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Development of Monoclonal Antibodies Against Human IRF-5 and Their Use in Identifying the Binding of IRF-5 to Nuclear Import Proteins Karyopherin-α1 and -β1

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Purpose: IRF-5 is a direct transducer of virus-mediated and TLR-mediated signaling pathways for the expression of cytokines and chemokines which form homodimers or heterodimers with IRF-7. However, direct IRF-5-specific monoclonal antibodies (mAbs) are not available at present. These could be used to further evaluate the functions of IRF-5. In this study, we produced and characterized three mouse mAbs to human IRF-5. The binding of IRF-5 to nuclear import proteins was first identified using a mAb. **Materials and Methods:** His-tagged human IRF-5 protein spanning amino acid residues 193-257 was used as an antigen and three mAbs were produced. The mAbs were tested with ELISA, Western blot analysis (WB), immunofluorescent staining (IF), and immunoprecipitation (IP). In addition, the nuclear import protein which carried phosphorylated IRF-5 was identified using one of these mAbs. **Results:** MAbs 5IRF8, 5IRF10 and 5IRF24 which reacted with the recombinant His-IRF-$5^{193-257}$ protein were produced. All mAbs bound to human IRF-5, but not to IRF-3 or IRF-7. They could be used for WB, IF, and IP studies. The binding of phosphorylated IRF-5 to karyopherin-α1 and -β1 was also identified. **Conclusion:** Human IRF-5-specific mAbs are produced for studying the immunologic roles related to IRF-5. Phosphorylated IRF-5 is transported to the nucleus by binding to nuclear import proteins karyopherin-α1 and -β1.

Key Words: Human IRF-5, monoclonal antibody, nuclear import protein, karyopherins

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INTRODUCTION

After first identifying interferon-regulatory factor 1 (IRF-1), nine additional IRF family proteins have been identified. IRF-1 binds to elements in the gene promoter that encodes for interferon-β (IFN-β).[1] Extensive studies of IRF family of proteins have revealed that they are involved in regulating virus-IFN axis, antigen presentation, nitric oxide production, and the cell cycle.[2,3] The IRF family of proteins also has an important role in the innate immune system, and the secondary response to cytokines. The signaling of toll-like receptors (TLRs) triggered by microbial components is important to the activation of innate and adaptive immune responses. For example, TLR-4 activation recruits the downstream adaptor molecule MyD88 and other signaling molecules for initial responses. IRF-3 and IRF-7 then interact with MyD88 and induce type I IFNs. In contrast, IRF-4 competes with IRF-5 and acts as a negative regulator for TLR signaling of the MyD88 interaction.[4] This suggests that there are complex regulatory mechanisms involved in MyD88 signaling. IRF-3 and IRF-7 function as direct transducers of virus-mediated signaling, and play a crucial role in the expression of type I IFN.[5-9]

IRF-5 is a recently characterized member of the IRF family. It encodes a ~61-kDa protein which was originally identified as a regulator of type I *IFN* gene expression.[10] Recent studies have indicated that it plays a role in host defense, including

the induction of multiple cytokines.[11] Similar to IRF-3 and IRF-7, IRF-5 is a direct transducer of virus-mediated signaling. However, this only occurs with specific viruses such as the Newcastle disease virus, vesiculostomatitis virus, and herpes simplex type 1 virus.[10,12] It also plays a role in the expression of cytokines and chemokines.[10,12,13] IRF-5 is a direct target of p53. Its expression is modulated by p53,[14] and it has a role in the p53-independent proapoptotic signaling pathway.[15,16] Recent studies have reported the association between IRF-5 and systemic lupus erythematosus.[17,18] In a gene chip study using overexpressed B cells which contained IRF-5 or IRF-7, the presence of IRF-5 was related to a strong immune response and adhesion genes. The presence of IRF-7, however, selectively upregulated the expression of mitochondrial genes and DNA repair genes.[19] This suggests a distinct role for IRF-5. The IRF family of proteins resides in the cytoplasm of resting cells. They are activated by phosphorylation on the C terminus, and are transported to the nucleus after homo- or heterodimerization.[10,13] IRF-5 dimerizes either with itself or with IRF-3, and activates *IFNA* gene transcription.[10,13] However, the heterodimerization of IRF-5 with IRF-7 represses *IFNA* transcription in virus-infected cells which were cotransfected with IRF-5 and IRF-7.[10,13] Recently, IRF-5 was found to have an important role in TLR signaling and the induction of proinflammatory cytokines such as interleukin (IL)-6, IL-12, and tumor necrosis factor (TNF)-α. It is impaired in cells from IRF-5-deficient mice,[11] suggesting that IRF-5 is generally involved downstream of the TLR signaling pathway. IRF-5 associates with both MyD88 and TRAF-6, and is translocated to the nucleus in a MyD88-dependent fashion.[11] However, many of the downstream mediators of the IRF-5 pathway need further identification.

