Anti-activin A Antibody (IgY) Specifically Neutralizes Various Activin A Activities

(43958)

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Abstract. **Activin A ($\beta_A\beta_A$), originally isolated from ovarian follicular fluids as a follicule-stimulating hormone (FSH) secretion stimulator, has also been identified as an erythroid differentiation factor (EDF), a neuron survival factor and a mesoderm-inducing factor. Thus, activin A is a multifunctional factor, and further studies on its physiological function are important. However, it is very difficult to produce a specific antibody to neutralize the activity of activin A because of its highly conserved amino acid sequence across mammalian species. In this study, we succeeded in generating an antibody against activin A, which can neutralize several activities of activin A, such as the stimulation of FSH secretion from pituitary cells and the induction of the differentiation of erythrocytes *in vitro*. This antibody did not affect the activity of activin B ($\beta_B\beta_B$), which induces the differentiation of erythrocytes *in vitro*, and the activity of inhibin A ($\alpha\beta_A$), which inhibits FSH secretion from pituitary *in vitro*, but slightly neutralized that of activin AB ($\beta_A\beta_B$). Western blotting analysis showed that this antibody recognized both dimeric and monomeric forms of the β_A subunit of activin and inhibin. These results suggest that this antibody recognizes the β_A subunit of activin and specifically neutralizes the activity of a dimer of the β_A subunit, activin A. Furthermore, by the addition of this antibody to the culture medium, the development of murine embryos was suppressed, suggesting that endogenous activin A plays an important role in murine development. These results indicate the usefulness of this antibody for studies of endogenous activin actions.** [P.S.E.B.M. 1996, Vol 211]

The activins, sulfhydryl-linked dimers comprised of two subunits (β_A and β_B), are structurally related to a functionally diverse group of growth and differentiation factors known as the transforming growth factor–β(TGFβ) family (1, 2). Activin A, activin AB, and activin B are the homo/heterodimers of subunits in the combination of $\beta_A\beta_A$, $\beta_A\beta_B$, and $\beta_B\beta_B$, respectively. Activin A and activin AB were originally isolated from ovarian follicular fluids as a follicule-stimulating hormone secretion stimulator (3, 4), and activin A ($\beta_A\beta_A$) has been identified as an erythroid differentiation factor (EDF), a neuron survival factor, and a mesoderm-inducing factor (5–7). Thus, activins are multifunctional factors, and further studies on their physiological functions are important.

Activin A is a highly conserved protein in mammals. There is no difference in amino acid sequence of the activin β_A subunit among humans, pigs, cattle, and rats (8). Therefore, it would be very difficult to produce a specific antibody to neutralize the activity of activin A using traditional mammalian hosts, such as

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Received May 12, 1995. [P.S.E.B.M. 1996, Vol 211]
Accepted September 6, 1995.

0037-9727/96/2111-0100$10.50/0

rabbits or mice. Antibodies against synthesized fragments of activin A conjugated with an antigenic protein such as bovine serum albumin (9, 10) or against the mixture of recombinant activin A with polyvinylpyrrolidone (11) are available for immunohistochemical approaches or immunoassays, but any neutralizing effect of these antibodies on activin A activity *in vitro* or *in vivo* has yet to be reported.

For production of a specific antibody against such a conserved mammalian protein, the immunization of an avian species is an alternative and useful approach (12). It is likely that at least part of the amino acid sequence will be different between birds and mammals. Immunoglobulin (Ig) species from the yolk of avian families are termed IgY, because the nature and yield of the avian Igs are slightly different from those of mammals, and antibodies are maternally transferred from serum to the egg yolk to give a protective effect from pathogens to the offspring.

Using this approach we have produced a antibody (IgY) against intact activin A using laying hens. The neutralizing effects of this antibody on activin activities were examined by using the abilities of activin to stimulate FSH secretion from pituitary cells *in vitro* and to induce the differentiation of erythrocytes *in vitro*. Furthermore, by using this antibody, the function of endogenous activin produced in murine embryos at an early stage *in vitro* was examined.

