Production of monoclonal antibodies to islet cell surface antigens using hybridization of spleen lymphocytes from non-obese diabetic mice

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Summary. Non-obese diabetic mice display a syndrome with dramatic clinical and pathological features similar to those of Type 1 (insulin-dependent) diabetes in man. Circulating auto-antibodies to the surface of islet cells were demonstrated in some of these mice by a protein A radioligand assay. To produce monoclonal antibodies to islet cell surface antigens, therefore, we took the spleens of non-obese diabetic mice, transferred the spleen cells into non-immunized recipient mice, which were made immunologically incompetent by a large dose of X-irradiation, and then fused their lymphocytes with FO mouse myeloma cells. After screening the resultant hybrids, one stable hybridoma (3A4) that produced a monoclonal antibody (IgG1) specifically bound to the surface of islet cells was obtained. The purified monoclonal antibody was bound to the surface of transplantable Syrian golden hamster insulinoma cells sevenfold more than control antibody. Adsorption of the antibody on mouse spleen lymphocytes or

thymocytes resulted in only a slight decrease in ^{125}I -protein A binding to insulinoma cells. This antibody also reacted with the surface of mouse and rat islet cells, but not with that of rat spleen cells or hepatocytes. A spectrophotometric assay for peroxidase activity demonstrated that six times more peroxidase bound to insulinoma cells incubated with the antibody than to cells treated with control antibody. Furthermore, this antibody could be visually detected in the immunoenzymatic labelling of the surface of insulinoma cells. In summary, we have developed a novel method of producing monoclonal antibodies to the surface of islet cells for probing into the pathogenesis of Type 1 diabetes.

Key words: Type 1 diabetes, autoimmunity, islet cell surface antibodies, non-obese diabetic mice, cell fusion, monoclonal antibodies, protein A radioassay, insulinoma cells.

The development of Type 1 diabetes often follows pathological processes that result in the destruction of the pancreatic B cells. Although its pathogenetic mechanism is not understood, the demonstration of insulinitis, [1, 2] and autoantibodies specific for pancreatic islet cells [3–5], has led to increased interest in possible autoimmune participation in Type 1 diabetes. The role of islet cell surface antibodies (ICSA) in the destruction of the pancreatic islets remains to be clarified. However, ICSA appear to be an important tool for elucidating the mechanism of both the aetiology and mechanisms of Type 1 diabetes. Studies defining the significance of ICSA have been relatively few because of the limited availability of the antibody. Eisenbarth et al., however, have reported the production of monoclonal antibodies that bind to surface antigens of islet cells by using mice immunized with intact cultured rat islet cells [6].

The study of animals displaying a spontaneous diabetic syndrome has contributed significantly to the understanding of Type 1 diabetes in man. However, to

date, most such animals have corresponded more to Type 2 (non-insulin-dependent) diabetes, demonstrating obesity as well as hyperglycaemia. Recently, non-obese diabetic (NOD) mice were established by Maki-no et al. in Japan. In 1974, a female mouse spontaneously exhibiting glycosuria accompanied with rapid weight loss was discovered in the CTS strain which was characterized by cataractous eyes and derived from ICR mice. After more than 20 generation of sister-brother mating, NOD mice were established in 1980 [7, 8]. NOD mice as well as BB Wistar rats [9] display a syndrome with dramatic clinical and pathological features similar to those of Type 1 diabetes in man. Since NOD mice suffer from severe insulinitis for long periods [7], circulating ICSA may be demonstrated in the serum of these mice.

In the present study, we have developed a method of producing monoclonal antibodies to islet cell surface antigens using ICSA-positive NOD mice.

Materials and methods

Animals

Female NOD mice (aged 22–28 weeks) were supplied by the Shionogi Research Laboratory, Osaka, Japan, and fed standard mouse chow and water ad libitum. Female ICR mice, female BALB/c mice and male Wistar rats were purchased from Clea, Osaka, Japan. Diabetic NOD mice were selected by testing for glycosuria with Tes-Tape (Shionogi Pharmaceuticals, Osaka, Japan). Blood samples were taken from the retro-orbital sinus and the serum was heat-inactivated at 56°C for 30 min.

