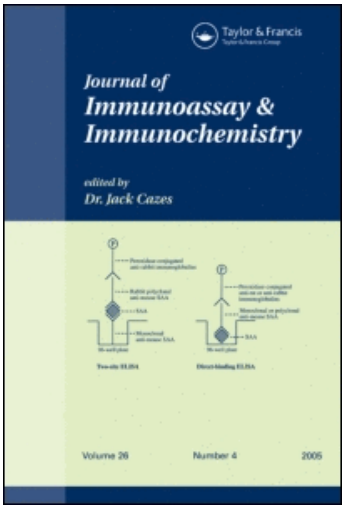

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Production and Characterization of a Monoclonal Antibody Specific for Ubiquitin-Like Polypeptide Responsible for Nonspecific Immune Suppression

Y. Nariai[a]; M. Nakamura[a]; T. Kondoh[a]; Y. Tanigawa[a]
[a] Department of Biochemistry, Shimane Medical University, Izumo, Japan

To cite this Article Nariai, Y. , Nakamura, M. , Kondoh, T. and Tanigawa, Y.(1998) 'Production and Characterization of a Monoclonal Antibody Specific for Ubiquitin-Like Polypeptide Responsible for Nonspecific Immune Suppression', Journal of Immunoassay and Immunochemistry, 19: 1, 49 — 62
To link to this Article: DOI: 10.1080/01971529808005471
URL: http://dx.doi.org/10.1080/01971529808005471

JOURNAL OF IMMUNOASSAY, 19(1), 49-62 (1998)

PRODUCTION AND CHARACTERIZATION OF A MONOCLONAL ANTIBODY SPECIFIC FOR UBIQUITIN-LIKE POLYPEPTIDE RESPONSIBLE FOR NONSPECIFIC IMMUNE SUPPRESSION

Nariai, Y, Nakamura, M, Kondoh, T, and Y. Tanigawa
Department of Biochemistry, Shimane Medical University, Izumo 693, Japan

ABSTRACT

Monoclonal nonspecific suppressor factor (MNSF) is a lymphokine product of a murine T cell hybridoma that inhibits the immune response in an antigen nonspecific manner. Recently, we found that a novel ubiquitin-like protein (Ubi-L), a subunit of MNSF, is responsible for its biological activity. We developed a monoclonal antibody with specific activity against Ubi-L. Inhibition experiments showed that this mAb, termed NA4, preferentially recognizes Ubi-L but not irrelevant proteins such as ubiquitin. With the use of NA4, we established an ELISA method for the quantitation of Ubi-L. By this ELISA system, approximately 40 ng/ml of MNSF was detected in the culture supernatants of concanavalin A (Con A)- or interferon gamma (IFNγ)-activated splenocytes, whereas MNSF in the supernatant of IFNα- and IFNβ-stimulated splenocytes was nil. In addition, NA4 could abrogate the action of Ubi-L. Thus NA4 was confirmed to be a pertinent tool for elucidation of the underlying mechanism of action of MNSF.
(KEY WORDS: ubiquitin, nonspecific, immune suppression)

INTRODUCTION

Monoclonal nonspecific suppressor factor (MNSF) is a lymphokine produced by Con A-activated murine T cell hybridoma and its biochemical and immunological characteristics have been elucidated (1). MNSF inhibits the generation of lipopolysaccharide (LPS)-induced immunoglobulin-secreting cells, proliferation of mitogen-activated T and B cells, and division in various tumor cell lines of murine

origin. It has been considered that MNSF consists of two isoforms, MNSFα and MNSFβ. Although no cDNA clone encoding MNSFα could be isolated, a cDNA clone encoding MNSFβ was isolated and characterized (2). The subunit, termed MNSFβ, encodes a protein of 133 amino acids consisting of an ubiquitin-like protein (36% identity with ubiquitin) fused to the ribosomal protein S30. Most recently, we have demonstrated that the recombinant form of the ubiquitin-like segment (Ubi-L) expressed in bacteria has biological activity similar to the hybridoma (E17)-derived 70 Kd MNSF, indicating that this polypeptide has potent immunoregulatory bioactivity (3). We have also isolated and characterized a human non-specific suppressor factor (hNSF), the putative human counterpart of MNSF, from a patient with systemic lupus erythematosus (4). The hNSF is serologically related to the T cell receptor α (TCRα) chain, indicating that MNSF may consist of TCRα or a related polypeptide.