The proteins in the KAP family act as shuttling receptors. They bind to the NLS motifs of cargo proteins to facilitate their import into the nucleus.[20] IRF-5 is phosphorylated by the stimulation of type I interferon and viral infections. It then enters the nucleus to regulate transcription.[10,12] IRF-5 has two nuclear localization signals (NLSs). These are found at residues 46 to 52 on a DNA binding domain, and on residues 448 to 454 on a transactivation domain. There is also one nuclear export signal (NES) on residues 150 to 160.[12,21] This implies its tight control of nuclear transport. IRF-5 is localized to the cytoplasm in an unstimulated state. It moves to the cytoplasm in a CRM1-dependent pathway after it is dephosphorylated in the nucleus.[21]

In this study we developed and characterized monoclonal antibodies (mAbs) to the human IRF-5 protein and tested their applicability of IRF-5-specific mAbs. Our mAbs were found to bind to human IRF-5, but not to human IRF-3 or IRF-7. We demonstrated the usefulness of these mAbs in Western blot, immunocytochemical, and immunoprecipitation analyses, as well as the import of IRF-5 to the nucleus. In addition, it was identified that IRF-5 is transported into the nucleus with the aid of the carrier proteins karyopherin (KAP)-α1 and -β1.

MATERIALS AND METHODS

Cell cultures and transfection

The human macrophage line THP-1, the human embryonic kidney cell line HEK293 (ATCC), and NIH3T3 cells were cultured. The culture was done at 37°C under 5% CO_2 in RPMI1640 supplemented with 10% FBS (Invitrogen Life Technologies, San Diego, CA, USA), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. For the transient transfection, FuGene6 (Roche Diagnostics GmbH, Mannheim, Germany) was used for the HA-tagged human IRF-3, HA-tagged IRF-5, GFP-tagged IRF-5, and Flag-tagged IRF-7 expressions.

DNA constructs and protein purification

We cloned the gene which encodes six His-tagged human IRF-5 protein spanning residues of 65 amino acids into a pRSET vector (Invitrogen) (His-IRF-$5^{192-256}$ in NCBI accession No. AAU 12877 which corresponds to His-IRF-$5^{176-240}$ in NCBI accession No. NP_116032.1). This is a region which is located between a DNA-binding domain and an IRF-association domain. It seems to be the least homologous with IRF-3 and IRF-7. cDNA of human peripheral blood mononuclear cells were

amplified by PCR using the sense primer 5′-GCT TGCGGATCCCCGCCCACTCTG-3′, and the antisense primer 5′-AGACTGGAATTCTAGATCAGC AGGTCTGG-3′. The restriction enzyme sites BamHI and EcoRI are underlined, respectively. The construct was confirmed by DNA sequencing (Applied Biosystems). The protein was produced in *E. coli* BL21(DE3) pLysS (Novagen) containing ampicillin (50 μg/mL) at 37°C. Isopropyl-β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM when the OD 600 of the culture broth had reached 0.5 - 0.6. The cells were harvested after 4 hours. The cell pellet was resuspended in 100 mL of lysis buffer (20 mM Tris, 1 mM DTT, 10% glycerol, 1 mM EDTA, pH 8.0). The bacterial lysis was sonicated with a Sonosmasher followed by centrifugation. The supernatant was loaded onto a Ni^{++}-NTA agarose resin (Peptron, Daejeon, Korea) which was equilibrated with a binding buffer (20 mM Tris pH 8.0, 5 mM imidazole, 0.5 M NaCl). The bound protein was washed with a washing buffer (20 mM Tris pH 8.0, 0.5 M NaCl, 20 mM imidazole) and eluted with an elution buffer (20 mM Tris pH 8.0, 0.5 M NaCl, 200 mM imidazole). For further purification of the protein, the eluent was concentrated with centricon (Vivaspin 20, VS2011) and applied to Sephacryl S200 gel chromatography (Amersham Biosciences, Uppsala, Sweden). The purity of the His-IRF-$5^{193-257}$ protein was more than 90% using SDS-PAGE. The molecular weight was measured using MALDI-TOF (Applied Biosystems).