Cells

FO cells are azaguanine-resistant BALB/c mouse myeloma, which were developed by de St. Groth and Scheidegger [10] and were supplied by Dr. R. A. Roth, Mount Zion Hospital and Medical Center, San Francisco, California, USA. Cultured insulinoma cells (In-111) are derived from the transplantable Syrian golden hamster insulinoma [11] and were supplied by Dr. S. Uchida, Department of Viral Infection, Institute of Medical Science, University of Tokyo, Japan. Cell lines were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum and supplemented with glutamine (1 mmol/l), sodium pyruvate (2 mmol/l), penicillin (50 U/ml), streptomycin (50 µg/ml) and 2-mercaptoethanol (50 µmol/l, Wako Pure Chemical Industries, Osaka, Japan). Serum and other supplements were obtained from Flow Laboratories, McLean, Virginia, USA. Normal mouse and rat islet cells were isolated by standard procedures [12, 13] with minor modifications. Briefly, pancreas from ICR mice or Wistar rats were digested with collagenase (Boehringer Mannheim, Mannheim, FRG), and the islets were dissociated into single cells by incubation with RPMI 1640 medium with 10 mmol/l Hepes containing 1% bovine serum albumin (BSA), 2 mmol/l EDTA and 100 U/ml dispase (bacterial neutral protease from *Bacillus polymyxa*, Godo Shusei, Tokyo, Japan) [14] for 30 min at 24°C. The islet cells were cultured in RPMI 1640 medium with 10% fetal calf serum at 37°C overnight. Spleen lymphocytes and thymocytes from NOD or ICR mice were prepared by chopping with fine scissors, and the cells were suspended in RPMI 1640 medium. Rat hepatocytes were isolated by the method of Seglen [15].

Hybridoma production

The method used involved the transfer of spleen lymphocytes from donor NOD mice into non-immunized recipient ICR mice which were made immunologically incompetent by a large dose of X-rays [16]. Ten female ICR mice used as recipients were exposed to total body X-irradiation of 600 rads 8 days before cell fusion. Four hours after irradiation, $5\text{--}8 \times 10^7$ spleen lymphocytes obtained from each of 10 NOD non-diabetic mice were injected into the tail vein of each of the 10 irradiated ICR mice. On the day before fusion, the serum of each irradiated mouse was tested for its ability to bind to the surface of In-111 cells as described below, and one mouse was selected for the cell fusion.

On the day of the fusion, the spleen lymphocytes from this mouse were fused to FO myeloma cells using polyethylene glycol 4000 (Sigma Chemicals, St. Louis, Missouri) according to the method of de St. Groth and Scheidegger [10]. The products of the fusion were aliquoted into 10, 24-well culture plates at 2×10^5 spleen cells, 2×10^5 FO cells and 2×10^4 mouse macrophages per well. When the hybrid cells were grown and semi-confluent, the medium was removed and assayed for antibody reacting with the surface of In-111 cells. Cells from the positive wells were cloned by limiting dilution and grown by mass culture or as ascites tumours in BALB/c mice primed with pristane (Aldrich Chemicals, Milwaukee, Wisconsin). The monoclonal antibodies from the positive hybrids were purified on a protein A-Sepharose column (Pharmacia Fine Chemicals, Piscataway, New Jersey) [17], and their immunoglobulin classes were identified by the use of

rabbit anti-mouse immunoglobulins (Miles Laboratories, Elkhart, Indiana) in Ouchterlony double immunodiffusion. Protein concentrations of purified monoclonal antibodies were measured by the method of Lowry et al. [18], using BSA as standard.

Protein A radioligand assay

Islet cell surface bound antibodies were determined by an ^{125}I -protein A assay as described by Lernmark et al. [19]. Briefly, various living cells in RPMI 1640 with 2% BSA were incubated first with mouse serum at 1:10 dilution and supernatant of hybridomas or various concentrations of purified antibodies for 30 min at 24°C. After washing four times by centrifugation, the cell pellets were incubated with ^{125}I -protein A ($0.7\text{--}1.4 \times 10^5$ cpm/tube, New England Nuclear, Boston, Massachusetts; spec act: 90–95 Ci/g) for 30 min at 24°C. The cells were washed four times with 2% BSA in RPMI 1640, and finally the radioactivity in the cell pellets was counted in a gamma counter (Aloka, Tokyo, Japan).

