Transplacental Transport of α 2-Macroglobulin (α 2M) and Induction of α 2M in Maternal and Neonatal Rats with Acute Inflammation

Makoto SHIMIZU¹⁾, Tomokazu JINBO¹⁾, Naomi KASHIWAZAKI²⁾, Takashi KURIBAYASHI¹⁾, Masanori NOMURA¹⁾, and Shizuo YAMAMOTO¹⁾

¹⁾Laboratory of Immunology, College of Environmental and Health Sciences, and ²⁾Laboratory of Animal Reproduction, School of Veterinary Medicine, Azabu University, 1–17–71 Fuchinobe, Sagamihara, Kanagawa 229-8501, Japan

Abstract: The aims of this study were to investigate transplacental transport of α 2-macroglobulin (α 2M) in rats and to examine the degree of α 2M induction in maternal and neonatal rats with acute inflammation. Serum was collected from healthy pregnant CD (IGS) rats, neonates of the pregnant rats and their cord blood. Additional serum samples were obtained from pregnant rats inoculated with an inflammatory agent, turpentine oil, their neonates and cord blood, and neonates inoculated with turpentine oil. The serum levels of α 2M were measured by means of an enzyme-linked immunosorbent assay. The average serum levels of α 2M in healthy neonates and cord blood were about 380 μ g/ml. Serum α 2M level in neonates inoculated with turpentine oil averaged about 580 μ g/ml. Serum α 2M levels in maternal rats inoculated with turpentine oil, neonates from those rats and their cord blood were elevated, the values being 2,000 μ g/ml or higher. It was demonstrated that induction of α 2M in neonatal rats was lower than in maternal rats when inoculated with turpentine oil. These results suggest that α 2M is transplacentally transported from maternal rats to fetal ones.

Key words: acute phase protein, α 2-macroglobulin, neonate, placenta, rat

Introduction

Rat α 2-macroglobulin (α 2M) is typically induced in the acute phase inflammation. α 2M exists in a small amount in the serum of healthy adult rats and is markedly increased in the serum of normal fetal, neonatal and pregnant rats, and rats with acute inflammation [1,

2, 13, 17]. Changes in the serum levels of α 2M in Buffalo rats during pregnancy and their neonates, obtained by using radioimmunoassay, have been reported [4, 10]. In these studies, the serum levels of α 2M in all pregnant rats peak just before whelping.

The transfer of hormones, growth factors and serum proteins between maternal-fetal organisms has been

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Address corresponding: S. Yamamoto, Laboratory of Immunology, College of Environmental and Health Sciences, Azabu University, 1–17–71 Fuchinobe, Sagamihara, Kanagawa 229–8501, Japan

demonstrated in the human haemochorial placenta [11]. $\alpha 2M$ exhibits immuno-suppression activity [3, 14]. $\alpha 2M$ from maternal rats might be a means of immuno-suppression in fetal rats. However, transplacental transport of $\alpha 2M$ in rats has not been reported.

The objectives of this study were to determine the serum levels of $\alpha 2M$ in healthy pregnant rats, neonates delivered from the pregnant rats and their cord blood; pregnant rats inoculated with turpentine oil as an inflammatory agent, their neonates and cord blood; and neonates inoculated with turpentine oil. Induction of $\alpha 2M$ in maternal and neonatal rats with acute inflammation was also compared. This study provides evidence that suggests the existence of transplacental transport of $\alpha 2M$ in rats.