MAb production and purification

BALB/c mice were intraperitoneally immunized with 20 μg of His-IRF-$5^{193-257}$ protein, which was emulsified in complete Freund's adjuvant and boosted twice with incomplete Freund's adjuvant at two week intervals. Finally, the mice were intravenously injected with 50 μg of His-IRF-$5^{193-257}$ protein three days before the fusion. Splenocytes were fused with P3-X63-Ag8.653 cells and selected with HAT medium (Sigma-Aldrich Co., St. Louis, MO, USA). The resulting hybridoma supernatants were screened using ELISA, as described below. The isotype of each mAb was determined using a mouse-hybridoma subtyping kit (Roche). The colonies which secreted anti-IRF-5 antibodies were cloned with limited dilution. The three hybridoma clones, 5IRF8, 5IRF10, and 5IRF24, were purified for further characterization using a protein G-Sepharose affinity column (Amersham).

ELISA

Microtiter plates (Corning Inc., Corning, NY, USA) were coated with 1 μg/mL of His-IRF-$5^{193-257}$ for mAb screening. The plates were washed with 0.05% Tween 20 in PBS (PBST) and blocked with 5% normal goat serum (NGS) in PBST. Hybridoma culture supernatants were added to the plates. The plates were then incubated for 1.5 hours at 37°C. After washing, horseradish peroxidase (HRP) labeled goat anti-mouse Ig (Sigma) was added. After 1 hour of incubation, o-phenylenediamine was added to the plates for color development. The ODs were measured at 490 nm.

Western blot analysis

Whole cell lysates (WCLs) from different cell lines were prepared using 1% Nonidet P-40 buffer which contained a protease inhibitor cocktail (Sigma). The protein concentrations were measured using a Bradford assay (Biorad) for the analysis of the WCLs. They were then electrophoresed in 10% SDS-PAGE. The proteins were transferred to a nitrocellulose membrane and probed with mAbs after blocking. HRP-conjugated rabbit anti-mouse Ig (Sigma) was used as a secondary antibody. The probe signals were revealed with enhanced chemiluminescence (ECL, Labfrontier Co., Seoul, Korea). Mouse anti-HA and anti-Flag M2 (Sigma) were used to detect the corresponding tag proteins.

Immunoprecipitation

Cells were lysed with a protease inhibitor cocktail. Cell homogenates were centrifuged at 20,000 g for 20 minutes and precleared by incubation with protein G-Sepharose (Amersham) at 4°C for 1 hour. The precleared extracts (500 μg) were incubated with purified mAbs. Protein G-Sepharose was then added and the samples were incubated for 1.5 hours at 4°C. Immune complexes were collected by centrifugation and washed with PBS. The collected complexes were fractionated using

SDS-PAGE, transferred to membranes, and blotted with anti-GFP (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). HRP-conjugated goat anti-mouse Ig (Sigma) was used to detect the probe signals.

Immunofluorescence and GFP imaging

To observe the applicability of our mAbs for use in an immunofluorescence assay, HEK293 cells which were transfected with an IRF-5-GFP plasmid were cultured in LabTek II chambers (Nunc). They were then fixed in 4% paraformaldehyde in PHEM buffer (60 mM PIPES, 25 mM HEPES, 10 mM EGTA, 4 mM $MgSO_4$, pH 7.0) for 10 minutes at room temperature. After fixation, the cells were washed with PBS and incubated for 3 minutes at 4°C with a HEPES-based permeabilization buffer containing 300 mM sucrose and 0.2% Triton X-100. The cells were blocked with 0.2% BSA in PBS for 15 minutes. They were then incubated with anti-IRF-5 mAbs for 1 hour at room temperature. PE-conjugated rabbit anti-mouse Ig (BD Pharmingen) was added after three washes. The cells were observed with a BX51 fluorescent microscope (Olympus). HEK 293 cells transfected with an IRF-5-HA plasmid were incubated for 24 hours and then treated with 20 nM of okadaic acid (Calbiochem) for 6 hours to investigate the movement of phosphorylated IRF-5 using our mAbs, Next, the cells were stained with 5IRF10 and FITC-conjugated anti-mouse Ig for tracing the movement of IRF-5 into the nucleus. The nuclei were stained with DAPI.

