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IMMUNOGLOBULINS IN MAMMARY SECRETIONS

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9.1 INTRODUCTION

Milk contains a range of factors which contribute to the protection of the neonate and the mammary gland from disease. Antibodies are an important component of the disease resistance function of mammary secretions. Unlike most other major organic components found in milk, immunoglobulins (Ig) are not synthesized by the mammary epithelial cells. Considerable diversity exists among mammalian species in the transport of Ig from mother to neonate, as well as in the implications of that transport. This chapter will discuss Ig found in mammary secretions in the context of their diversity of structure, origin, transfer and function. A number of recent reviews can provide more detailed discussions of milk Ig and immunocytes (Telemo and Hanson, 1996; Butler, 1998, 1999), mucosal immunity (Chernishov and Slukvin, 1990; Brandtzaeg *et al.*, 1991; Cummins and Thompson, 1997), mammary gland immunobiology (Lee *et al.*, 1992; Sordillo *et al.*, 1997) and evolution of immunologic functions of the mammary gland (Goldman *et al.*, 1998). The disease resistance function of Ig typically occurs in concert with a range of protective factors and several other reviews address the spectrum of anti-infective components of milk (Goldman, 1993; Xanthou *et al.*, 1995; Xanthou, 1998; Goldman and Ogra, 1999).

9.2 IMMUNITY AND IMMUNOGLOBULINS

The animal's immune system is composed of two interrelated components, humoral immunity composed of soluble protective components and cellular

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immunity composed of leucocytes. Early investigations of the immune properties of milk focused on the presence of antibodies in mammary secretions and the transfer of humoral immunity to the neonate (Brambell, 1970; Butler, 1974), while early investigation of mammary cellular immunity focused primarily on changes in the concentrations of milk leucocytes and associated changes in the composition of milk caused by bacterial infection during mastitis (Paape *et al.*, 1981). More recent research often has been based upon a growing appreciation of the importance of immunobiology for understanding mammary gland function and for protection against disease (Sordillo *et al.*, 1997).

9.2.1 Humoral immunity and immunoglobulins

Humoral-mediated immunity of the mammary gland consists primarily of antibodies in the form of Ig. Milk antibodies play an important role in immune protection of the mammary gland and the neonate (Xanthou *et al.*, 1995; Telemo and Hansen, 1996; Sordillo *et al.*, 1997; Xanthou, 1998). Humoral defenses of the immune system also include the complement system; however, complement is particularly low in milk (Targowski, 1983) and complement-Ig interactions would not be expected to be significant in mammary secretions.

(a) Classes and structure of immunoglobulins

Synthesis of Ig occurs by a complex process of gene rearrangement and combinatorial joining of gene segments, addition or removal of nucleotides at the point of joining (junctional diversity), and somatic hypermutation of variable region gene segments (Butler, 1998; Marchalonis *et al.*, 1998). Accounting for the number of Ig genes identified and for the gene processing steps in Ig synthesis, an antibody repertoire of greater than 10^{12} may be expected (Butler, 1998); however, considerable variability exists among species in how these mechanisms of creating antibody diversity are employed (Meyer *et al.*, 1997; Butler, 1998; Marchalonis *et al.*, 1998).

Antibodies can be divided into five classes or isotypes, IgG, IgA, IgM, IgE and IgD. All monomeric Ig molecules consist of a similar basic structure composed of four subunit polypeptides (Figure 9.1), including two identical heavy chains and two identical light chains, with a total molecular mass of approximately 160 kDa. Both heavy and light chains are composed of domains referred to as variable (V_H, V_L) and constant (C_H, C_L) regions. Disulfide bonds link each heavy and light chain pair, as well as linking the two heavy chains, resulting in a Y-shaped molecule (Figure 9.1) with two antigen-binding sites. The number and position of disulfide bonds linking heavy chains varies with Ig isotype. Immunoglobulins are glycoproteins with the carbohydrate groups linked to the constant regions of the heavy chains (Figure 9.1).

