TWO EPITOPES OF BLOOD SERUM IMMUNOGLOBULINS AS INDICATORS OF PHYSIOLOGICAL ANTIBODY DEFICIENCY IN LAMBS*

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Abstract

A group of 104 lambs of Kołuda sheep (prolific milk line) were investigated. Observations were made on changes in the level of IgG immunoglobulins with IghgA1 and IghgA2 epitopes in 15 and 23 informative animals, respectively, during the first two months of their life. The lambs both absorbed the antibodies of mothers (already after colostrum intake) and produced their own immunoglobulins (from three or four weeks of age). Colostral antibodies that carried both markers began to disintegrate earlier than 14 or 28 days of age. From this time, the immunoglobulin levels were observed to decrease temporarily: proteins with IghgA1 marker to 28 and 42 days, and globulins with IghgA2 epitope to 42 days of age of sucklings. The results obtained show that the biosynthesis of the animals' own immunoglobulins, which gradually developed during this period, was insufficient to make up for the loss of colostral antibodies catabolized. The determinations indicate that immune protein deficiency may lead to increased susceptibility of hypogammaglobulinemic lambs to different infections. Antigenic markers of immunoglobulins seem to be a useful tool for physiological tests of antibody deficiency, the accuracy of identification being determined only by shortening the blood sampling intervals in different periods of lamb life.

Key words: lambs, IgG immunoglobulins, physiological deficiency of antibodies

Newborn lambs are usually deficient in antibodies, which is due to the structure and properties of the syndesmochorial placenta in sheep. This five-layer barrier efficiently prevents the transfer of maternal antibodies to the offspring during the entire prenatal period (Chełmońska-Soyta and Nikołajczuk, 2000). In addition, uninduced ovine fetuses show no or little synthesis of their own immune bodies (Deptuła and Buczek, 1998). Therefore, neonates obtain the entire pool of antibodies from mothers'

colostrum (and, to a very limited extent, from their milk). This is the only natural and rich source of immunoglobulins, easily available to neonates directly after birth. Immune proteins absorbed from colostrum quickly provide lambs with protection from pathogens, but the activity of the non-renewable pool of maternal immunoglobulins is limited in time due to the gradual biodegradation of these proteins and the elimination of these from the blood system (Skiba and Węgrzyn, 2001).

An alternative source of immune globulins for a young organism is production of own immunoglobulins, induced by the expression of genes that control this production. However, only small amounts of these proteins are synthesized initially. The intensity of production increases with time, until a "fluid balance" between the synthesis and catabolism of the animal's own antibodies is reached (Holmes and Lunn, 1991; Butler, 1995).

Both the absorption and metabolism of colostral antibodies as well the endoproduction of the animal's own immune proteins are physiological processes and as such they show unique dynamics. In the initial period of animal growth, they may overlap and at the same time influence (to different extents) changes in the total quantity of plasma proteins (White, 1993; Hein, 1995). If the synthesis of the animal's own antibodies does not compensate for the natural loss of maternal immunoglobulins, this results in a transitory decrease in the total plasma level of immune proteins, known as physiologic hypogammaglobulinemia.

The aim of our study was to test the suitability of two epitopes of IgG immunoglobulins for determining physiological hypogammaglobulinemia in lambs of the prolific-dairy Kołuda line over the first two months of age.

Material and methods

A total of 104 Kołuda lambs and their parents (15 rams and 46 ewes) were investigated. Blood samples were collected from the lambs within 5-10 minutes of birth (before colostrum ingestion), at 24 h of age and at ~14, 28, 42 and 60 days of age. The first blood sample was collected simultaneously with the blood of parents and colostrum of ewes. During the first 24–36 hours after lambing, each mother and its offspring stayed in a separate pen. Fat was removed from colostrum by centrifugation at +4°C and RCF 8000.

Blood and serum immunoglobulins were determined using anti-IghgA1 and anti-IghgA2 alloantibodies that identify the markers of IgG immunoglobulins (IghgA1 and IghgA2, respectively). These markers are conditioned by allelic, codominant genes *IGHGA1* and *IGHGA2* (Skiba and Węgrzyn, 1993; Skiba et al., 2000). The antigenic determinants were determined using a double immunodiffusion test in agar gel (TPI) according to Ouchterlony (1953). TPI results were used to conclude the presence or absence of the analysed immunoglobulins as well as changes in the level of these proteins in consecutive serum samples. To genotype lambs and their parents, genetic relationships of the *IGHGA1* and *IGHGA2* genes between one another and with the IgM immunoglobulin gene were used (Skiba and Węgrzyn, 1993).

Results

Physiological deficiency of the immune proteins was observed only in those animals which both absorbed immunoglobulins from colostrum and synthesized the same proteins on their own. In addition, the depletion of serum antibodies could not be balanced by the flow of immunoglobulins produced by the lambs. As regards the analysed proteins that carried IghgA1 and IghgA2 epitopes, the above conditions were fulfilled by 15 and 23 animals, respectively out of the 104 lambs examined.