Materials and Methods

Materials. Recombinant human activin A was purified from the culture supernatant of Chinese hamster ovary cells bearing an expression vector for the cDNA of the β_A subunit of activin (5, 13). Activin AB and activin B purified from porcine ovaries were kindly provided by Dr. H. Sugino (Tokushima University, Tokushima, Japan) (14). Inhibin A, a dimer of the β_A subunit of activin and an α subunit, purified from bovine ovarian follicular fluids, was kindly provided by Dr. Y. Hasegawa (Kitasato University, Towada, Japan) (15).

Immunization. Recombinant human activin A was dissolved in saline and emulsified with the same volume of Freund's complete adjuvant (DIFCO, Detroit, MI). This mixture was injected intradermally (1 mg activin A/hen) into the breast of three adult white Leghorn hens, weighing between 1.6 and 2.1 kg (Shiraishi, Koshigaya, Japan). Additional booster injections (0.5 mg activin A/hen) were given at 2-week intervals until the development of a significant titer.

Purification of IgY from Egg Yolk. IgY was purified from egg yolk by the method of Hatta *et al.* (16). Egg yolk was removed and diluted with an equal amount of 20 m*M* phosphate buffer (pH 8.0), and then homogenized briefly. A 2-fold volume of 0.15% λ-carrageenan solution (Wako Chemicals, Osaka, Japan) was added, and the mixture was allowed to settle for 30 min at room temperature, followed by centrifugation at 10,000*g* for 15 min. The supernatant was filtered through filter paper No. 2 (Advantec, Tokyo, Japan) and the filtrate adjusted to pH 8.0 by the dropwise addition of 3 *N* HCl. The solution was poured onto a DEAE-Sephacel column (ϕ5.0 × 10.0 cm; Pharmacia-LKB, Uppsala, Sweden) that had previously been equilibrated with 20 m*M* phosphate buffer (pH 8.0) at a flow rate of 5.0 ml/min. After washing the column with the same buffer (20 volumes of the column), the adsorbed protein was eluted with 0.2 *M* phosphate buffer (pH 8.0). The fractions were collected by monitoring the optical absorbance at 280 nm. Sodium sulfate, anhydrous powder, was added to the eluate at 15% concentration (w/v) at 20°C. The solution was gently stirred for 30 min and then centrifuged at 10,000*g* for 15 min. The resulting precipitate was dissolved in 100 ml of phosphate buffer (100 m*M*, pH 8.0). This salting-out procedure was repeated twice more. The final precipitate was dissolved in 100 ml of phosphate buffer (10 m*M*, pH 8.0) and dialyzed against the same buffer. The dialyzate was filtered through a membrane filter (pore size is 0.45 μm) (Millipore, Bedford, MA). The filtrate was used as the IgY solution for titration and the measurement of neutralizing activity and for Western blot analysis. IgY solutions showing high titer and neutralizing activity were mixed and lyophilized. This lyophilized IgY was dissolved in medium which is suitable for *in vitro* each culture assay.

Enzyme-Linked Immunosorbent Assay for Activin A. Fifteen microliters of 0.1 *M* $NaHCO_3$ containing 500 ng activin A was added to each well of a microtiter plate (Labsystems Japan, Tokyo, Japan) and incubated for 2 hr at 37°C. This plate was then washed twice with phosphate buffered saline with 0.05% Tween 20 (pH 7.4) (PBST). Subsequently, each well was filled with 200 μl of blocking solution (Block Ace; Snow Brand Milk Product, Dainippon Pharm. Co., Ltd., Osaka, Japan) to minimize nonspecific binding of antibodies, then incubated at 4°C overnight. After washing three times with PBST, 50 μl of IgY solution diluted 1:100 in 0.1 *M* $NaHCO_3$ was added to each well, and the plate incubated for 2 hr at 37°C. After washing three times with PBST again, 100 μl of horseradish peroxidase-labeled rabbit anti-chicken IgG solution was added to each well and the plate incubated for 2 hr at 37°C. After washing four times with PBST, the enzyme remaining in individual wells was determined by adding 100 μl of 50 m*M* citrate buffer (pH 5.0) with 4% o-phenylendiamine and 30% H_2O_2. The reaction was stopped by adding 10 μl of 2 *N* H_2SO_4. Absorbance of each well was measured at 492 nm.