Genes encoding the constant region of heavy chains are the primary determinants characterizing discrete Ig classes and subclasses. Multiple genes encode the constant regions of subclasses of IgG gamma heavy chain (γ-chain). The number of IgG subclasses varies among species (Butler, 1998). In contrast to IgG, only one gene encoding the constant region of the μ-chain of IgM has been identified and typically only one gene encodes for the α-chain of IgA and one gene for the ε-chain of IgE (Bulter, 1998). Light chain constant regions of antibodies are encoded by two separate genes, kappa (κ) and lambda (λ). The frequency of usage of κ- and λ-chains varies with species (Butler, 1998).

The structure of the constant regions of bovine heavy chain genes has been characterized for γ-chains (Symons *et al.*, 1989), α-chain (Brown *et al.*, 1997),

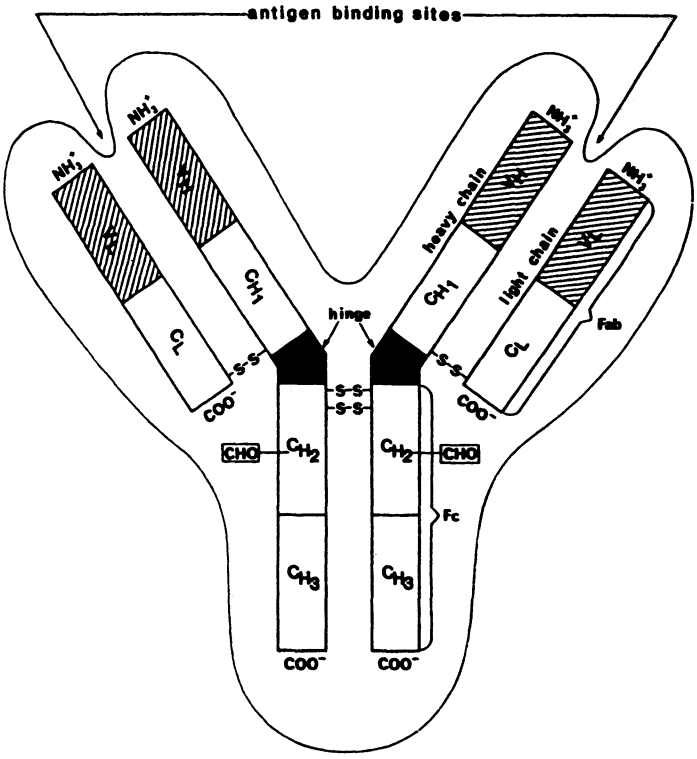

Figure 9.1 Schematic diagram of a basic immunoglobulin molecule showing two heavy and two light chains joined by disulfide bonds: V = variable region; C = constant region; L = light chain; H = heavy chain; Subscripts 1, 2 and 3 refer to the three constant regions of the heavy chains; CHO = carbohydrate groups; Fab refers to the antigen-specific (top) portion of the Ig molecule; Fc refers to the cell-binding effector portion of the Ig molecule. (From Guidry, 1985, reprinted by permission from *Lactation*, B.L. Larson, ed., 1985, by Iowa State University Press, Ames.)

μ-chain (Mousavi *et al.*, 1998) and ε-chain (Mousavi *et al.*, 1997). The bovine lacks IgD (Butler, 1998). The reader is referred to other reviews for characterization of heavy chain genes in other species (Butler and Brown, 1994; Butler, 1998), comparisons of α-chain gene structure among species (Mestecky *et al.*, 1999b) and Ig light chain expression (Gorman and Alt, 1998).

The N-terminal portion of the Ig molecule is the antigen binding region (Figure 9.1). Antigen binding occurs through interactions of the antigen with the variable regions of heavy and light chains. Digestion of the IgG molecule with papain hydrolyses the heavy chain at the hinge region and releases two identical antigen binding fragments (Fab) and the constant portion of the molecule (Fc). The Fab consists of V_H and C_{H1} domains of the heavy chain and V_L and C_L domains of the light chain (either κ or λ). The Fc portion of the IgG molecule consists of the C_{H2} and C_{H3} domains. The Fc fragment of an antibody contains the portion responsible for many of the biological activities of the antibody molecule, including complement activation, recognition by Fc-receptors on leucocytes and epithelial cells, transport through epithelial cells and recognition by bacterial Ig-binding proteins.