Table 1. Absorption and probable* synthesis of IgG immunoglobulins with IghgA1 marker in 15 lambs with physiological deficiency of these antibodies

Genotypes of parents (locus IGHGA) ♂ × ♀	No. of lambs	Genotypes of lambs (locus IGHGA)	Reactions of lamb serum samples with anti-IghgA1 antibodies**					
			before colostrum ingestion	24 hrs	14 days	28 days	42 days	60 days
				after first colostrum ingestion				
			↓	↓	↓	↓	↓	↓
A1/A2 × A1/A2	1	A1/A1						
A1/A2 × A1/A2	3	A1/A2	–	++	[+]	++	++	+++
A1/A2 × A1/A2	1	A1/A1						
A1/A2 × A1/A2	4	A1/A2	–	++	[+]	[+]	++	+++
A2/A2 × A1/A2	4	A1/A2						
A1/A2 × A1/A2	2	A1/A2	–	++	++	[+]	++	+++

■ IgG absorbed from colostrum   ┈┈ probably own IgG

* Based on the course of synthesis of immunoglobulins with this epitope in the other seven lambs, A1/A2 heterozygotes.

** Symbols "+" or "–" designate presence or absence of trait;
reaction strength: "+"   – weak;
                          "++"   – average;
                          "+++"   – strong.

The results of the determinations of both immunoglobulin markers in consecutive serum samples are shown in Table 1 for the IghgA1 marker and in Table 2 for the IghgA2 epitope.

Table 2. Absorption and probable* synthesis of IgG immunoglobulins with IghgA2 marker in 23 lambs with physiological deficiency of these antibodies

Genotypes of parents (locus IGHGA) ♂ × ♀	No. of lambs	Genotypes of lambs (locus IGHGA)	Reactions of lamb serum samples with anti-IghgA2 antibodies**					
			before colostrum ingestion	24 hrs	14 days	28 days	42 days	60 days
				after first colostrum ingestion				
				↓	↓	↓	↓	↓
A1/A2 × A1/A2	9	A1/A2	–	++	+	+	++	+++
A2/A2 × A1/A2	2	A2/A2						
A1/A2 × A1/A2	6	A1/A2	–	+++	+	++	+++	+++
A2/A2 × A1/A2	1	A1/A2						
A1/A2 × A1/A2	4	A1/A2	–	++	++	+	++	+++
	1	A2/A2						

* Based on the course of synthesis of immunoglobulins with this epitope in the other seven lambs, A1/A2 hetero-zygotes.

Other assumptions and symbols as under Table 1.

Globulins with IghgA1 marker

At birth, none of the fifteen neonates had proteins with IghgA1 marker in blood (Table 1). These proteins were found to occur in the blood collected 24 h after the first ingestion of colostrum. A temporary decrease in the level of globulins was found in 4 neonates on day 14, in other 9 neonates on days 14 and 28, and in the remaining 2 animals on day 28. In the first four lambs, IgG levels returned to the level obtained on day 28 (and persisted to 42 days of age), whereas in the other 11 animals this oc-curred on day 42. In the next tests, proteins with IghgA1 epitope were observed to increase in the blood of all lambs. At 60 days of age they reached a level comparable with the level of globulins in mothers' colostrum.

Globulins with IghgA2 marker

Immunoglobulins with this epitope were first detected in the blood of 23 lambs, collected 24 h after colostrum ingestion (Table 2). In 9 neonates, the level of these proteins was similar to that found in the colostrum of mothers, and in the other 14 neonates it was slightly lower. In 5 animals, this level persisted until the next test at 14 days of age. A temporary decrease in the level of immunoglobulins was observed in them in a sample taken on day 28. In the other 18 lambs, IgG level decreased on both day 14 and 28. The level from day one was resumed in all animals on day 42. In the next samples, the level of proteins with IghgA2 epitope in blood was observed to increase.

Discussion

Lambs in which temporary antibody deficiency was observed, had inherited from their parents genes that control the production of these proteins. However, at birth their blood contained no immunoglobulins with the epitopes studied (none of the 15 neonates for the IghgA1 marker, and none of the 23 animals for the IghgA2 marker). This observation shows that *IGHGA1* and *IGHGA2* genes were not expressed during the fetal life of these lambs. However, during the first day of life, following ingestion of colostrum rich in these proteins, they appeared in considerable quantity in animals' blood. Both the sudden appearance of immunoglobulins in blood and the fact that from the beginning they appeared in large quantities (similar to those determined in mothers' colostrum) clearly indicate that they originated from colostrum. Thus, neonates deprived of the protection of their own immune bodies at birth, were quick to absorb mothers' immunoglobulins from the colostrum consumed.