In the present study, we prepared a murine mAb against Ubi-L, termed NA4, and the characterization of this mAb was performed to demonstrate its specific reactivity with Ubi-L. We also tested the possibility that this mAb is a pertinent tool for quantitation of Ubi-L in culture supernatants by ELISA system. We will show that the sensitive ELISA using NA4 mAb is useful for elucidation of the mode of action of the antigen non-specific suppressor factor in the immune response.

MATERIALS AND METHODS

Animals. BALB/c mice were obtained from Clea Japan, Inc. (Osaka) and were reared in the laboratory of the Institute for Experimental Animals, Shimane Medical University and used when 8-12 weeks of age.

Materials. MNSF was purified by affinity chromatography with the use of the anti-MNSF mAb MO6 from the ascites of F1 mice (AKR/J x BALB/c) after injection of MNSF-producing hybridoma (E17) into the peritoneal cavity (1). Recombinant

MNSFβ was prepared as described previously (2). Briefly, MNSFβ was expressed as a fusion protein with glutathione S-transferase (GST) by using the pGEX-2T vector (Pharmacia, Sweden). The rMNSFβ fusion protein was expressed in *Escherichia coli*, extracted, and purified by glutathione-Sepharose 4B. Recombinant GST-Ubi-L was also prepared as described (3). Specific polyclonal Ab against synthetic peptide (GQETVAQIKDHV) corresponding to the ubiquitin-like region (PU1) was elicited in rabbits as described (2). Bovine ubiquitin was purchased from Sigma.

Production of anti-ubiquitin like segment of MNSFβ (Ubi-L) mAb. For immunization, 0.33 mg of rMNSFβ were emulsified in complete Freund's adjuvant, and were given subcutaneously to female BALB/c mice (8-12 weeks old) biweekly for 3 months. Three days after the last injection, the mice were sacrificed by cervical dislocation, spleens removed, and then teased apart with forceps to prepare a single cell suspension. The cells were fused with mouse myeloma cells, SP2/O as described previously (1). Wells were screened by ELISA to test production of the anti-MNSFβ mAb as described below. Positive wells were expanded, and were cloned by a limiting dilution. Two types hybridoma cells producing anti-MNSFβ or anti-GST mAb were obtained and were cloned three times. Isotyping of mAb was performed by isotyping kit (Amersham, Japan). The hybridoma cells ($1x10^7$ cells) were injected into the peritoneal cavity of pristane (Aldrich, WI)-primed BALB/c mice. The ascitic fluid was centrifuged at 3000 x g and gel filtration (Sephadex G-200) was employed to prepare mAb against Ubi-L, because this mAb (termed NA4) was IgM. NA4 was further purified by ammonium sulfate precipitation.

ELISA for reactivity and specificity of NA4. To clarify the reactivity of NA4 with MNSFβ, the ELISA method and antibody dilution tests were used. First, purified recombinant GST-MNSFβ (12 μg/well), GST (10 μg/well) and ubiquitin (8 μg/well) were coated onto a 96-well flat-bottomed microtiter plate (Nunc, Denmark) for 48 hr