(b) Physical properties of immunoglobulins

Molecular components and physical characteristics of Ig from bovine mammary secretions are summarized in Table 9.1. Immunoglobulins are not distributed randomly among fractions of milk (Frenyo *et al.*, 1986). Ultracentrifugation of milk results in preferential association of IgM and IgA with the fat (cream) fraction and association of IgM and IgG_2 with the casein pellet. However, the major portions of IgG_1, IgG_2, IgA and IgM are found in the whey fraction (Frenyo *et al.*, 1986). Some milk Ig is associated with the cell pellet in fractionated milk.

Concentrated Ig in bovine colostrum stores well at refrigerated temperatures or frozen. This has significant practical value to the dairy industry for the storage of colostrum containing high Ig concentrations for feeding newborn calves. However, milk Ig is heat-labile (Goldsmith *et al.*, 1983; Larson, 1992; Fukumoto *et al.*, 1994; Lindstrom *et al.*, 1994), which is of particular importance in situations where colostrum or milk is pasteurized for use to treat or control disease. Heat denaturation resulting in loss of antigen binding or in the loss of the biological activities of Ig is of particular concern in designing heat treatments of milk or colostrum (Dominguez *et al.*, 1997).

9.2.2 Immunoglobulins in mammary secretions

Immunoglobulin G is found only in the monomeric form in blood or milk, whereas IgA and IgM are present in polymeric forms in both blood and

TABLE 9.1
Properties of the immunoglobulins of bovine mammary secretions[a]

Property	*Immunoglobulin or component*						
	IgG_1	*IgG_2*	*sIgA*	*IgM*	*L-chain*[b]	*J-chain*	*Secretory component*
Electrophoretic fraction[c]	γ_1 and β_2	γ and γ_2	β_2	β_2	n/a	n/a[2]	β_2
Molecular mass (kDa):							
Intact	146–163	146–154	385–430	1030	22–27	16.5	70–96
Heavy chain	56–59	54–59	61–63	62–76	n/a	n/a	n/a
Heavy chain type	γ_1	γ_2	α	μ	n/a	n/a	n/a
Carbohydrate:							
Total	2.8–3.1	2.6–3.0	6–10	10–12	0.5	–	3.1–5.9
Hexose[d]	1–1.5	1–1.5	5.6	5–5.5	–		2.6–2.9
Hexosamine	0.9–1.7	0.8–1.6	–	2–3	–	–	2.5–3
Fucose	0.1–0.3	0.2–0.3	–	1.25	–	–	0.2–0.8
Sialic acid	0.3	0.2	–	1.4	–		0.4
$S_{w,20}$[e]	6.3–7	6.5–7.1	10.8–11	18–20	–	–	4–4.9
Isoelectric point	5.5–6.8	7.5–8.3	–	–	–	–	–
$A_{1\%,1\ cm}$[f]	12.1, 13.5	12.0, 12.1	–	11.8	–	–	–

[a]Adapted from data compiled by Butler (1983, 1986) and Eigel *et al.* (1984). n/a = Not applicable.
[b]L-chains (κ and λ) predominantly of the λ type.
[c]Important for relating older literature to current nomenclature. Relative location in classical electrophoretic pattern of blood serum proteins (serum albumin; α-, β (β_1 + β_2)- and γ (γ_1 + γ_2)-globulins) in order of decreasing mobility; γ_1 was also called 'Fast γ'.
[d]Hexoses of IgG_1 and IgG_2 are mannose, galactose and glucose, present in approximately similar amounts; hexoses of SC are predominantly mannose.
[e]Sedimentation constant in Svedberg units ($\times 10^{-13}$ s).
[f]Absorptivity of a 1% solution in a 1 cm light path at 280 nm.
(Adapted from Larson, B.L., Immunoglobulins of the Mammary Secretions, in *Advanced Dairy Chemistry-1: Proteins,* 2nd edn., P.F. Fox, ed., p. 241, 1992, Elsevier Applied Science Publishers, London.)

milk. Most serum IgA is monomeric (Mestecky *et al.*, 1999b), while most IgA in external secretions is di- or tetrameric IgA and contains the J chain (~15 kDa) which links monomers together near the C-terminal of the heavy chains (Brandtzaeg, 1985). The mass of dimeric IgA, including the J chain, is approximately 370 kDa.