The first decreases in the level of antibodies with IghgA1 and IghgA2 markers, found in the majority of lambs at 14 days of age, are evidence that globulin catabolism had already progressed during that time. The unchanged initial level of antibodies persisted for over 14 days only in two animals for proteins with IghgA1 epitope, and in five animals for globulins with IghgA2 marker. These findings correspond with the observations in lambs of the Olkuska sheep (Krzyścin, 2004), in which analogous antibodies began to disintegrate at two and three weeks of age.

The observed immunoglobulin deficiency was transitory. The increase in the level of antibodies with IghgA1 epitope at 28 or 42 days of age (to the level found at one day of age) was most probably the result of the inflow of the animals' own proteins. Both homozygous (A1/A1) and heterozygous (A1/A2) offspring inherited *IGHGA1* genes, but their expression took place after birth and not during fetal life. The hypothetic course (here masked by colostral antibodies) of the postnatal synthesis of immunoglobulins with IghgA1 marker was assumed based on the observation of this process in six other A1/A2 heterozygotes. These lambs began IgG endosynthesis at three or four weeks of age.

Similar conclusions were drawn for proteins with IghgA2 markers. All of 23 lambs inherited *IGHGA2* genes, but before birth they did not synthesize the proteins controlled by these genes. Gene expression and immunoglobulin production took place later, as indicated by an increase in the level of these antibodies found for 9 lambs at 28 and 42 days of age and for the other 14 animals at 42 and 60 days of age. The hypothetic course of synthesis of antibodies carrying the IghgA2 markers was assumed based on observations in the other seven heterozygous (A1/A2) animals.

Analysis of the last samples of blood, which showed that the level of immunoglobulins with both markers remained similar until the end of the experiment, confirms the continued endoproduction of these antibodies by lambs and the constant flow of these antibodies to the blood.

The observed pool of immunoglobulins (both those with IghgA1 marker and those with IghgA2 epitope) was dominated first by antibodies inherited by lambs from mothers' colostrum. Previous research (Krzyścin et al., 2002) showed that these proteins can remain in the blood of lambs for over three months after absorption. Then,

the progressing catabolism of acquired immunoglobulins was paralleled by IgG deficiency in the body. In most lambs, the deficiency of immune bodies with both markers started before the end of the second week and ended at five or six weeks of age, spanning a period of three or four weeks. This deficiency was gradually eliminated when animals' own antibodies began to be produced at three or four weeks of age, the rate of this process being dependent on the efficiency of IgG synthesis. The animals' own immunoglobulins came to increasingly dominate over the successive weeks of the experiment.

Over the years, immunologically determined and transitory deficiency of immunoglobulins was reported in sheep by Sawyer et al. (1977), Perryman (1979), Klobasa et al. (1992), Waelchli et al. (1994) and McMurray (1999), who showed that hypogammaglobulinemic lambs are more susceptible to various infections, especially to recurrent or chronic respiratory infections that are particularly dangerous during the first weeks of life.

It seems that antigenic markers, which find application in studies on the absorption, synthesis and catabolism of antibodies in sheep (Węgrzyn et al., 2001; Krzyścin et al., 2002), can also be used to identify physiological immunoglobulin deficiency in lambs. The accuracy of the identification is determined only by reducing the time intervals between collection of successive blood samples in appropriate periods of animals' lives.

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Dwa epitopy immunoglobulin surowicy krwi jako wskaźniki fizjologicznych niedoborów przeciwciał u jagniąt

STRESZCZENIE

Badaniami objęto 104 jagnięta plenno-mlecznej owcy kołudzkiej. Obserwowano zmiany poziomu immunoglobulin klasy IgG z epitopami IghgA1 i IghgA2 u, odpowiednio 15 i 23 zwierząt w pierwszych dwóch miesiącach ich życia. Jagnięta zarówno absorbowały przeciwciała, pochodzące od matek (po wypiciu siary), jak i wytwarzały własne immunoglobuliny (od trzeciego lub czwartego tygodnia życia). Rozpad przeciwciał siarowych niosących oba oznaczane markery rozpoczął się przed 14. lub 28. dniem życia. Od tego czasu notowano przejściowe obniżenie się poziomu badanych immunoglobulin: białka z markerem IghgA1 – do 28. i 42. dnia, a globulin z epitopem IghgA2 – do 42. dnia życia osesków. Uzyskane wyniki wskazują, że stopniowo rozwijająca się w tym okresie biosynteza własnych immunoglobulin nie była w stanie uzupełnić ubytków katabolizowanych przeciwciał siarowych.

Takie czasowe niedobory białek odpornościowych mogą być przyczyną zwiększonej podatności jagniąt na różnorodne infekcje. Wydaje się, że antygenowe markery immunoglobulin mogą być wykorzystane w badaniach fizjologicznych niedoborów przeciwciał, a o dokładności rozpoznania decyduje jedynie zawężenie przedziałów czasowych pobierania kolejnych prób krwi w odpowiednich okresach życia jagniąt.