The binding of IRF-5 to KAP proteins

GST-KAP fusion proteins-α1, -α2, -α3, -α4, -α5, -α6, and -β1 were produced as previously described for the assay involving the KAP protein binding to the cargo protein.[22] HEK293 cells were transfected with an HA-tagged IRF-5 plasmid. WCLs were prepared after 24 hours and allowed to bind to Sepharose 4B-immobilized GST-KAPs-α1, -α2, -α3, -α4, -α5, -α6, and -β1 at 4°C overnight. Unbound proteins were washed. Sepharose-bound proteins were dissolved in a Laemmli sample buffer, separated on 12% SDS-PAGE, and subjected to Western blotting. The membrane was blotted with 5IRF10 mAb and then reblotted with anti-GST to visualize the amount of Sepharose-immobilized GST-KAP proteins.

RESULTS

Generation of mAbs and antigen reactivity

Recombinant human His-IRF-$5^{193-257}$ protein was produced in *E. coli* and purified using an affinity column for immunogens. This region is located between a DNA-binding domain and an IRF-association domain. The recombinant His-IRF-$5^{193-257}$

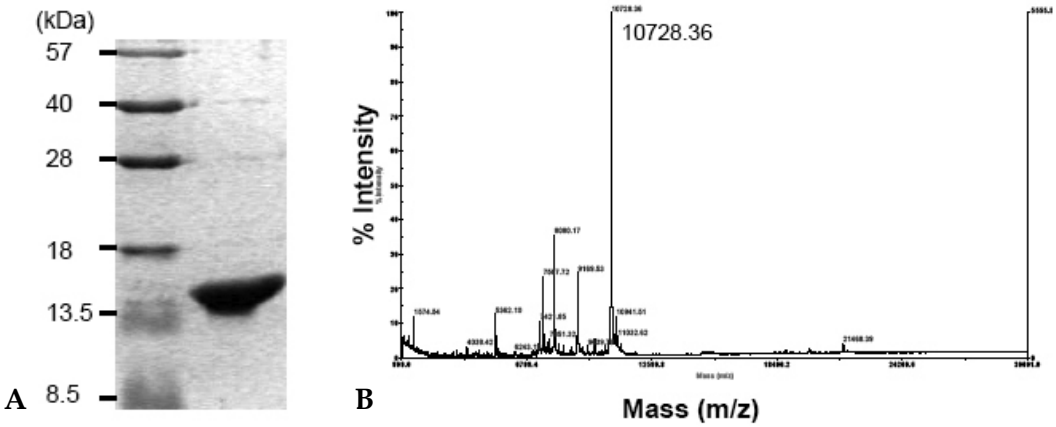

Fig. 1. SDS-PAGE and MALDI-TOF mass spectrum of recombinant His-IRF-$5^{193-257}$. (A) SDS-PAGE analysis of purified His-IRF-$5^{193-257}$. His-IRF-$5^{193-257}$ was eluted with 200 mM imidazole on a Ni^{++}-NTA agarose resin and purifed by Sephacryl S-200 gel chromatography. (B) MALDI-TOF mass spectrum analysis of purified His-IRF-$5^{193-257}$ protein. The molecular weight (MW) was determined to be 10728.36 Da using MALDI-TOF mass spectrum. This was very close to the theoretical MW of 10721.1 Da.

protein was purified and identified using SDS-PAGE (Fig. 1A). The molecular weight was determined to be 10728.36 Da using MALDI-TOF mass spectrum (Fig. 1B). This was very similar to the theoretical molecular weight of 10721.1 Da, implying the exact fragment of IRF-5 protein.

We obtained clones of three hybridomas; 5IRF8, 5IRF10, and 5IRF24. The culture supernatants were tested for their specific antigen-binding capacity using ELISA. This was done by serially diluting the culture supernatant (Fig. 2A) or His-IRF-5$^{193-257}$ antigen (Fig. 2B) to test whether these mAbs recognize the specific antigen. The serially diluted mAbs were shown to bind in a dose-dependent manner at a fixed antigen coating concentration of 1 μg/mL. MAbs also bound to antigen in a dose-dependent manner when the antigen coating concentration was increased from 0.001 to 10 μg/mL. These mAbs demonstrated no binding to irrelevant protein α-synuclein at a concentration of 10 μg/mL. All isotypes of the mAbs were found to be IgG1 (κ).