Intraepithelial transport of dimeric IgA occurs by binding to a polymeric Ig receptor (pIgR; Mostov and Kaetzel, 1999). Binding of polymeric IgA to the pIgR involves the constant regions of the IgA heavy chains and the J chain. The IgA complex remains bound to the receptor during intracellular transport, but the receptor is subsequently hydrolysed, leaving a portion of the receptor, called secretory component (SC; ~75 kDa), attached to the polymeric IgA molecule (Mostov, 1994; Hunziker and Kraehenbuhl, 1998; Mostov and Kaetzel, 1999) and resulting in secretory IgA (sIgA) containing the J chain and bound to SC. The ratio of dimeric to tetrameric sIgA in milk and saliva is about 3:2 (Mestecky *et al.*, 1999b), while monomeric IgA in milk and saliva represents about 5 to 10% of total IgA, respectively. Dimeric and tetrameric forms of secretory IgA increase the number of antigen-binding sites of each molecule and increase the multivalency of the sIgA.

Serum and milk IgM are complex molecules composed of five monomers of IgM linked by disulfide bonds and containing one J chain. This complex has a molecular mass of approximately 1000 kDa. The pentameric structure of IgM gives each molecule 10 antigen-binding sites. Transport of IgM through epithelial cells occurs *via* the same pIgR mechanism as secretory IgA and much of secretory IgM in milk is associated with SC.

A summary of the concentrations of Ig in blood, colostrum and milk of various species is given in Table 9.2. Immunoglobulin G is the predominant Ig in blood. The predominant colostral Ig depends on the species and particularly on the route of transfer of passive immunity from mother to offspring. Concentrations of IgG are greatest in the colostrum of ruminants and other ungulates (Table 9.2). The highest concentrations of Ig in mammary secretions are found in bovine mammary secretions removed immediately after parturition (Guidry *et al.*, 1980; Larson *et al.*, 1980; Larson, 1992). Total quantity of IgG_1 in mammary secretions of the dairy cow during the peripartum period can exceed 2 kg and can significantly reduce the concentration of IgG_1 in maternal blood serum (Larson *et al.*, 1980; Larson, 1992). Estimates of concentrations of Ig in colostrum and milk are quite variable and can be affected by parity, genetics, stage of lactation and management of the animal studied (Newstead, 1976; Oyeniyi and Hunter, 1978; Guidry *et al.*, 1980; Muller and Ellinger, 1981; Norman and Hohenboken, 1981; Devery-Pocius and Larson, 1983; Guidry and Miller, 1986; Caffin and Poutrel, 1988; Gilbert *et al.*, 1988; Pritchett *et al.*, 1991; Quigley *et al.*, 1994). Immunoglobulin G is the major isotype in bovine milk.

TABLE 9.2
Concentration of immunoglobulins and percentage of the major components in serum and mammary secretions of several species[a]

Species	*Immunoglobulin*	*Concentration (mglmL)*			*% Major component immunoglobulin*		
		Blood serum	*Colostrum*	*Milk*	*Serum*	*Colostrum*	*Milk*
Cow	IgG-total	25.0	32–212	0.72	88	85	66
(*Bos taurus*)	IgG_1	14.0	20–200	0.6			
	lgG_2	11.0	12.0	0.12			
	IgA	0.4	3.5	0.13			
	IgM	3.1	8.7	0.04			
	FSC		0.5	0.2			
Horse	IgG-total	21.9	113.4	0.39	89	88	
	IgG(T)	8.2	15.2	0.09			
	IgA	1.5	10.7	0.48			43
	IgM	1.2	5.4	0.03			
Pig	IgG	21.5	58.7	3.0	89	80	
	IgA	1.8	10.7	7.7			
	IgM	1.1	3.2	0.3			70
Dog[b]	IgG	11.1	23.4	0.24	81	68	
	IgA	0.7	9.8	2.63			85
	IgM	1.7	0.8	0.22			
Rat[c]	IgG-total	24.6	2.6	–[d]	96	76	–[d]
	IgG_{2a}	8.0	0.9	1.53			
	IgA	0.15	0.8	0.59			
	IgM	0.77	ND[e]	ND[e]			

