski R. and Briggs R.C. (1989) J. Cell. Physiol. 141, 148-153.

17. Goldberger A., Hnilica A.S., Casey S.B. and Briggs R.C. (1986) J. Biol. Chem. 261, 4726-4731.