Assay for Erythroid Differentiation Factor (EDF) Activity. Serially diluted samples were added to cultures of Friend F5-5 cells (1×10^4 cells/ml) in

Ham's F-12 medium (Gibco-BRL, Gaithersburg, MD) with 10% fetal bovine serum (FBS). For the measurement of the neutralizing activity of IgY solution to activin, the diluted IgY solutions (1:50) containing 2 ng/ml activin A were added to the culture of the Friend cells. After culture for 6 days at 37°C in a humidified incubator containing 5% CO_2, hemoglobin in Friend cells of each well was stained with dianisidine solution (Sigma Chemical Co., St. Louis, MO). Hemoglobin-positive cells (differentiated cells) were counted under a microscope and the percentage of such cells was calculated.

Western Blotting Analysis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 12.5% polyacrylamide gels by the method of Laemmli (17), and both the transfer of proteins to membrane and the reaction of protein with antibodies after electrophoresis were performed as previously described (18). Recombinant human activin A (300 ng) or purified bovine inhibin A (300 ng) was loaded after the incubation with or without 4% (w/v) 2-mercaptoethanol (2ME) for 5 min at 94°C. After electrophoresis and the transfer of proteins to an Immobilon membrane (Millipore, Bedford, MA) with a semi-dry blotting apparatus (BioRad Lab., Richmond, CA), the membranes were blocked with skim milk (Block Ace; Snow Brand Milk Product) for 1 hr, and incubated with anti-activin IgY solution at 1:1500 dilutions with Tris-buffered saline (10 m*M* Tris-HCl, 140 m*M* NaCl, and 0.05% Tween 20) (TBST) at 4°C for 16 hr. The membranes were then washed with TBST for 40 min, and reacted with peroxidase-conjugated anti-chicken IgG antibody for 40 min at room temperature. After washing with TBST for 40 min, the anti-chicken IgG antibody which bound to the anti-activin IgY antibodies on the membrane was visualized by a commercial kit (ECL detection kit, Amersham, United Kingdom) using enhanced chemiluminescence reagent. IgY solution from Hen #2 collected at Day 42 after immunization (#2–42) was used for the anti-activin A IgY solution.

Culture of Anterior Pituitary Cells. Anterior pituitary lobes were collected from 22-day-old female Sprague-Dawley rats (Charles River Japan, Atsugi, Japan) (19) and dispersed enzymatically as previously described (20). The cells were seeded into 24-well tissue plates (Coaster, Cambridge, MA) with Dulbecco's Modified Eagle's medium (DMEM) (Gibco-BRL) supplemented with 10% FBS. On the second day of culture, the medium was removed and samples of activin A or inhibin A with or without anti-activin A IgY solution, dissolved in 1 ml of DMEM with 1% FBS, were added. After 48 hr of incubation, the medium was collected for measurement of FSH by radioimmunoassay using a kit provided by Dr. A. F. Parlow through the National Hormone and Pituitary Program (National Institute of Diabetes and Digestive and Kidney Disease) (20). FSH-RP-2 was used as a standard. The mixture of anti-activin A IgY solution from hen #2 from days 41 to 45 after immunization was used as the anti-activin A IgY solution for this and the following studies. One unit per milliliter is defined as the activity which neutralizes an EDF activity of 1 ng/ml activin A, and 2.5 U/ml or 5 U/ml of anti-activin IgY solutions were added with or without 2.5 ng/ml of activin or inhibin.