Table 9.2 (Continued)

Species	*Immunoglobulin*	*Concentration (mglmL)*			*% Major component immunoglobulin*		
		Blood serum	*Colostrum*	*Milk*	*Serum*	*Colostrum*	*Milk*
Human	IgG	12.1	0.43	0.04	78		
	IgA	2.5	17.35	1.00		90	87
	IgM	0.93	1.59	0.10			
	FSC		2.09	0.02			

[a]Approximate values, some from limited observations. Data compiled and calculated from: human and pig (Butler, 1974); rat (Stechschulte and Austen, 1970; Bazin *et al.*, 1974; McGhee *et al.*, 1975; Michalek *et al.*, 1975; Rousseaux and Bazin, 1979); dog (Vaerman and Heremans, 1969; Heddle and Rowley, 1975); horse (Rouse and Ingram, 1970; Vaerman *et al.*, 1971; McGuire and Crawford, 1972); cow (Butler, 1981,1983; Devery-Pocius and Larson, 1983). Certain IgG subclasses for rat, horse and cow are shown and included in the total IgG. Where subclasses in other species were reported, they are grouped in the total for the class.
[b]See Larson (1992) for discussion of dog colostral Ig concentration reported by others.
[c]Data for the rat are inconsistent. Values given are average from several studies. See Larson (1992).
[d]Data not available but total IgG estimated to be >1.53 mg/ml and 72% (Larson, 1992).
[e]ND = Not consistently detected.
(Adapted from Larson, B.L., Immunoglobulins of the Mammary Secretions, in *Advanced Dairy Chemistry-1: Proteins,* 2nd edn., P.F. Fox, ed., p. 245, 1992, Elseiver Applied Science Publishers, London.)

The primary Ig isotype in human colostrum and milk is IgA (Table 9.2). Combined with high concentrations of lactoferrin (Chapter 10) and high activity of lysozyme, human milk has a particularly high antimicrobial activity (Goldman, 1993; Xanthou *et al.*, 1995; Goldman and Ogra, 1999). Data on Ig concentrations in rat mammary secretions are less consistent and may represent differences due to several factors (discussed by Larson, 1992). Because most estimates of rat IgG have focused on the IgG_{2a} subclass, total IgG is difficult to estimate. Nevertheless, the amount of IgG_{2a} exceeds the concentrations reported for rat colostral IgA, and presumably IgG is the major colostral isotype (Table 9.2). This would be consistent with the much studied specific intestinal absorption of IgG by the neonatal rat intestine.

(a) Nutritional value of colostral immunoglobulin

Estimates of amino acid compositional data of colostral IgG has been summarized by Larson (1992). The true nutritional value of colostral Ig to the neonate is determined by the concentration of Ig in colostrum, the rate of gastric emptying and the susceptibility of the Ig to digestion (Yvon *et al.*, 1993). Bovine IgG_1 is hydrolyzed extensively by trypsin, but little by chymotrypsin (Brock *et al.*, 1977). In the newborn lamb, bovine IgG has a high gastric emptying rate, but as a group, Ig are only poorly digested in the gastrointestinal tract. Nevertheless, because IgG is the major protein in bovine colostrum, it does serve as a major source of amino acids for the neonate, along with β-lactoglobulin and casein (Yvon *et al.*, 1993).