at 4 °C and then the unreacted sites of wells were saturated with 0.5% ovalbumin (OVA)/phosphate buffered saline (PBS)/ 0.05% Tween 20 for 1 hr at room temperature (RT). The reciprocally 2 to 2^{10} times diluted NA4 were added to wells and incubated for 2 hr at RT. Then, peroxidase-conjugated IgG of rabbit anti-mouse IgM (seroTec, England) was added to each well and incubated for 90 min at RT. The plates were washed 5 times with PBS/0.05% Tween 20 each step of this procedure. The peroxidase substrate was o-phenylenediamine (0.8 mg/ml) in 0.1 M citrate, pH 4.0, with 0.015% hydrogen peroxide. After incubation for 30 min at RT, the reaction was halted by the addition of 0.5 M NaF, and the OD of the mixture was determined by using ELISA reader. According to the results obtained, the optimal dilution of anti-MNSFβ mAb was determined and used for the following inhibition test. To test the specific reactivity of NA4 with MNSFβ and Ubi-L, the cross-reactivity test was done by using recombinant MNSFβ, Ubi-L, GST and ubiquitin. Various amounts of the substances were first incubated with the diluted NA4 (80 ng/ml) for 16 hr at 4 °C, after which the mixture was added to wells of the ELISA plate previously coated with MNSFβ. The ELISA reading was conducted as described above.

Sandwich ELISA for MNSF. First, purified NA4 (0.16 μg/well) was coated onto a 96 well flat-bottomed microtiter plate and then various amounts of GST-Ubi-L (1 to 1000 x 10^{-11} M) were added. In some experiments, the supernatants of Con A (3 μg/ml)-, LPS (20 μg/ml)-, IFNγ (20 U/ml)-, or IL-2 (50 U/ml)-activated murine splenocytes were added. After incubation, the plates were incubated with the anti-PU1 Ab (0.22 μg/well). The peroxidase-conjugated goat affinity purified Ab to rabbit IgG (Cappel, PA) was used for the third Ab. ELISA technique was the same as described in "ELISA for reactivity and specificity of NA4".

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting of NA4 with Ubi-L. SDS-PAGE was performed on GST-Ubi-L and GST, under reducing conditions in 15% polyacrylamide gels (5). GST-Ubi-L or GST migrating

on the gels were blotted electrophoretically on nitrocellulose membrane (6), which was subsequently blocked with PBS/Tween containing 0.5% ovalbumin. The membrane was incubated with NA4. After incubation, the membrane was incubated with the biotinylated sheep anti-mouse Ig (Amersham), which was subsequently incubated with the streptavidin-biotinylated peroxidase complex (Amersham). Locations of GST-Ubi-L or GST conjugated with NA4 were visualized after incubation with 3,3'-diaminobenzidine (DAB; Nacalai Tesque, Japan) by using the method of Grass et al. (7). The membrane was washed with PBS/Tween at each step of this procedure. In some experiments, Ubi-L was cleaved from the GST by thrombin (2).

RESULTS

The supernatants of many hybridoma clones strongly reacted with the recombinant GST-MNSFβ on screening. All were cloned three times by limiting dilution. Except one clone, designated NA4, all hybridomas produced mAb recognizing recombinant GST. The NA4 mAb was purified from mouse ascitic fluids, and was found to be of the IgM isotype and to have a κ light chain. Fig. 1 shows the representative results of antibody dilution tests. Among a range from 32 to 512 times dilution of NA4 with a steep declining slope, 128 dilution (80 ng/ml) was considered to be the most suitable, hence was used for studies of the next cross reactivity test. NA4 did not recognize such irrelevant proteins as ubiquitin and GST (Fig.1). To further confirm the specificity of NA4, inhibition experiments were performed. As shown in Fig. 2, both GST-MNSFβ and GST-Ubi-L exclusively inhibited, in a dose dependent manner, the binding of NA4 to MNSFβ coated on the ELISA plate. In contrast, ubiquitin and GST showed no cross-reactivity, thereby suggesting the specific reactivity of NA4 with Ubi-L and MNSFβ. Accordingly, NA4 recognizes the ubiquitin-like moiety of MNSFβ, but not ribosomal protein S30.