For the adsorption of purified monoclonal antibody, aliquots of antibody were resuspended with various cell numbers of mouse spleen lymphocytes, thymocytes or islet cells and incubated for 16 h at 4°C. To equalize the influence of collagenase and dispase on cell membranes, spleen lymphocytes and thymocytes also were exposed to these enzymes during their isolation. The antibody was retrieved by centrifugation (10000 g for 5 min) and tested for the ability to react with the surface of In-111 cells as described above.

Immunoenzymatic labelling of islet cell surface antigens on In-111 cells

Various concentrations of purified antibody or normal mouse IgG (Sigma), which served as control, were added to In-111 cells (2×10^6 cells/ml) and incubated for 30 min at 24°C. After incubation, the cell suspension was centrifuged at 50 g for 10 min, and the cell pellets were washed twice with 10 mmol/l phosphate buffered saline (PBS, pH 7.4) and resuspended in galactosidase-linked goat anti-mouse immunoglobulin (BRL, Gaithersburg, Maryland) at a final dilution of 1:200 in PBS with 1% BSA. After incubation for 60 min at 24°C, the cells were centrifuged and washed as described above. The cell pellets were resuspended in buffer containing 3 mmol/l o-nitrophenyl- β -D-galactopyranoside (Sigma), a chromogenic substrate for galactosidase, and 0.18 mmol/l mercaptoethanol [20]. The enzymic reaction was allowed to proceed for 20 min at 24°C and then was stopped by cooling the reaction tubes at 4°C and centrifuging the cells. Na_2CO_3 (0.15 mol/l) was added to the supernatant, and the absorbance was read at 420 nm.

Staining of In-111 cells labelled with peroxidase-linked antoglobulin

To visualize the monoclonal antibody bound to the surface of In-111 cells, a histochemical staining for peroxidase activity was utilized [21]. In-111 cells (5×10^5 cells/50 µl) were incubated with 5 µg of purified monoclonal antibody or normal mouse IgG for 30 min at 4°C. After the cells were washed, peroxidase-linked rabbit anti-mouse IgG (Dako, Denmark) diluted at 1:2 was added to the cells and incubated for 15 min at 4°C. After washing four times, the cells were resuspended in 15 mmol/l Hepes (pH 7.4) containing 0.15 mol/l NaCl, 5 mmol/l KCl and 1 mg/ml gelatin, and the cell suspensions were applied to a microscope slide precoated with poly-L-lysine (Sigma). After removal of the unbound cells, a solution containing 1.4 mmol/l 3,3'-diaminobenzidine tetrahydrochloride (Aldrich) and 0.12 µl/ml H_2O_2 was added and left for 10 min at 24°C. After the cells were washed five times, 2% OsO_4 (Sigma) was added, and the cells were incubated in the dark for 20 min at 4°C. The cells were washed and fixed with 0.025% glutaraldehyde in 50% glycerin and examined under a light microscope.

Table 1. Binding of mouse serum to In-111 cells by protein A radioligand assay

Mice	No.	¹²⁵ I-Protein A radioactivity (% of total)	ICSA	Mice	No.	¹²⁵ I-Protein A Radioactivity (% of total)	ICSA
NOD non-diabetic (<i>n</i> = 15)	1	1.03	(-)	ICR transferred NOD spleen cells (<i>n</i> = 10)	1	1.31	(-)
	2	3.83	(+)		②	4.85	(+)
	3	0.90	(-)		3	0.98	(-)
	4	0.84	(-)		4	1.08	(-)
	5	3.28	(+)		5	0.92	(-)
	6	0.81	(-)				
	7	0.93	(-)				
	8	0.92	(-)				
	9	0.93	(-)				
	10	0.92	(-)				
	11	2.52	(+)		6	0.75	(-)
	12	2.06	(+)		7	2.23	(+)
	13	1.26	(-)		8	0.98	(-)
	14	0.83	(-)		9	0.72	(-)
	15	1.11	(-)		10	1.09	(-)
NOD diabetic (<i>n</i> = 5)	1	0.71	(-)				
	2	0.93	(-)				
	3	1.13	(-)				
	4	0.98	(-)				
	5	3.78	(+)				
ICR non-treated (<i>n</i> = 5)		0.83 ± 0.09		ICR radiated (<i>n</i> = 5)		0.74 ± 0.08	