Western blot analysis

We next tested the reactivity of our mAbs to His-IRF-5$^{193-257}$ protein using Western blot analysis to determine the antigen reactivity. All three mAbs

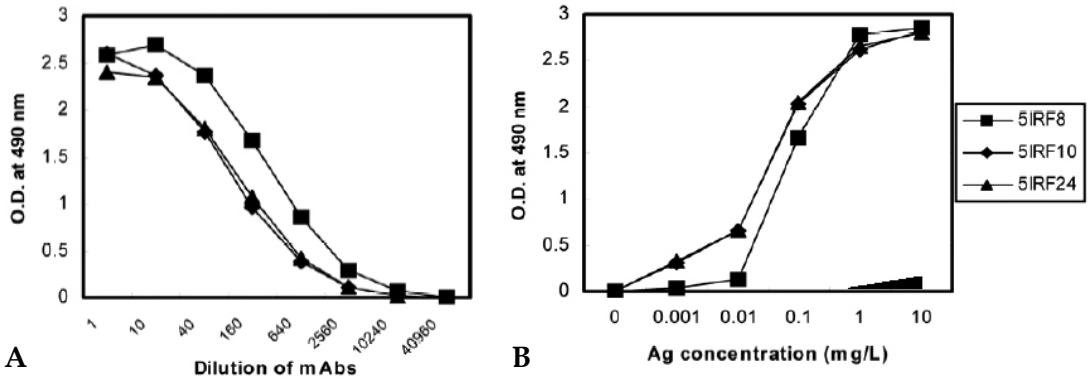

Fig. 2. Binding curve between the anti-IRF-5 monoclonal antibodies and the His-IRF-5$^{193-257}$ protein. (A) ELISA was performed to the wells. The wells were coated with 1 μg/mL of His-IRF-5$^{193-257}$ protein using the various dilutions of culture supernatants of our three mAbs. (B) ELISA was performed with mAbs at a fixed dilution to the wells which were coated with various concentrations of the His-IRF-5$^{193-257}$ protein. The bindings of all three mAbs to the control protein of α-synuclein are shown inside the black triangle.

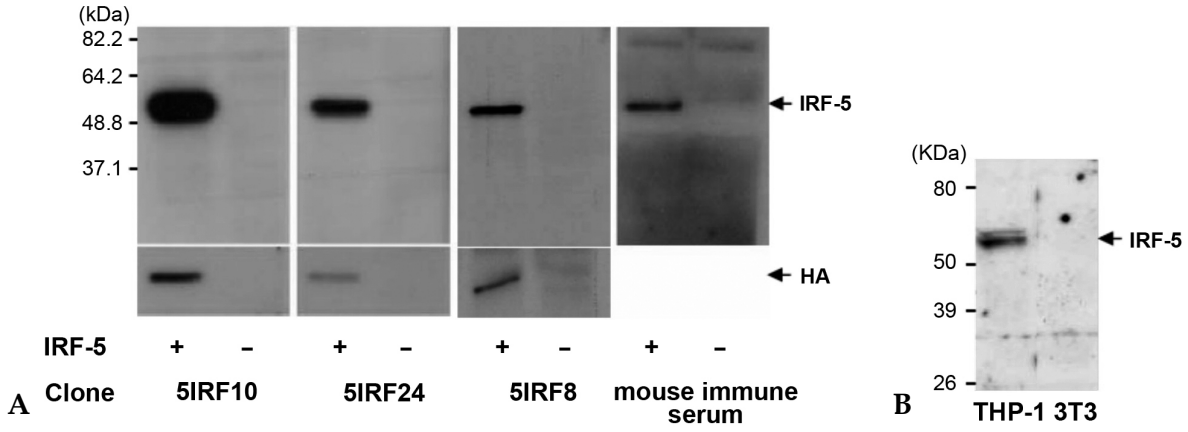

Fig. 3. Western blot analysis for antigen specificity. (A) An HA-tagged IRF-5 plasmid was transfected into HEK293 cells and the WCL was separated. The reactivity of mAbs to IRF-5 was tested. The expression of IRF-5 was tested with anti-HA. Mouse immune serum was used as a positive control Ab. (B) Detection of endogenous IRF-5. WCLs of THP-1 and NIH3T3 cells were separated and immunoblotted with 5IRF10 as a representative study. NIH3T3 cell lysate was used as a negative control.

detected the purified His-IRF-5$^{193-257}$ protein (data not shown). HEK293 cells were transfected with an HA-tagged IRF-5 plasmid, and WCLs were harvested to determine whether these mAbs could recognize the wild type IRF-5 protein. All three mAbs readily recognized the wild type IRF-5 and did not bind to non-transfected control WCLs (Fig. 3A). IRF-5 is constitutively expressed by B cells, monocytes, and dendritic cells.[10,23] We tested the binding of 5IRF10 as a representative to the endogenous IRF-5 using WCLs of human cell lines of THP-1 cells. 5IRF10 recognized two major IRF-5 bands at ~61 kDa (Fig. 3B).