Materials and Methods

Rats: Thirty-two (16 males and 16 females) specific pathogen free/virus antibody free CD (IGS) rats were purchased from Charles River Japan Inc. (Kanagawa, Japan). The rats were examined and confirmed to be free of *Corynebacterium kutscheri*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Pasteurella pneumotropica*, *Streptococcus pneumoniae*, *Bordetella bronchiseptica*, *Mycoplasma pulmonis* and parasites, and antibodies to Tyzzer's organism, Sendai virus, mouse hepatitis virus, pneumonia virus of mice, mouse polio virus, reovirus type 3, lymphocytic choriomeningitis virus, mouse adenovirus, rat coronavirus/Sialodacryoadenitis virus, Toolan's H-1 virus, Kilham rat virus and Hantaan virus. These rats were reared under sterile room conditions, and received a diet of sterile CRF-1 and water until being sent to the Inoue Experimental Animals Center Co., Ltd. (Kumamoto, Japan). After arrival, the rats were reared at this institute, individually housed in cages (W 280 $\times$ D 440 $\times$ H 180 mm) in an animal room adjusted to a temperature of $22 \pm 2^\circ\text{C}$, relative humidity level of $60 \pm 10\%$, and illumination for 12 h (7:00–19:00 h). Air was exchanged 15 times/h. The animals were fed a solid stock food (MF; Oriental Yeast Co., Ltd., Tokyo, Japan), and allowed free access to water. All experiments conformed to the Japanese regulation concerning animal care and use, following the *Guideline for Animal Experimentation* [6], and were approved by the Institutional Animal Care and Use Committee of the Inoue Experimental Animals Center

Co., Ltd.

Collection of sera from pregnant rats, neonates and cord blood: Sixteen female rats were copulated with male rats of the same strain. Pregnancy was confirmed by the finding of spermatozoa in the vagina on Giemsa staining of a vaginal smear. Blood was successively collected from a subclavian vein of ten pregnant rats under ether anesthesia. For one female rat spermatozoa were found in the vagina but the rat did not become pregnant. One pregnant rat died just before whelping. Just after whelping, blood was collected from sixteen neonatal rats (two from each of eight maternal rats) and from cord blood.

Immediately after whelping, four other neonates were inoculated intramuscularly with 0.1 ml/kg of turpentine oil (Wako Pure Chemical Industries Ltd. Osaka, Japan). Blood was collected from the rats 2 days after inoculation.

Six pregnant rats were inoculated intramuscularly with 0.1 ml/kg of turpentine oil (Wako Pure Chemical Industries Ltd.) 20 days after copulation. Just after whelping (1 or 2 days after inoculation of turpentine oil), blood was collected from the female rats, neonates and cord. Calculation of the day of ovulation was determined retrospectively as the 21st day before whelping.

Quantification of $\alpha 2M$: Concentrations of $\alpha 2M$ in the serum were determined by means of a capture ELISA with an anti-rat $\alpha 2M$ monoclonal antibody [8]. Anti-rat $\alpha 2M$ monoclonal antibodies, adjusted with 0.05 M sodium bicarbonate buffer (pH 9.6) to 1.0 $\mu\text{g}/\text{ml}$, were incubated at 100 $\mu\text{l}/\text{well}$ at 37°C for 1 h. After blocking with 1% bovine serum albumin in sodium bicarbonate buffer (pH 9.6), 100 $\mu\text{l}/\text{well}$ of serially diluted rat serum with a known $\alpha 2M$ concentration (2,520 μg of $\alpha 2M$ per ml) and the test sera were added. The plates were incubated at 37°C for 2 h. Peroxidase (Sigma Chemical Co., St. Louis, Mo, USA)-conjugated anti-rat $\alpha 2M$ monoclonal antibodies were prepared by using the method of Nakane and Kawaoi [12], and used at 20.0 $\mu\text{g}/\text{ml}$; the plates were incubated at 37°C for 2 h. The substrate used was 0.05% 2,2-azino-di-(3-ethylbenzthiazoline sulphonic acid-6; ABTS) (Zymed Laboratories, South San Francisco, CA, USA). After incubation at room temperature for 50 min, A_{415} was measured. The results were compared with a standard curve obtained with serially diluted specimens from rats

Fig. 1. Changes in the serum α 2M level in four pregnant rats.

containing known α 2M concentrations.

Statistical analysis: Statistical analysis of the data was carried out by means of the paired Student's *t*-test.

Results

Changes in the serum α 2M levels in pregnant rats: Changes in serum α 2M levels in pregnant rats are shown in Figs. 1 and 2. Two patterns of changes in the serum α 2M levels in the pregnant rats were discerned. Four pregnant rats showed peak serum α 2M levels at 10 or 14 days after ovulation. The peak level was 400 μ g/ml or more. Five other pregnant rats showed peak serum α 2M levels just before whelping. The peak level was 300 μ g/ml or more. The serum α 2M level in a non-pregnant rat was not altered.