Values for ICR non-treated mice are expressed as mean ± SD. ¹²⁵I-protein A ($1-1.4 \times 10^5$ cpm) was added to 2×10^5 In-111 cells that had been pre-incubated with a 1:10 dilution of mouse serum. Results are expressed as the percentage bound of total radioactivity. Bound radioactivity exceeding twofold the mean control value defined ICSA-positive serum

Fig. 1. Screening of hybridomas by the protein A radioligand assay. The binding of supernatants to 2×10^5 In-111 cells was measured with ¹²⁵I-protein A (1×10^5 cpm). Results from positive hybridomas and negative controls are expressed as mean + SD of triplicate determinations. A representative experiment of the three experiments performed is shown

Results

Hybridoma production

Twenty female NOD mice (aged 22-28 weeks) were divided into 15 non-diabetic and five diabetic mice by testing for the presence of glycosuria. Sera from these mice were evaluated for the presence of ICSA by the protein A radioligand assay. In four of the 15 non-diabetic and one of the five diabetic mice, the binding of ¹²⁵I-protein A to In-111 cells exceeded the mean of that

in non-treated ICR mice twofold ($0.83 \pm 0.09\%$; Table 1). Eight days before fusion, each of the 10 spleens obtained from 10 non-diabetic NOD mice (nos. 1-5 and 11-15) was transferred into each of the 10 recipient ICR mice (nos. 1-10), which had previously been exposed to X-irradiation. On the day before fusion, two transferred mice (nos. 2 and 7) showed more than double the values of ¹²⁵I-protein A radioactivity of the ICR irradiated control mice ($0.74 \pm 0.08\%$). In transferred mice (nos. 5 and 6), which received spleen lymphocytes from ICSA-positive NOD mice (nos. 5 and

Fig. 2. Binding of purified monoclonal antibodies to In-111 cells by the protein A radioligand assay. In-111 cells (2×10^5) were first incubated with the indicated amounts of either 3A4 (●), 4A1 (▲) or normal mouse IgG (○) and the binding assayed with ^{125}I -protein A (1.2×10^5 cpm). Results are expressed as mean $\pm$ SD of three different experiments

Fig. 3. Quantitative adsorption of purified monoclonal antibody 3A4 with mouse spleen lymphocytes and islet cells. 3A4 (100 $\mu\text{g}/\text{ml}$) was adsorbed with the indicated cell numbers of either spleen cells (●) or islet cells (■) and the binding of adsorbed antibody to 2×10^5 In-111 cells was estimated with ^{125}I -protein A (0.7×10^5 cpm). The bindings of ^{125}I -protein A to In-111 cells after incubation with unabsorbed antibody (○) or with normal mouse IgG (100 $\mu\text{g}/\text{ml}$) (△) and of mouse IgG adsorbed with spleen lymphocytes (▲) are shown also. Results are the mean values of two or three different experiments

11), however, the binding of ^{125}I -protein A to In-111 cells showed normal values in samples taken 7 days after the transfer of spleen lymphocytes. Since the radioactivity in transferred mouse no.2 was nearly seven times higher than that in irradiated control mice, we chose this mouse for the cell fusion. Fourteen days after fusion, 24 of the 240 culture wells showed growth of hybridomas. The results of a screening assay by ^{125}I -protein A binding to In-111 cells are shown in Figure 1. Significant antibody binding, which was more than twice the mean of binding with negative control FO supernatant (838 ± 75 cpm/ 2×10^5 cells), was found in only two hybridomas (3A4; 4796 ± 98 cpm and 4A1; 1962 ± 67 cpm). These two hybridomas were grown in

the mass culture and as ascites tumours, and the monoclonal antibodies were purified by protein A-Sepharose. Both antibodies were identified as an IgG1 by Ouchterlony double immunodiffusion. Monoclonal antibody purified from 3A4 has been mainly used for further investigations.

Binding of ^{125}I -protein A

Serial dilutions of purified antibody 3A4 were incubated with In-111 cells and the binding of antibody detected with ^{125}I -protein A. The binding of antibody to In-111 cells was increased in proportion to increasing concentrations of antibody, and the binding of ^{125}I -protein A to In-111 cells incubated with 100 $\mu\text{g}/\text{ml}$ of antibody 3A4 was sevenfold that of the cells incubated with the same amount of normal mouse IgG. Purified antibody 4A1 was bound about threefold more than control IgG to In-111 cells (Fig. 2).