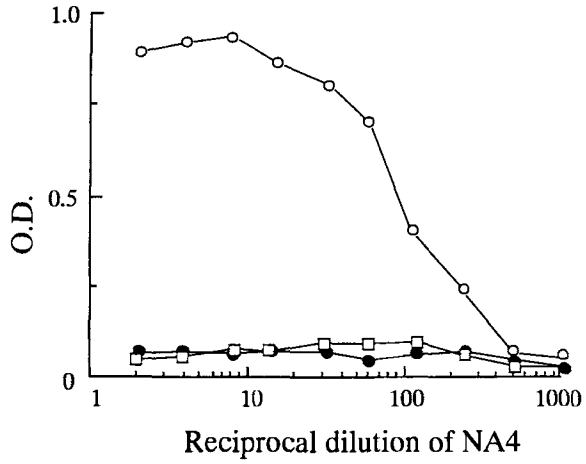

FIGURE 1. Reactivity of NA4 mAb with GST-MNSFβ. NA4 purified by gel filtration chromatography was tested for binding to GST-MNSFβ. Dilutions of the Ab were added to wells coated with GST-MNSFβ (○), GST (●) and ubiquitin (□) and the reactivity was determined by ELISA as described under Materials and Methods.

Fig. 3 shows the results of blotting analysis using NA4 labeling. GST-Ubi-L and GST on the nitrocellulose membrane were stained by amide black (lanes B and C). Lanes D and E show immunostaining of GST-Ubi-L. In the case of lane E, GST was partially cleaved from Ubi-L by the use of thrombin before the application to SDS/PAGE. NA4 detected 8 Kd Ubi-L but not 25.5 Kd GST, confirming that this mAb specifically reacted with Ubi-L antigen. Intact GST-Ubi-L band migrated at the position of 33.5 Kd was also detected (lane E). NA4 did not react with GST (lane F). Special emphasis was placed on testing the reactivity of NA4 with ubiquitin, because the amino acids of Ubi-L present a 36% similarity with ubiquitin (2). NA4 failed to react with ubiquitin (1 μg; lane G), indicative of the specificity of NA4. We next investigated whether NA4 mAb recognizes hybridoma-derived 70-Kd MNSF. NA4 could detect the authentic 70 Kd-MNSF (lane H), implying that Ubi-L is a component of native MNSF as previously described (2,3). As most often IgM monoclonal

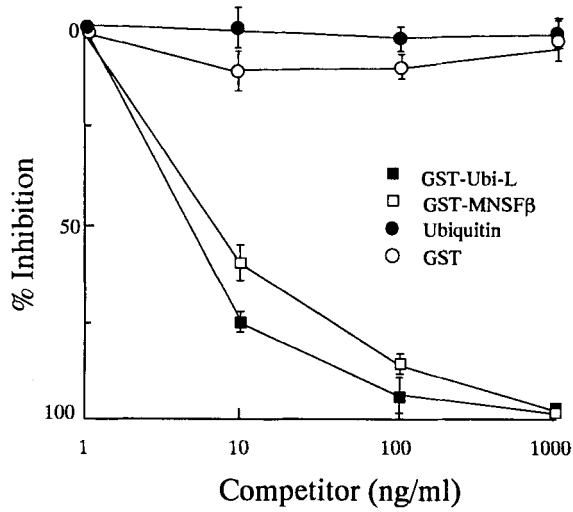

FIGURE 2. Cross-reactivity of NA4 with various proteins. NA4 (80 ng/ml) was incubated with various amounts of antigens (1 to 1,000 ng/ml), and then the residual reactivity of the antibody with MNSFβ coated on ELISA plate was determined as described under Materials and Methods.