Cross-reactivity analysis

The spanning region of recombinant His-IRF-5$^{193-257}$ protein was chosen because it has a very low amino acid homology with human IRF-3 and IRF-7. In order to exclude cross-reactivity with other proteins in the IRF family, we determined the binding specificity of all three mAbs to IRF-3 and IRF-7. For this study, HEK293 cells were transfected with each plasmid containing IRF-3, IRF-5, and IRF-7. The WCL proteins were separated using SDS-PAGE and immunoblotted with each mAb. This was followed by reblotting with anti-HA for IRF-3 and IRF-5, and with anti-Flag for IRF-7. All three mAbs showed specific binding to IRF-5 protein, but not to IRF-3 and IRF-7 proteins. This indicates that our mAbs bind specifically to IRF-5. We used 5IRF10 data as a representative

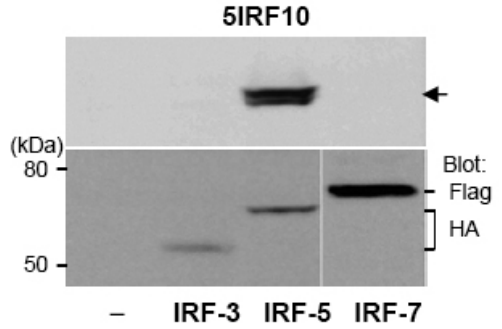

Fig. 4. Cross-reactive binding study. HA-tagged IRF-3, HA-tagged IRF-5, and Flag-tagged IRF-7 plasmids were transfected into HEK293 cells. WCLs were separated and the cross-reactivity of 5IRF10 to IRF-3 and IRF-7 was tested as a representative study. The membranes were reblotted with anti-HA or anti-Flag depending on the tagged protein. Arrow: IRF-5.

(Fig. 4).

Immunofluorescent analysis

Using immunofluorescent staining, three mAbs were examined for their ability to detect intracellular IRF-5 protein. HEK293 cells, which do not express the detectable endogenous IRF-5 through Western blotting (Fig. 4), were transfected with an IRF-5-GFP plasmid. Indirect immunofluorescent staining was also performed to observe the location of the IRF-5. This was done using each mAb and PE-conjugated anti-mouse Ig as the primary and secondary antibodies, respectively. Green fluorescence was observed in the cytoplasm in an unstimulated state. IRF-5 protein stained intensively in the cytoplasm with all three mAbs (Fig. 5). When the two images were merged, the staining image of IRF-5 using each mAb was co-localized to the GFP expression image. This result demonstrates that our mAbs can be used for the immunofluorescent staining of IRF-5.

Immunoprecipitation

HEK293 cells were transfected with an IRF-5-GFP plasmid, and the WCLs were subjected to immunoprecipitation using our mAbs to identify the applicability of these mAbs to the immunopre cipitation procedure. The WCLs were immunoprecipitated with 5IRF10 mAb as a representative sample. The precipitates were analyzed with a Western blot using anti-GFP. IRF-5-GFP protein was observed at the expected molecular weight of ~88 kDa (Fig. 6A). The immunoprecipitation study was also performed against WCLs of THP-1 cells to determine whether 5IRF10 can immunoprecipitate endogenous IRF-5 protein. The membrane was probed with anti-GFP. 5IRF10 could immunoprecipitate endogenous IRF-5 protein (Fig. 6B).

Import of phosphorylated IRF-5 into the nucleus

The nuclear import study of IRF-5 was performed to further evaluate the functions of our mAbs. The movement of phosphorylated IRF-5 was detected after transfection. To do this, HEK 293 cells were transfected with an HA-IRF-5 plasmid, stained with 5IRF10. Okadaic acid is a type 1/2A

protein phosphatase inhibitor[24] for forcibly inducing phosphorylation of proteins. Okadaic acid was used at a low concentration of 20 nM for 6 hours to block entry into and the cell cycle.[25] IRF-5 was located in the cytoplasm in an unstimulated state and then moved to the nucleus after phosphorylation (Fig. 7A). This confirms the results from a previous report.[10]

The KAP family proteins serve important functions as shuttling receptors. They bind to the NLS motifs of cargo proteins to facilitate their movement into the nucleus.[20] We produced the GST-KAPs-α1, -α2, -α3, -α4, -α5, -α6, and -β1 in *E. coli* in order to investigate which KAP protein binds to IRF-5 to facilitate the transport of IRF-5 into the nucleus. WCLs were prepared from HEK 293 cells,