Adsorption of the purified antibody 3A4 to mouse spleen or islet cells is shown in Figure 3. The adsorption curve for spleen lymphocytes declined gradually and reached an apparent maximum with 2×10^7 cells. On the other hand, ^{125}I -protein A binding to In-111 cells decreased from 7.5% to 1.2% (almost the same percentage as that shown for the non-specific binding of normal mouse IgG) by the adsorption of 3A4 to mouse islet cells at 7×10^5 cells.

The effect of mouse thymocytes at concentrations of 1.5 – 25×10^6 cells on the adsorption of 3A4 was similar to that of spleen lymphocytes (data not shown). Direct binding of 3A4 to various living cells is shown in Figure 4. The binding of ^{125}I -protein A to In-111 cells incubated with 100 $\mu\text{g}/\text{ml}$ of antibody 3A4 was 19331 ± 1582 cpm/ 2×10^5 cells compared with 1565 ± 203 cpm/ 2×10^5 cells when incubated with 100 $\mu\text{g}/\text{ml}$ of normal mouse IgG. At the same concentrations of 3A4 and normal mouse IgG, the binding to mouse and

Fig. 4. Binding of purified monoclonal antibody 3A4 to the surface of various cells by the protein A radioligand assay. Antibody 3A4 (100 µg/ml) (■) or normal mouse IgG (100 µg/ml) (□) was incubated with either 2×10^5 In-111 cells, 3×10^4 mouse islet cells, 1.5×10^4 rat islet cells, 2×10^5 rat hepatocytes or 2×10^6 rat spleen cells. The binding was assayed with ^{125}I -protein A (1.2×10^5 cpm) and the results are expressed as mean + SD of triplicate determinations. ** $p < 0.001$, * $p < 0.01$

Fig. 5. Immunoenzymatic labelling of In-111 cells with purified monoclonal antibody 3A4. In-111 cells were incubated with the indicated amounts of either 3A4 (●) or normal mouse IgG (○) and then with a galactosidase-linked second antibody, and assayed spectrophotometrically. One experiment representative of three is shown

Fig. 6. Visual detection of islet cell surface antigens on In-111 cells. In-111 cells were incubated with either normal mouse IgG (A) or purified antibody 3A4 (B) and treated with a peroxidase-linked second antibody and 3,3'-diamino-benzidine tetrahydrochloride. One experiment representative of four is shown

rat islet cells incubated with 3A4 was 6089 ± 146 cpm/ 3×10^4 cells and 4780 ± 611 cpm/ 1.5×10^4 cells compared with 1178 ± 110 cpm/ 3×10^4 cells and 1563 ± 203 cpm/ 1.5×10^4 cells incubated with control mouse IgG, respectively. However, the binding to rat hepatocytes (2×10^5) and spleen cells (2×10^6) showed no significant difference between 3A4 and normal mouse IgG.

Immunoenzymatic labelling of surface of In-111 cells

Either purified monoclonal antibody 3A4 or normal mouse IgG was incubated with In-111 cells and binding

to the cells was quantified using a second antibody coupled to galactosidase. With increasing quantities of antibody 3A4, increased galactosidase activity was bound to the cells (Fig. 5). In-111 cells incubated with 100 µg/ml of antibody 3A4 had sixfold more galactosidase activity than cells incubated with the same amount of normal mouse IgG.

To visualize the binding of antibody to the surface of In-111 cells, the histochemical stain for peroxidase activity, 3,3'-diamino-benzidine tetrahydrochloride, was utilized. In-111 cells treated first with normal mouse IgG showed no apparent staining. In contrast, cell sus-

pensions treated with antibody 3A4 contained many stained cells that had a black ring around the cell periphery (Fig. 6).