Culture of Murine Embryos. Fertilized embryos (15–20 embryos/dish) were collected and cultured as previously described (21). Female C57/BL mice, superovulated by the administration of pregnant mare's serum gonadotropin followed by human chorionic gonadotropin injection (hCG), were mated with C57/BL male mice. Embryos were collected 24 hr after hCG injection and cultured for 72 hr in Whitten's medium containing bovine serum albumin with or without IgY solution in an atmosphere of 5% CO_2 in air at 37°C. After the incubation, the numbers of embryos at stages of two-cell or from four-cell to eight-cell (four- to eight-cell), morula or blastocyst, and degenerated embryos were counted and expressed as the percentage of the total number of embryos cultured in a dish. Two different doses of anti-activin IgY solution (12.8 U/ml and 64 U/ml) were added to culture medium. IgY purified from nonimmunized chick egg was used as control IgY solution, and two different concentrations of control IgY were adjusted to be at the same protein level with those of anti-activin A IgY. The percentage of developed embryos incubated with cultured medium only is indicated as control.

Statistical Analysis. Data were analyzed by unpaired Student's *t* test or by one-way analysis of variance followed by Duncan's test when differences were significant.

Results

Developments of Titer and Neutralizing Effect on Activin Activity. Activin A was administered to three hens at 2-week intervals, and developments of titer and neutralizing activity of anti-activin A IgY to activin A were monitored by enzyme-linked immunosorbent assay (ELISA) and Friend cell assay. Clear increments of both titer and neutralizing activity were observed in anti-activin A IgY solution obtained from Hen #2 around 6 weeks (between 39 and 45 days) after immunization. Generally, the neutralizing activity of the antibodies was coincident with that of titer. In Hen #1 and #3, there were small increases of titer after immunization, and neutralizing activities were much lower than those of Hen #2. When 1 unit/ml of neutralizing activity is defined as the activity which neutralizes an EDF activity of 1 ng/ml activin A, the activity of anti-activin A IgY solution collected from Hen

#2 at Day 42 after immunization (#2–42) was 320 U/ml.

Western Blotting Analysis. Activin A (25–28 kDa) and inhibin A (32 kDa) are a homodimer of β_A subunits and a heterodimer of α and β_A subunits, respectively, under nonreducing conditions. Under reducing conditions, by the addition of 2-mercaptoethanol, activin A is divided into two β_A subunits (14–15 kDa) and inhibin A is divided into β_A and α subunits (18 kDa). Anti-activin A IgY from Hen #2 recognized recombinant human activin A and purified bovine inhibin A in nonreducing conditions at 28 and 32 kDa, respectively, and also recognized monomeric the β_A subunit of activin/inhibin at 15 kDa (Fig. 1). Anti-activin A IgY did not recognize the α subunit of inhibin under reducing conditions. These results indicated that anti-activin IgY from Hen #2 recognized the β_A subunit of activin A ($\beta_A\beta_A$) and inhibin A ($\alpha\beta_A$) in dimeric and monomeric forms using Western blotting analysis.

Cross-Reactivity to Activin AB or Activin B. Cross-reactivity of anti-activin A IgY to activins AB ($\beta_A\beta_B$) and B ($\beta_B\beta_B$) was examined by comparing the neutralizing activity of anti-activin A IgY against activins AB and B with that of activin A (Fig. 2). Differentiation of the Friend cells was induced by activin A, activin AB, and activin B from 0.625 ng/ml to 10 ng/ml of the activins. Maximal differentiation induced by 10 ng/ml of each activin is indicated as 100% differentiation (Fig. 2). The anti-activin A IgY solution (6.4 U/ml) collected from Hen #2 at Day 42 after immunization (#2–42) neutralized activin A ($\beta_A\beta_A$) activity completely at 10 ng/ml, but did not show a neutralizing effect on activin B ($\beta_B\beta_B$). The anti-activin A IgY so-