9.2.3 Cell-mediated immunity

Mammary gland and milk leucocytes play important roles in mammary immunobiology (Newby *et al.*, 1982; Paape and Capuco, 1997; Sordillo *et al.*, 1997) and in immunity of the neonate (Slade and Schwartz, 1987; Xanthou *et al.*, 1995; Xanthou, 1998). Leucocyte concentration in mammary secretions varies considerably with species, stage of lactation, and physiological and pathological states of the mammary gland (Hurley, 1989; Goldman, 1993; Sordillo *et al.*, 1997; Maunsell *et al.*, 1998). Somatic cell count in milk from bovine mammary glands with bacterial infections can increase rapidly within a few hours of infection (Harmon *et al.*, 1976). In the dairy industry, milk leucocyte concentration (somatic cell count) has been the basis for recognizing and quantifying mammary gland inflammatory responses. Milk leucocytes primarily consist of neutrophils, macrophages and lymphocytes. Milk also may contain a small percentage of epithelial cells.

Neutrophils generally are not considered to play a role in the cellular immunity of the mammary gland; rather, they offer a phagocytic defense against infection (Paape *et al.*, 1981; Sordillo *et al.*, 1997). Milk macrophages

are also phagocytic and are capable of ingesting bacterial cells, milk components and cellular debris. Phagocytosis by macrophages is increased by opsonization of antigens with specific antibodies. Macrophages also play an important role in cellular-mediated immunity through their antigen processing and presentation functions (Sordillo *et al.*, 1997). Intracellular processing of ingested antigen results in the appearance of antigen at the macrophage cell surface in association with major histocompatibility complex (MHC) class II antigens. Specific immunity arises from the interaction of antigen-presenting cells, such as macrophages, with lymphocytes.

(a) Mammary lymphocytes

Lymphocytes are divided into two distinct types, T and B cells. Populations of T lymphocytes include several subtypes, including αβ and γβ T lymphocytes. The αβ T lymphocytes include subpopulations of T-helper (CD4+) lymphocytes and T-cytotoxic or T-suppressor (CD8+) lymphocytes. The CD4+ T-helper-1 cells promote cellular responses against intracellular pathogens and viruses and the T-helper-2 cells promote humoral immunity. Cytotoxic CD8+ cells act as scavengers of old or damaged cells in tissues. Suppressor CD8+ cells can modulate the immune response by suppressing host immune responses. The γδ T lymphocytes are associated with the protection of epithelial surfaces. Another lymphocyte involved in host immune responses is the natural killer (NK) cell which has Fc receptors. Binding of antibody to NK cells results in antibody-dependent, cell-mediated cytotoxicity. This NK-like activity includes antibacterial properties (Sordillo *et al.*, 1997).

Recognition of antigen by B lymphocyte surface receptors results in internalization of bound antigen. Antigen is processed and presented at the cell surface associated with MHC class II antigens. These complexes stimulate T-helper cells to secrete a cytokine which causes B lymphocytes to proliferate and differentiate into either antibody-producing plasma cells or memory cells. Percentages of B lymphocytes in mammary tissue and in milk remain relatively constant between stages of lactation (Shafer-Weaver *et al.*, 1996), although total numbers of plasma cells and proportions of Ig isotype-specific B lymphocytes are altered during mammary infection (Nickerson and Heald, 1982). In contrast, T lymphocyte populations and their functions in mammary tissue change with stage of lactation (Sordillo *et al.*, 1997). The ratio of CD4+ to CD8+ cells in mammary tissue and mammary secretions is generally less than one, with CD8+ cells predominating (Sordillo *et al.*, 1997; Yamaguchi *et al.*, 1999). This is in contrast to blood in which the CD4+ to CD8+ ratio is greater than one. Bovine mammary tissue CD4+ lymphocytes are predominantly the T-helper-2 type in the immediate post-partum period and T-helper-1 type in mid and late lactation (Shafer-Weaver *et al.*, 1999).