Serum α 2M levels in neonates delivered from pregnant rats and their cord blood: Serum α 2M level in healthy neonates, cord blood and neonates inoculated with turpentine oil are shown in Table 1. There was no significant difference in serum α 2M levels between the healthy neonates and cord blood ($p > 0.05$).

Serum α 2M levels in pregnant rats inoculated with turpentine oil, their neonates and their cord blood: The serum α 2M levels in maternal rats inoculated with turpentine oil, and their neonates and cord blood are shown

in Table 2. The neonates whose mothers were inoculated with turpentine oil had significantly greater α 2M levels than the healthy neonates ($p > 0.05$).

There were no significant differences among the pregnant rats inoculated with turpentine oil, and their neonates and cord blood ($p > 0.05$).

The serum α 2M levels in the neonates delivered from maternal rats inoculated with turpentine oil were significantly greater than in the neonates inoculated with turpentine oil ($p > 0.05$).

Discussion

The pattern of changes in the serum α 2M levels in rats during pregnancy has been reported previously [4, 10]. The peak α 2M levels were found in late gestation in the previous studies. In the current study, two patterns of change of the serum levels of α 2M in pregnant rats were demonstrated. The peak α 2M levels were found in pregnant rats in mid- or late gestation. The peak α 2M levels found in late gestation in the current study showed the same pattern as those reported in previous studies [4, 10]. However, it is difficult to come to any conclusion as to why the α 2M levels exhibited two patterns in the results of this study alone. α 2M exhibits immunosuppression activity [3, 14]. An

Fig. 2. Changes in the serum α 2M level in the remaining five pregnant rats.

Table 1. Serum α 2M levels (μ g/ml) in healthy neonates and cord blood, and neonates inoculated with turpentine oil

	n	Mean	SD	Range
Neonates	16	383	64	303–522
From Rat 1–4	8	362	48	303–443
*From Rat 5–9	8	405	74	303–522
Cord blood	75	384	69	298–502
From Rat 1–4	42	396	51	298–461
From Rat 5–9	33	369	80	336–502
Neonates inoculated with turpentine oil	4	583	133	483–778

*Without Rat 7.

increase in serum α 2M might be a means of immunosuppression in pregnant rats.

The average serum α 2M level in healthy neonates was about 380 μ g/ml. This value was greater than that of healthy adult rats whose α 2M level is about 40 μ g/ml [8]. The synthesis and secretion of α 2M in the visceral yolk sac of fetal rats have been reported [16]. High serum α 2M levels in neonatal rats might reflect α 2M secreted from the visceral yolk sac. Consistent with those observations, when turpentine oil was inoculated, induction of α 2M in neonatal rats was lower than that in the maternal rats. α 2M is synthesized by the liver [9, 15]. The synthesis of α 2M by the liver in maternal rats might be greater than that in neonatal

Table 2. Serum α 2M levels (μ g/ml) in maternal rats inoculated with turpentine oil, and their neonates and cord blood. One or two days before whelping, maternal rats were inoculated with turpentine oil. Sera from maternal rats, neonates and cord blood were collected just after whelping

	n	Mean	SD	Range
Maternal rats	6	3229	490	2316–3539
Neonates	17	2954	1310	1473–5494
Cord blood	43	3296	153	3076–3415

rats.

The human placenta has specific binding sites for a large number of maternal plasma proteins. Such receptors may recognize hormones, growth factors, immunoglobulin G and proteins that transfer important nutrients across the placenta. A receptor for the α 2M-proteinase complex on human placental syncytiotrophoblasts has been demonstrated [7]. This may indicate that human α 2M undergoes transplacental transport. Serum α 2M levels in the neonatal rats inoculated with turpentine oil increased slightly, the peak values being 800 μ g/ml or less. Serum α 2M levels in maternal rats inoculated with turpentine oil, neonates from those rats and their cord blood were elevated, the values being 2,000 μ g/ml or more. These results suggest that α 2M is transplacentally transported from maternal rats to fetal rats. It has been considered that interleukin-6 is a major inducer of α 2M in rats [5]. If interleukin-6 is transplacentally transported, it may influence the induction of α 2M. However, transplacental transportation of interleukin-6 has not been reported.

The demonstration of the existence of an α 2M receptor on rat placental syncytiotrophoblasts would provide additional evidence of the transplacental transport of α 2M in rats.

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