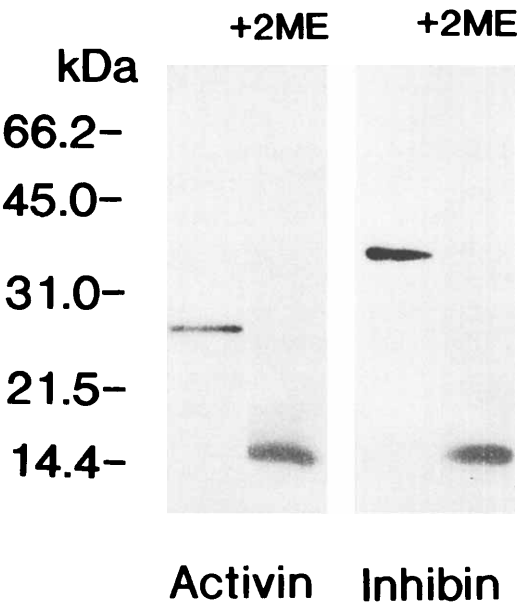

Figure 1. Immunoblotting analysis of activin A with anti-activin A IgY solution from Hen #2. Recombinant human activin A (300 ng) or purified bovine inhibin A (300 ng) was subjected to SDS-PAGE after incubation with or without 4% (w/v) 2-mercaptoethanol (2ME) for 5 min at 94°C, then transferred to an Immobilon membrane and reacted with anti-activin A IgY prepared from Hen #2 (1:1500 dilution). The proteins which reacted with anti-activin A IgY antibodies on the membrane were visualized by a commercial kit (ECL detection kit, Amersham, United Kingdom) using enhanced chemiluminescence reagent. IgY solution from Hen #2 collected at Day 42 after immunization (#2–42) was used for the anti-activin A IgY solution.

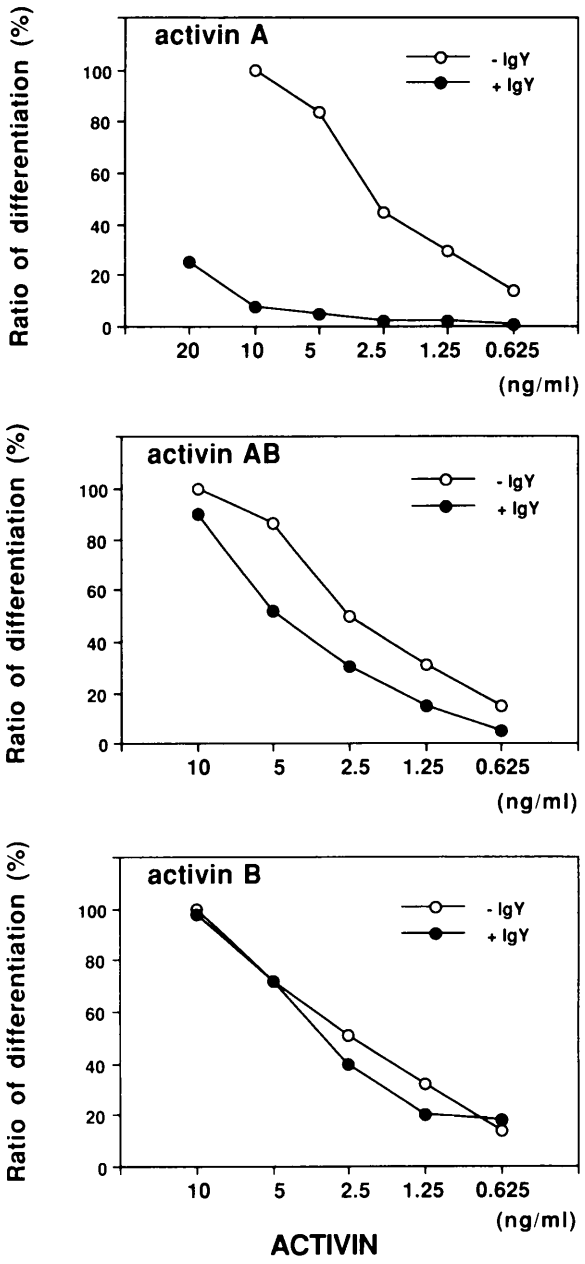

Figure 2. Effects of anti-activin A IgY solution from Hen #2 on the differentiation of the Friend cells induced by activin A ($\beta_A\beta_A$), activin AB ($\beta_A\beta_B$) and activin B ($\beta_B\beta_B$). Activin AB and activin B were purified from porcine ovaries (14). The Friend cells were incubated for 6 days with various doses of activins and anti-activin A IgY solution (#2–42) diluted 1:50. Each point represents the mean of duplicate values.