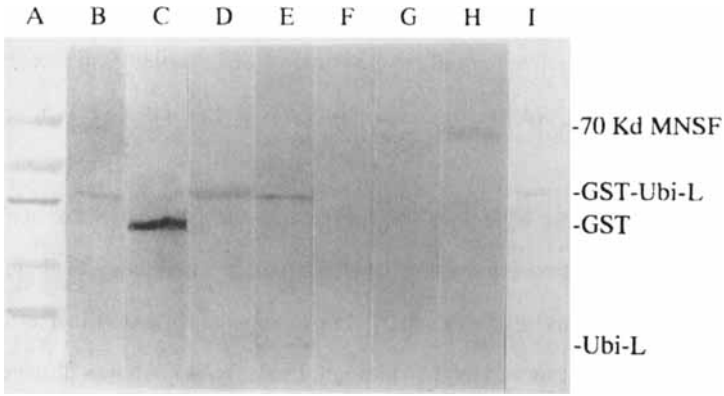

FIGURE 3. Immunoreactivity of NA4 with Ubi-L on immunoblots. Purified GST-Ubi-L was separated on 15% SDS-polyacrylamide gels. The proteins were blotted on nitrocellulose membranes and were allowed react with NA4. The membrane was incubated with the biotinylated sheep anti-mouse Ig, then with the streptavidin-biotinylated peroxidase complex. Antigenic bands were visualized by incubation with diaminobenzidine (lanes D to H). Lanes A, B and C were stained with amide black. Lane A contained the molecular weight standards: lanes B and D, the purified GST-Ubi-L; lanes C and F, GST; lane E, GST-Ubi-L treated with thrombin; lane G, ubiquitin; lane H, authentic 70 Kd-MNSF; lane I, crude Ubi-L recombinant preparations.

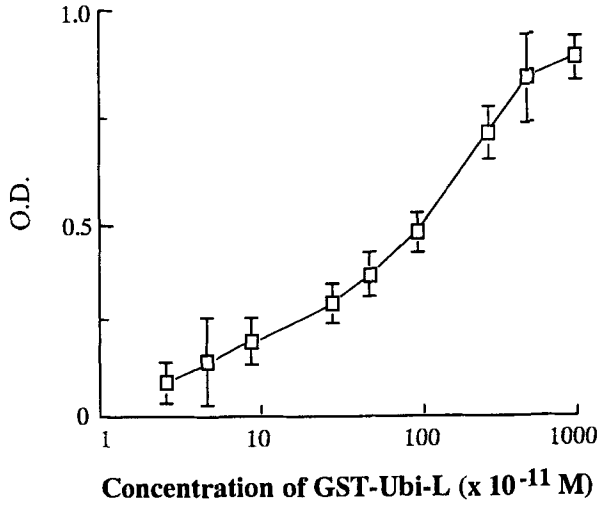

FIGURE 4. Sandwich ELISA for Ubi-L. NA4 mAb were coated onto ELISA plates and then various amounts of GST-Ubi-L (1 to 1, 000 $x10^{-11}$ M) were added. After incubation, the plates were incubated with the anti-PU1 Ab. The peroxidase-conjugated goat affinity purified Ab to rabbit IgG was used for the third Ab as described under Materials and Methods.

antibodies are polyreactive we tested NA4 on crude Ubi-L recombinant preparations. As can be seen in lane I, NA4 recognized only GST-Ubi-L, suggestive of no reaction with other proteins.

In a previous study, we have developed polyclonal anti-PU1 Ab, raised against a synthetic internal 12-amino acid peptide of Ubi-L with low homology to ubiquitin (8). The availability of the anti-PU1 Ab and NA4 mAb allowed us to establish an ELISA to facilitate the quantitation of Ubi-L in biologic fluids. NA4 mAb was utilized as the capture antibody and the anti-PU1 Ab as the detecting reagent as described under Materials and Methods. Fig. 4 shows the representative standard curve obtained with recombinant GST-Ubi-L as a standard. The ELISA displayed a minimum sensitivity of 30 pM of GST-Ubi-L protein. To show the precision of the ELISA, recovery tests

TABLE 1. Validation of the sandwich ELISA*

GST-Ubi-L added (ng/ml)	GST-Ubi-L detected** (ng/ml)
1	0.8±0.2
5	5.5±1.1
10	11.3±1.7
50	48.8±3.3

*Various amounts of GST-Ubi-L were added to the medium containing 10% FBS and detected by the ELISA.

**The results represent the mean ± SD of three separate experiments.

TABLE 2. Determination of MNSF in the supernatant of Con A or IFNγ-activated splenocytes.

Factors*	GST-Ubi-L (pM)	MNSF (ng/ml)**
Medium	< 50	< 5
Con A (3 μg/ml)	610±40	43
IFNα (100 U/ml)	< 50	< 5
IFNβ (100 U/ml)	< 50	< 5
IFNγ (20 U/ml)	530±50	37
LPS (20 μg/ml)	< 50	< 5
IL-2 (50 U/ml)	< 50	< 5

*BALB/c splenocytes ($1x10^8$ cells) were incubated with or without factors for 48 hr, and culture supernatant was harvested.