Mammary tissue CD8+ lymphocytes are localized primarily in contact with the basal surface of the alveoli and between epithelial cells, while CD4+ cells are distributed equally in epithelial and connective tissue areas (Yamaguchi *et al.*, 1999). The CD8+ lymphocytes localized between epithelial cells have been referred to as mammary intraepithelial lymphocytes (mIEL) and may function to protect the integrity of the mammary epithelium (Yamaguchi *et al.*, 1999). The mIEL may function in a manner similar to intestinal intraepithelial lymphocytes (iIEL). The role of iIEL in the intestine is an area of active debate (Kelsall and Strober, 1999). Further study of the mIEL is required to understand their role in mammary immunity.

9.3 Transfer of passive immunity

The mammalian immune system develops slowly and the offspring are initially dependent upon maternal antibodies for disease protection. A considerable range of mechanisms of transport of passive immunity from mother to neonate exists among mammalian species (Table 9.3). The neonate of ungulates is born essentially agammaglobulinemic and requires absorption of substantial amounts of maternal antibodies from colostrum to attain sufficient systemic immunity to protect it from disease during early postnatal development. In these species, IgG_1 is typically the major Ig found in colostrum. The presence of IgG_1 in high concentrations in the first mammary secretions consumed by the neonate coincides with extensive, but short-lived macromolecular absorption by the neonate intestine. In contrast, the human fetus acquires systemic IgG primarily during the last trimester of gestation *via* transport across the placental membrane. The variation among species in the mechanism of transport of immunity from mother to neonate is one particularly interesting aspect of the role of Ig in mammary secretions.

9.3.1 Immunoglobulin transport systems

The major portion of Ig found in the alveolar lumen is transported across the mammary epithelial cells by a receptor-mediated transport system (Table 9.3). In the pre-partum cow, this transport appears to occur through a Fc receptor-mediated endocytic mechanism (Lascelles, 1979; Leary and Larson, 1982) which has a high affinity for IgG_1 (Sasaki *et al.*, 1977). This high affinity mechanism accounts for the particularly high concentration of IgG_1 in bovine colostrum. During the formation of colostrum, bovine mammary epithelial cells rapidly take up IgG_1 at their basolateral membrane surface and large amounts of IgG_1 can be observed both in the cells and accumulated in the lumen at the time of maximum transport just preceding parturition (Leary *et al.*, 1982; Larson, 1985). Binding of IgG_1 to epithelial cells during lactation also might be responsible for the low

TABLE 9.3
Routes of transmission of immunoglobulins from mother to neonate

	Species	*Primary route of transmission*	*Primary colostral Ig*	*Neonate intestinal selectivity*[a]	*Absorption period*	*Primary impact of colostral Ig*[b]
Ungulates	Cow Goat Pig Horse	Fcγ-receptor, Mammary gland	IgG_1	Non-selective	24 h 24 h 36 h 36 h	Systemic immunity
Carnivores	Dog Cat	Fcγ-receptor, Placenta/yolk sac	IgG	Non-selective, (minor absorption)	brief, variable	Intestinal protection
Rodents	Rat Mouse	Fcγ-receptor, Yolk sac (minor)	IgG	Selective for IgG, Fc_γ-receptor	21 days	Systemic immunity
Lagomorphs	Rabbit	Fcγ-receptor, Yolk sac	IgA	(minor absorption)	–	Intestinal protection
Primates	Human	Fcγ-receptor, placenta	IgA	(trace absorption)	–	Intestinal protection

[a]Intestinal absorption occurs in the jejunum (Staley and Bush, 1985).
[b]Indicates the most significant impact of colostral Ig on the neonate. Intestinal protection by colostral Ig probably occurs in all species. Colostral Ig may affect development of neonate immune system (see text, Section 9.3.4).

concentrations of the IgG found in bovine milk (Sasaki *et al.*, 1977); however, mammary tissue leucocytes constitute the primary IgG_1-binding cell type rather than the epithelial cells (Barrington *et al.*, 1997a). The transepithelial transport mechanism in the mammary gland requires further investigation to understand the specific molecular entity of these Fc receptors, the intracellular route of IgG_1 transport and the control of receptor expression.