Discussion

The present study demonstrates that the production of monoclonal antibodies reacting with the surface of islet cells is successfully achieved when spleen cells of NOD mice are fused with FO myeloma cells. Based on the data in Table 1 it was assumed that the production of auto-antibodies to islet cells in NOD mice was present before diagnosis and in fact may be diminished at the time of overt diabetes. Therefore, we selected non-diabetic NOD mice for the production of monoclonal antibodies to islet cell surface antigens. In preliminary experiments, we took the spleens of NOD mice and fused their lymphocytes directly with FO myeloma cells. However, we were unable to obtain hybridoma production from four such fusions. It is difficult to explain why these direct fusions were unsuccessful. We could not produce hybridomas by the fusion of spleen cells from non-immunized mice and FO cells using the same technique as described here, whereas high frequencies of hybridomas were obtained when spleen cells from mice immunized with sheep red blood cells were used (unpublished data). Several lines of indirect evidence suggest that activated B cells fuse preferentially with myeloma cells, or at least result in a higher frequency of viable hybridomas [22]. Mosmann et al. also reported that the total number of hybridomas obtained, using spleen cells from non-immunized mice, was higher after stimulation of spleen cells with lipopolysaccharide and lipopolysaccharide stimulation could increase the frequency of antigen-specific hybridomas from immunized mice [23]. Since preliminary fusions employed spleen lymphocytes from autoimmune mice that were not pre-immunized with antigens, there must have been low numbers of blast lymphocytes in these spleen cells.

Thus, the spleen cells from NOD mice were transferred into heavily irradiated ICR mice. This method is based on the fact that the intravenous injection of spleen cells into isologous hosts previously exposed to total-body irradiation leads to the formation of colonies of proliferating cells in the spleens of these animals [24]. We could actually observe whitish multiple colonies in the spleens of ICR mice with transferred spleen cells from NOD mice. Under these conditions, the antibody formed in the recipient is derived from donor-primed cells alone, and not from the recipient [16]. As shown in Table 1, ICSEA was demonstrated in two of four ICR mice with transferred spleen cells from ICSEA-positive NOD mice at 7 days after transfusion. The results of fusion using such an *in vivo* culture technique showed that the frequency of hybridomas was considerably lower than that reported for other conventional fusions [6, 25, 26]. These results appear to be due to employing

spleen cells from non-immunized mice for cell fusion, because high frequencies of specific hybridomas are dependent on the increased frequency of specific B-blast cells at the time of fusion these being obtained by frequent immunization with high doses of antigens [23, 27]. Therefore, high frequencies of antigen-specific hybridomas are expected by fusing *in vitro* lipopolysaccharide-stimulated spleen cells from ICSEA-positive NOD mice with myeloma cells and this fusion procedure is now in progress.

Five different experimental approaches indicated that the monoclonal antibody 3A4 was specifically bound to the surface of islet cells. Firstly, purified antibody 3A4 was bound to the surface of cultured cells of transplantable Syrian golden hamster insulinoma, In-111, sevenfold more than control antibody, using the protein A radioligand assay. Secondly, the binding of ^{125}I -protein A to In-111 cells was significantly reduced by adsorption of antibody 3A4 to 7×10^5 mouse islet cells, whereas adsorption of 3A4 to as many as 4×10^7 mouse spleen lymphocytes only slightly decreased the binding of ^{125}I -protein A to In-111 cells. Because both insulin and glucagon are secreted by In-111 cells and their insulin content is almost the same as that of normal hamster pancreas, it is assumed that In-111 cells comprise at least A and B cells [28]. Our result also indicates that In-111 cells may express a large number of surface antigens, including tumour-specific antigens, but share the surface antigens of normal pancreatic islet cells and thus are useful for detecting ICSEA. Thirdly, this antibody also reacted with the surface of mouse and rat islet cells, but not with that of rat hepatocytes or spleen cells. Although ^{125}I -protein A binding to rat hepatocytes showed a high value, indicating non-specific binding or trapping of immunoglobulin to the cells, these results suggest that this antibody binds to the surface of various species of islet cells. Fourthly, a spectrophotometric assay for galactosidase activity demonstrated that galactosidase bound to In-111 cells incubated with antibody 3A4 six times more than to cells incubated with control antibody. And fifthly, antibody 3A4 could be visually detected on the surface of the In-111 cells after incubation with a peroxidase-linked antibody directed against the first antibody and histochemical staining for peroxidase activity.

In summary, we have obtained hybridomas that produce monoclonal antibodies to the surface of islet cells by using hybridization of spleen lymphocytes from non-immunized NOD mice. Since antibody 3A4 specifically binds to islet cell surface antigens, this antibody provides a useful tool for studies on purification of antigens and for clarification of the pathogenesis of Type 1 diabetes. Finally, the method described here should be applicable to other autoimmune disorders to produce monoclonal antibodies against the autoantigens.

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