**Ubi-L in the supernatant was measured by the developed ELISA, and then the amount of MNSF was converted.

were performed. As shown in Table 1, various concentration of GST-Ubi-L added to the medium containing 10% FBS could be detected precisely. The validity of this assay was also established by demonstrating that the Ubi-L levels in supernatants of different cell types such as Con A or IFNγ activated splenocytes could be quantitated by the ELISA. 610±40 and 530±50 pM Ubi-L was detected in the supernatant of Con A (3 μg/ml) or IFNγ (20 U/ml) -stimulated splenocytes, respectively (Table 2). These

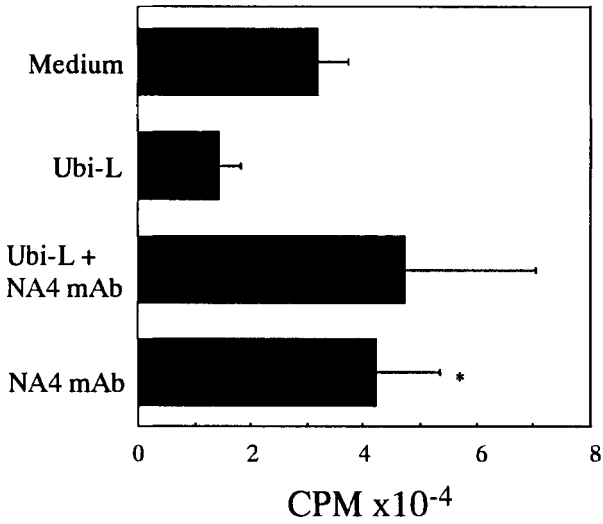

FIGURE 5. The effect of NA4 mAb on Ubi-L action. Splenocytes ($2x10^5$/well) were stimulated with 3 μg/ml of Con A for 72 hr in the presence or absence of Ubi-L (20 U/ml). In neutralizing tests, NA4 (100 μg/ml) and Ubi-L were added at the initiation of the culture. The proliferative response was estimated by pulsing [^{3}H]thymidine (1 μCi/well), in the last 16 hr. Incorporation of thymidine was measured by liquid scintillation counting techniques. *Significantly different from controls at $p< 0.07$.

values are converted into 43 and 37 ng/ml of 70 Kd-MNSF in good agreement with the previous observations that Con A and IFNγ can induce MNSF (1) probably because IFNγ is produced during immune reactions by Con A-activated T cells (9). Thus, the ELISA using NA4 will replace the bioassay for MNSF activity. We also investigated whether other types of IFNs might be involved in MNSF production. As shown in Table 2, a saturating amount of neither IFNα nor IFNβ could induce Ubi-L in the culture supernatants. Ubi-L amounts detected in the supernatants of LPS-, IL-2-stimulated or unstimulated splenocytes were less than 50 pM.

We next examined the possibility of NA4 mAb to abrogate Ubi-L activity. As can be seen in Fig. 5, NA4 remarkably neutralized the action of Ubi-L on the proliferation of Con A-activated splenocytes, suggesting that this mAb may recognize the active

site of Ubi-L. Mouse monoclonal IgM did not affect the action of Ubi-L (data not shown). It should be noted that NA4 alone caused a significant augmentation of the proliferation.

DISCUSSION

A problem in research on lymphokines is that bioassays are often required for purposes of quantitation and identification. As these assays are rather tedious, we were motivated to develop simpler and more rapid assay system useful in evaluating the presence or absence of MNSF in various biological samples. A number of mAb have been used for development of sensitive immunoassays to replace bioassays (10-12). We attempted to develop mAb toward various epitope domains of MNSFβ, a subunit of 70 Kd-MNSF that consists of 8 Kd ubiquitin-like segment (Ubi-L) coexpressed with the ribosomal protein S30 (6.5 Kd). We demonstrated that one mAb, designated NA4, recognizes Ubi-L responsible for MNSF activity (3) and is employable for a sandwich ELISA. This ELISA system revealed that Con A or IFNγ activated splenocytes produce MNSF. The finding that IFNα could not induce MNSF is interesting, because both IFNα and IFNγ show similar synergic action with Ubi-L (13). Despite repeated attempts, mAb to ribosomal protein S30 could not be developed. With the use of rat myeloma cell line, we were under way to isolate mAb to the other epitope domains of MNSFβ.