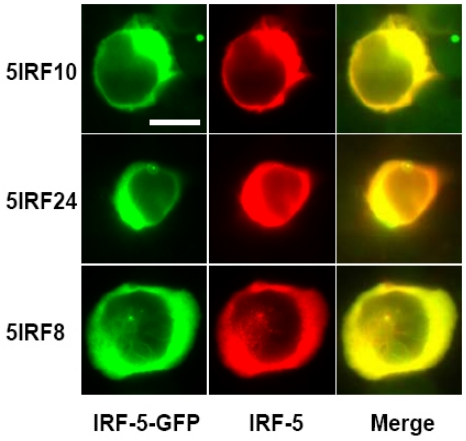

Fig. 5. Immunofluorescence staining of IRF-5. HEK293 cells were transfected with a GFP-tagged IRF-5 plasmid. The cells were fixed and stained with the indicated mAbs for the His-IRF-5$^{193-257}$ protein. PE-labeded anti-mouse Ig was added to the cells. The merged images are shown. Bar: 10 μm.

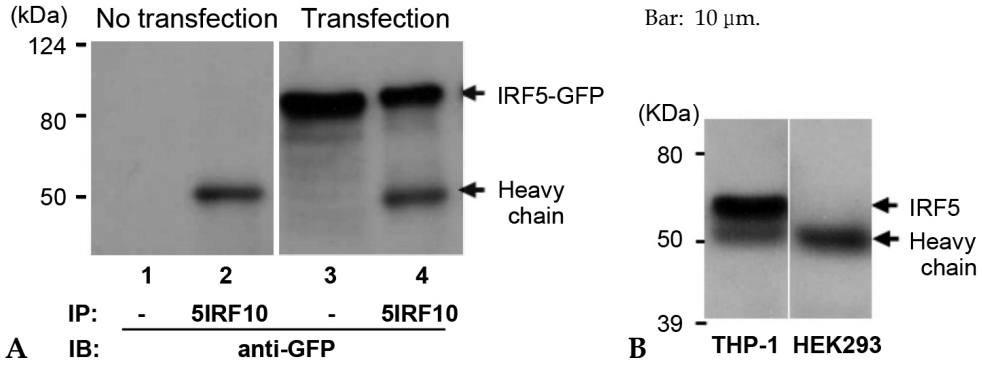

Fig. 6. Immunoprecipitation. (A) HEK293 cells were transfected with a GFP-tagged IRF-5 plasmid. The WCL was immunoprecipitated with 5IRF10. The bound proteins were separated and the membrane was blotted with anti-GFP. WCLs were used as controls (lanes 1 and 3). (B) Immunoprecipitation of endogenous IRF-5 protein. WCLs of human cell lines of THP-1 and HEK293 were immunoprecipitated with 5IRF10. They were separated and blotted with 5IRF24. HEK293 cell lysate was used as a negative control.