lution neutralized activin AB ($\beta_A\beta_B$) only slightly. ED_{50} of activin AB was changed from 2.52 ng/ml to 3.32 ng/ml by the addition of anti-activin A IgY solution.

Cross-Reactivity to Inhibin A. Cross-reactivity of anti-activin A IgY to inhibin A was examined by using the anterior pituitary primary culture system. Activin A (2.5 ng/ml) stimulated FSH secretion from cultured pituitary cells, while inhibin (2.5 ng/ml) inhibited it (Table I). Treatment of the assay system with anti-activin A IgY solution (5 U/ml) significantly suppressed FSH secretion stimulated by activin A (2.5 ng/ml), but did not affect FSH secretion inhibited by inhibin (2.5 ng/ml and 5 ng/ml) (Table I). The treatment with anti-activin A IgY (5 U/ml) did not affect basal FSH secretion during a 48-hr incubation.

Effects of IgY Solution on the Development of Murine Embryos. The treatment of embryos of C57/BL mice with a high concentration of anti-activin A IgY (64 U/ml) markedly increased the percentage of two-cell compared with total embryos and blocked embryo development after the eight-cell stage while those of control groups developed to morula and blastocyst stages (Table II). Addition of control IgY (purified IgY from normal chick) and a low concentration of anti-IgY solution did not affect the percentage of two-cell, higher stage, or degenerated embryos compared with control. In addition, the neutralization of activin A in embryo culture with anti-activin A IgY caused a higher incidence of embryonic degeneration.

Table I. Effects of Anti-Activin A IgY Solution from Hen #2 on Activin- or Inhibin-Modulated FSH Secretion from Cultured Anterior Pituitary Cells

Treatments[a]		FSH concentration (ng/ml)[b]
Control		64.2 ± 1.57
Anti-activin A IgY	5 U/ml[c]	64.6 ± 2.97
Activin 2.5 ng/ml		89.8 ± 0.96[d]
Activin 2.5 ng/ml + anti-activin A IgY	5 U/ml	67.2 ± 1.31[e]
Inhibin 2.5 ng/ml		40.0 ± 2.38[d]
Inhibin 2.5 ng/ml + anti-activin A IgY	2.5 U/ml	41.1 ± 1.40[d]
Inhibin 2.5 ng/ml + anti-activin A IgY	5 U/ml	41.3 ± 1.34[d]

[a] Culture of enzymatically dispersed anterior pituitary lobes collected from 22-day-old female Sprague-Dawley rats were treated on the second day of culture with activin A (2.5 ng/ml) or inhibin (2.5 ng/ml) with or without anti-activin A IgY solution.
[b] At 48 hr of incubation after the treatments, the medium was collected and the concentration of FSH in medium were measured by radioimmunoassay. Data are the means ± SEM of two experiments performed in triplicate.
[c] One unit per milliliter is defined as the activity which neutralizes an EDF activity of 1 ng/ml activin A. A mixture of anti-activin A IgY solution from Hen #2 from Day 41 to 45 after immunization was used as the anti-activin A IgY solution.
[d,e] Data were statistically evaluated by Student's *t* test: $^dP < 0.01$, versus Control and $^eP < 0.01$, versus activin only.