Ubiquitin, a highly conserved 76-amino acid protein present in all eukaryotic cells is involved in the degradation of short-lived or structurally abnormal proteins (14). Conjugation of ubiquitin (ubiquitination) to cellular proteins plays a role in a variety of cellular processes such as apoptosis (15), DNA repair (16), and cell cycle control (17). In addition, ubiquitin may have immunoregulatory functions (3, 18). NA4 mAb does not recognize ubiquitin (Fig. 1, 2 and 3), indicative of the irrelevance of ubiquitin in our ELISA system.

In this study, GST-Ubi-L was used for standard, because 8 Kd-Ubi-L tends to self-aggregate. After cleavage of Ubi-L from GST by the treatment of thrombin, Ubi-L forms self-aggregates during incubation. Therefore GST-Ubi-L was partially treated (10 min at 20 °C; Fig. 3). It is possible that the self-aggregation due to its strong hydrophobicity, results in a change of its antigenicity, and/or the decrease of Ubi-L's activity. Alternatively, Ubi-L might conjugate to itself in a similar way for polyubiquitination. The aggregation and the association of Ubi-L to other component(s) are common characteristics among suppressor factors (19-22). We have previously generated and characterized mAb (designated MO6) to T cell hybridoma (E17) derived-MNSF. MO6 fails to recognize MNSF β (2), suggesting that MO6 may react with the other subunit, MNSFα (23). In this context, MNSFα seems to be responsible for the stability as well as the secretion of Ubi-L. Therefore, a combination of both MO6 and NA4 mAbs could provide some insight into the structure of MNSF. In a previous study, anti-PU1 Ab was made against synthetic peptides, termed PU1, representing Ubi-L (2). NA4 mAb might recognize an epitope domain other than the PU1 region, because NA4 does not cover this domain when used as a first Ab for ELISA (Fig. 4), and because NA4 does not react with the PU1 peptide on ELISA plate (data not shown). Both NA4 and anti-PU1 Abs may be useful for epitope analysis of Ubi-L by two-dimensional electrophoresis. NA4 mAb can neutralize MNSF activity (Fig. 5), thereby indicating that this mAb recognizes the active site of Ubi-L. It is interesting that NA4 alone did augment the proliferation of Con A-activated splenocytes, presumably because this mAb might inhibit the action of Con A-induced MNSF, i.e. Ubi-L. Supporting evidence for this notion is the fact that NA4 does not augment the proliferation of LPS-activated splenocytes (data not shown). Most recently, we observed that NA4 neutralizes the effect of Ubi-L *in vivo* (unpublished data). Therefore, NA4 might be useful for investigation of Ubi-L action

in vivo as well as *in vitro*. Further investigations with the use of NA4 mAb are underway to clarify the mode of action of MNSF.

Correspondence: Morihiko Nakamura, Department of Biochemistry, Shimane Medical University, Izumo 693, Japan.

REFERENCES

1. Nakamura M Ogawa H and Tsunematsu T. Characterization of monoclonal nonspecific suppressor factor (MNSF) with the use of a monoclonal antibody. J. Immunol. 1987; 138: 1799-03.

2. Nakamura M. Xavier R.M. Tsunematsu T. and Tanigawa Y. Molecular cloning and characterization of a cDNA encoding monoclonal nonspecific suppressor factor. Proc. Natl. Acad. Sci. USA 1995; 92: 3463-67.

3. Nakamura M Xavier R.M and Tanigawa T. Ubiquitin-like moiety of the monoclonal nonspecific suppressor factor β is responsible for its activity. J. Immunol 1996; 156:533-38.