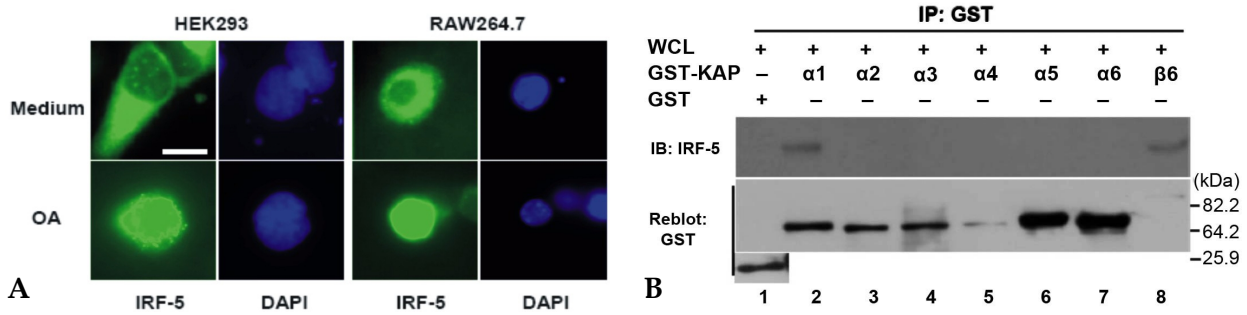

Fig. 7. Import of phosphorylated IRF-5 to the nucleus. (A) The localization of phosphorylated IRF-5 to the nucleus. HEK293T cells and RAW264.7 cells were transfected with an IRF-5 plasmid and treated with 20 nM OA for 6 h after transfection. The cells were stained with 5IRF10, followed by FITC-conjugated anti-mouse Ig. DAPI was used for staining the nucleus. Bar: 10 μm. (B) The binding of IRF-5 to KAP-α1 and -β1. GST-KAPs-α1, -α2, -α3, -α4, -α5, -α6 and -β1 fusion proteins immobilized on glutathione-Sepharose 4B beads were incubated overnight at 4°C with WCLs of HEK293 cells transfected with an IRF-5 plasmid. Sepharose-bound proteins were separated and the membrane was blotted with 5IRF10. It was then reblotted with anti-GST.

which were transfected with HA-tagged IRF-5 plasmids, for an *in vitro* protein-protein interaction study. The WCLs were incubated overnight with Sepharose-immobilized GST-KAPs-α1, -α2, -α3, -α4, -α5, -α6, and -β1 at 4°C. Sepharose-bound proteins were analysed using a Western blot. The membrane was blotted with 5IRF10. It was then reblotted with anti-GST to visualize the amount of Sepharose-immobilized GST-KAP proteins in the sample. We identified that KAP-α1 and -β1 were involved in the transport of the IRF-5 protein (Fig. 7B).

DISCUSSION

The His-IRF-5$^{193-257}$ protein region was selected to generate IRF-5-specific mAbs for the further functional study of IRF-5. This region was chosen because it belongs to a regulatory domain which has the least amino acid homology with IRF-3 and IRF-7. We produced three mAbs against human IRF-5. These mAbs are useful for performing Western blots, in addition to immunocytochemical, and immunoprecipitation analyses. They were very specific to His-IRF-5$^{193-257}$ protein and recognized the endogenous IRF-5 and did not show cross-reaction to IRF-3 and IRF-7 proteins. Using an overexpression study, there are at least nine reported human IRF-5 variant isoforms. Variants 1 through 6 encode ~61 kDa isoforms. Variants 7 and 8 encode ~47 kDa isoforms. Variant 9 encodes a ~25 kDa isoform.[17,23] Among these isoforms, variants 1 through 7, but not variants 8 and 9, include the IRF-5$^{193-257}$ protein region that was used as an immunogen in this study. In our data, two major bands at ~61 kDa could be observed by Western blot using 5IRF10 in whole cell lysates of THP-1 cells. However, endogenous ~47-kDa protein could not be found. This may be due to the low expression of IRF-5 variant 7 since this expression has only been studied in overexpressed cells.[23] Human IRF-5 has about an 87% homology with mouse IRF-5. The region which codes for the His-IRF-5$^{193-257}$ protein has a 72% (47/65) amino acid sequence homology with mouse IRF-5. Using a Western blot, we observed that 5IRF10 bound to ~61 kDa mouse endogenous IRF-5 using mouse B cell lymphoma cell line A20.3[26] (data not shown). Further evaluation is needed to confirm the cross-reactivity with mouse IRF-5 by transfecting mouse IRF-5 genes.

IRF-5 is localized to the cytoplasm in an unstimulated state. It is moved to the nucleus by an inducible phosphorylation, such as a viral infection.[10,12] It is then relocalized to the cytoplasm in a CRM1-dependent pathway.[21] The transport of IRF-3 into the nucleus is mediated by KAP-α3 and KAP-α4[27] and the transport out of the nucleus is mediated by a CRM1-dependent pathway. This study is the first to identify that IRF-5 is bound to KAP-α1 and -β1 during transport into the nucleus. This result suggests that the import of the IRF family of proteins into the nucleus has different controls.

The importance of IRF-5 is increasing. It is one factor in the susceptibility to autoimmune diseases such as systemic lupus erythematosus[17] and rheumatoid arthritis.[28] It functions as a tumor suppressor and in antiviral immunity by inducing IRF-5-dependent apoptosis of virus-infected cells.[15] IRF-5 also plays an important role in TLR signaling and in the induction of the proinflammatory cytokines IL-6 and IL-12.[11] It is involved in Fas-induced apoptosis.[29] However, many of the downstream mediators in the IRF-5 pathway need further identification for the understanding of autoimmune diseases and host defense mechanisms and these mAbs may be helpful for the study.

In summary, we developed anti-IRF-5 mAbs for IRF-5-specific detection. We demonstrated that KAP-α1 and -β1 are the nuclear carrier proteins for IRF-5.

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