Discussion

In this study, we succeeded in generating a polyclonal antibody against activin A. This anti-activin A IgY was able to neutralize several biological activities of activin A, such as the stimulation of FSH secretion from pituitary cells and the induction of the differentiation of erythrocytes *in vitro*. This antibody did not affect the EDF activity of activin B, which induces the differentiation of erythrocytes *in vitro*, but slightly neutralized the action of activin AB. Inhibin A is a heterodimer of the β_A subunit of activin and another subunit, α subunit, which inhibits FSH secretion from pituitary cells (22–25). The IgY antibody did not affect this biological activity of inhibin. Western blotting analysis showed that this antibody recognized both dimeric and monomeric forms of the β_A subunit of activin. Therefore, these results found that this anti-activin A IgY recognizes the β_A subunit of activin and specifically neutralizes the activity of a dimer of β_A subunit, activin A.

Although the anti-activin A IgY recognized not only the β_A subunit of activin but also that of inhibin A in Western blotting analysis, the biological activity of inhibin A ($\alpha\beta_A$) was not affected by treatment with the anti-activin A IgY. Since addition to the culture medium of an antibody against the N-terminus of the α subunit of inhibin neutralized the inhibitory effect of inhibin on FSH secretion from pituitary cells *in vitro* (25), the α subunit of inhibin might be more important than the β subunit for the expression of inhibin activity. On the other hand, the β subunit of inhibin also seems to be necessary for the expression of inhibin activity because any inhibin-like biological activity of the inhibin α subunit monomer, isolated from bovine follicular fluid, has not been reported (26–28). Thus, the roles of the β subunit of activin and inhibin may be different for the expression of each biological activity. Further study is needed to test this hypothesis.

The amino acid sequence of the chicken activin β_A subunit has not been completely determined, but a partial nucleotide and deduced amino acid sequence of the chicken activin β_A subunit has been determined using the polymerase chain reaction on chicken genomic DNA. This assay revealed an 85% and 94% homology in the activin β_A subunit between chicken and humans at the DNA and the amino acid levels, respectively (29). The difficulty in generating an antibody which has a neutralizing activity against activin A action may be reflected in this homology.

Interestingly, the increase of titer was observed as a transient peak during a limited period. Recombinant human activin A has been shown to induce the forma-

Table II. Effect of Anti-Activin A IgY Solution from Hen #2 on the Development of Murine Embryos

	No. of experiments	Embryo development (%)[a]			Embryo degenerated (%)
		Two-cell	Four- to eight-cell	Morula or blastocyst	
Control[b]	3	0	12.9 ± 14.5	76.8 ± 18.3	10.3 ± 3.7
Control IgY (low)[c]	1	0	5.0	90.0	5.0
Control IgY (high)	1	5.0	0	85.0	10.0
Anti-activin IgY (low)[d]	3	20.3 ± 18.5	18.8 ± 16.0	40.0 ± 35.0	20.9 ± 5.2[e]
Anti-activin IgY (high)	3	57.9 ± 2.0[e]	21.5 ± 6.1	0[e]	20.6 ± 5.8[e]

[a] After incubation of fertilized embryos (15–20 embryos per dish) for 72 hr in Whitten's medium containing bovine serum albumin with or without IgY solution in an atmosphere of 5% CO_2 in air at 37°C, the numbers of embryos at two-cell, four- to eight-cell, morula or blastocyst were counted and expressed as the percentage of the total number of embryos cultured in a dish. Data are the mean ± SEM of three experiments in control and anti-activin IgY groups. One or two dishes were cultured for each group of experiments.
[b] The percentage of developed embryos incubated with cultured medium only is indicated as control.
[c] IgY purified from nonimmunized chick egg was used as control IgY solution, and two different concentrations, low and high, of control IgY were adjusted to be at the same protein level with those of anti-activin A IgY.
[d] Low and high concentrations of anti-activin IgY solutions were 12.8 U/ml and 64 U/ml, respectively. One unit/ml is defined as the activity which neutralizes an EDF activity of 1 ng/ml activin A. A mixture of anti-activin A IgY solution from Hen #2 from Day 41 to 45 after immunization was used as the anti-activin A IgY solution.
[e] Data were statistically evaluated by Student's *t* test: $P < 0.05$, versus corresponding control.

tion of axial structures in the chick (29), and chicken embryos contained activin A-like activity, as detected by EDF bioassay using murine Friend cells (30). These facts suggest that the structure of the active site of chicken activin A is the same or very similar to that of mammalian activin A. Therefore, an antibody against activin A generated in hen may neutralize endogenous activin action, especially on immune function, and affect antibody generation, resulting in the observed sudden decrease of titer after its initiation.