4. Xavier R.M, Nakamura M. and Tsunematsu T. Isolation and characterization of a human nonspecific suppressor factor from ascitic fluid of systemic lupus erythematosus:Evidence for a human counterpart of the monoclonal nonspecific suppressor factor and relationship to the T cell receptor α-chain. J. Immunol. 1994; 152:2624-32.

5. Laemmli U.K. Cleavage of the structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227:680-5.

6. Towbin H Staehelin T and Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some application. Proc. Natl. Acad. Sci. USA. 1979; 76, 4350-4.

7. Glass W.F Briggs R.C and Hnilica L.S. Identification of tissue-specific nuclear antigens transferred to nitrocellulose from polyacrylamide gels. Science 1981; 211: 70-2.

8. Schlesinger D.H Goldstein D. and Niall H.D. The complete amino acid sequence of ubiquitin, an adenylate cyclase stimulating polypeptide probably universal in living cells. Biochemistry 1975; 14: 2214-8.

9. Johnson H.M and Torres B.A. Recombinant mouse interferon-gamma regulation of antibody production. Infect. Immun. 1983; 41: 546-8.

10. De Groote D. Marchant A. Fauchet F. et al. Characterization of monoclonal antibodies against human interleukin-10 and their use in an ELISA for the measurement of this cytokine. J. Immunol. Method 1994; 177: 225-34.

11. Asseman C. Pancre V. Delanoye A. Capron A. and Auriault C. A radioimmunoassay for the quantification of human ubiquitin in biological fluids: application to parasitic and allergic diseases. J. Immunol. Method 1994; 173: 93-101.

12. Ohkaru Y. Asayama K. Ishii H. et al. Development of a sandwich enzyme-linked immunosorbent assay for the determination of human heart type fatty ac d-binding protein in plasma and urine by using two different monoclonal antibodies specific for human heart fatty acid-binding protein. J. Immunol. Method 1995; 178: 99-111.

13. Nakamura M, Nagata T, Xavier R.M, Tanigawa T. Ubiquitin-like polypeptide inhibits the IgE response of lipopolysaccharide-activated B cells. Int. Immunol. 1996; 8:1659-65.

14. Hershko, A. Ubiquitin-mediated protein degradation. J. Biol. Chem. 1988; 263:15237-40.

15. Xu C. Meikrantz W. Schlegel R. and Sanger R. The human papilloma virus 16E6 gene sensitizes human mammary epithelial cells to apoptosis induced by DNA damage. Proc. Natl. Acad. Sci. USA. 1995; 92: 7829-33.

16. Jentsch S. MacGrath J.P and Varshavsky A. The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. Nature 1987; 329:131-4.

17. Glotzer M. Murray A.W. and Kirschner M.W. Cyclin is degraded by the ubiquitin pathway. Nature 1991; 349:100-1.

18. Pancre V. Auriault C. Joseph M. Cesbron J.Y. Kusnierz J.P. and Capron A. A suppressive lymphokine of platelet cytotoxic functions. J. Immunol. 1986; 137: 585-91.

19. Dorf M.E. Kuchroo V.K. and Collins M. Suppressor T cells: some answers but more questions. Immunol. Today 1992; 13:241-3.

20. Iwata T. Katamura K. Kubo R.T. Grey H.M. and Ishizaka K. Relationship between T cell receptors and antigen-binding factors: production and analysis of in vitro modulating properties. J. Immunol. 1989; 143: 3917-24.

21. Fairchild R.L. Kubo R.T. and Moorhead J.W. DNP-specific/class I MHC-restricted suppressor molecules bear determinants of the T cell receptor alpha- and beta-chains. The V beta 8+ chain dictates restriction to either K or D. J. Immunol. 1990; 145, 2001-9.

22. Zheng H. Boyer M. Fotedar A. Singh B. and Green D.R. An antigen-specific helper T cell hybridoma produces an antigen-specific suppressor inducer molecule with identical antigenic fine specificity. J. Immunol. 1988; 140:1351-8.

23. Nakamura M. Ogawa H. and Tsunematsu T. Characterization of N-terminal amino acid sequence of monoclonal nonspecific suppressor factor. Cell Immunol. 1992; 139:139-44.