A spontaneous arrest of cleavage occurs at an early stage of embryonic development in most mammalian species, with some exceptions, when their embryos are cultured *in vitro* (31–33). The occurrence of the two-cell block in cultured mouse embryos has been considered to be due to the lack of some factor(s) derived from the oviduct. The two-cell block, which occurs in rodent embryos cultured *in vitro*, was overcome by the addition of activin A (34). The immunohistochemical localization of activin/inhibin β subunits and the expression of mRNAs of these subunits and the activin receptor have been demonstrated both in two-cell stage embryos and in the oviduct containing two-cell stage embryos (35). Thus, activin appears to be the factor responsible for breaking the two-cell block. These observations raise the interesting question of whether embryos of a nonblocking strain of mouse, which develop *in vitro* without a two-cell block, can produce activin by themselves and pass through the two-cell stage under culture conditions. It is expected that if activin A is responsible for the development of the two-cell stage and if the anti-activin A IgY developed in this study can neutralize this biological activity, the development of cultured embryos of the nonblocking strain mouse, C57/BL, should be arrested at the two-cell stage by adding the anti-activin A IgY. As shown in Table II, this turns out to be the case.

The expression of mRNAs of the β subunits, β_A and β_B, was different among tissues. In brain and bone marrow, β_A subunit was highly expressed while in pituitary it was less (36). Therefore, to clarify the physiological significance of the different types of activins, specific neutralization of each activin action is important. In several studies, follistatin, an activin binding protein, has been used for the neutralization of activin activities, such as the stimulation of FSH secretion from pituitary cells (37), the induction of differentiation of Friend cells (38), the induction of mesodermal tissue formation in Xenopus embryos (39), and stimulation of FSH-induced differentiation of ovarian granulosa cells (40, 41). However, it is difficult to discriminate actions of activins, because follistatin binds to all three types of activins, activin A, activin AB and activin B (14). Furthermore, the binding affinity of follistatin to heparan sulfate proteoglycan on the cell surface suggested that follistatin binding on the cell surface may play an important role in the autocrine or paracrine regulation of activin actions in activin-targeted cells by trapping the activins (42, 43). The generation of a specific monoclonal antibody, which could neutralize activin B activity, by immunizing mice with human recombinant activin B has been reported (44, 45). Since there is a difference of three amino acids in the 114 amino acid sequence of the β_B subunit between humans and rats, it is likely that this small difference accounted for this success. However, there is no difference among human, porcine, bovine or rat β_A subunits. In this study, we have demonstrated the generation of specific IgY antibody against activin A, an evolutionarily highly conserved peptide, using hen as a host and neutralizing activity of the

antibody against activin A action was observed using several assay systems. The generation of the specific antibody that neutralizes activin A action in this study further demonstrates the usefulness of the hen for generating antibodies against mammalian highly conserved proteins and will contribute to research on the multiple actions of endogenous activin A, especially in brain or bone marrow, and discrimination of specific biological actions among activin A, activin AB, and activin B.

We thank Dr. Y. Hasegawa, Kitasato University, and Dr. H. Sugino, Tokushima University, for kindly providing purified inhibin and activins, respectively; Dr. K. Shiota, University of Tokyo, for the advice for the culture of pituitary cells; and Dr. A. F. Palow for kindly providing FSH kit. We thank particularly Dr. J. G. Brand, Monell Chemical Senses Center, for careful review and helpful advice on the manuscript. This study was supported by the Research Development Corporation of Japan.

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