Transport of IgG across the placental barrier during pregnancy in primates has been studied more than the transport of IgG in the mammary gland. Several Fcγ receptors (FcγR) have been studied for their potential role in transplacental IgG transport (Simister and Story, 1997). Macrophages localized in the placental stroma contain several FcγR which may be involved in clearing immune complexes transported across the syncytiotrophoblast (Simister, 1998). Immune cell FcγR also have been identified in placenta and are detectable in other tissues (de Haas *et al.*, 1995; Daeron, 1997; Simister and Story, 1997). The neonatal Fc receptor (FcRn) is the most probable candidate for the transplacental transport of IgG (Simister and Ahouse, 1996; Simister and Story, 1997; Hunziker and Kraehenbuhl, 1998). This receptor is found in syncytiotrophoblast cells and fetal endothelial cells. The FcRn has been identified as an Fc receptor from the neonatal rat intestine (Rodewald and Kraehenbuhl, 1984; Simister and Mostov, 1989), rat yolk sac (Roberts *et al.*, 1990), and mouse yolk sac and intestine (Ahouse *et al.*, 1993). The FcRn is composed of two polypeptide chains. The MHC class I protein, β_2-microglobulin, is the smaller subunit (Hunziker and Kraehenbuhl, 1998). The larger subunit of FcRn is an integral membrane protein structurally related to MHC class I α chains (Simister and Mostov, 1989; Burmeister *et al.*, 1994; Ghetie and Ward, 1997). Human syncytiotrophoblast contains FcRn messenger RNA and protein, as well as β_2-microglobulin. Of the placental Fc receptors, only FcRn has a known role in IgG transport (Simister and Story, 1997; Hunziker and Kraehenbuhl, 1998). However, mice in which the β_2-microglobulin gene was deleted still have normal concentrations of IgG in milk (Velin *et al.*, 1996). A role for FcRn in mammary gland IgG transport remains to be demonstrated. The FcRn is found in other tissues in adult rodents and humans and may function to remove circulating IgG from degradation (Simister *et al.*, 1997).

In species where IgG transport occurs during gestation, IgA generally is the major colostral and milk Ig (Tables 9.2 and 9.3). Transport of IgA through mammary epithelial cells appears to be similar to other secretory tissue sources (Brandtzaeg, 1997). pIgR (see Section 9.2.2) is a transmembrane glycoprotein synthesized in the mammary epithelial cell. The pIgR binds dimeric IgA or pentameric IgM at the basolateral membrane (Mostov, 1994; Morton *et al.*, 1996; Raghavan and Bjorkman, 1996; Mostov and Kaetzel, 1999). The receptor-IgA or receptor-IgM complex moves through

the mammary secretory cell *via* an endocytic pathway to the apical surface (Hunziker and Kraehenbuhl, 1998; Mostov and Kaetzel, 1999). In contrast to the MHC class I-related FcRn, pIgR has homology with the variable domains of Ig (Krajci *et al.*, 1995; Mostov and Kaetzel, 1999). Binding of dimeric IgA to plgR activates a signalling pathway involving protein kinase C and inositol-4,5-triphosphate and an increase in intracellular calcium (Hunziker and Kraehenbuhl, 1998; Mostov and Kaetzel, 1999). This receptor is responsible for transepithelial transport of secretory IgA in tissues like intestine and mammary gland. In contrast, the myeloid FcαR is found on neutrophils and monocytes and is structurally related to FcγR (Kerr and Woof, 1999).

9.3.2 Mammary plasma cells and colostral immunoglobulins

The extreme quantities of Ig in the colostrum of ungulates is accounted for primarily by transepithelial transport from the maternal serum. However, the contribution of Ig synthesized by local plasma cells in the mammary tissue should be considered also. Mammary gland plasma cells lie adjacent to the mammary alveolar epithelial cells (Nickerson and Heald, 1982; Sordillo and Nickerson, 1988). Plasma cells producing IgG, IgA and IgM isotypes are present in bovine mammary tissue, with IgG-producing plasma cells predominating during lactation and mammary gland involution (Yurchak *et al.*, 1971; Sordillo and Nickerson, 1988). The numbers of mammary tissue plasma cells can increase during infection, with largest increases occurring in the IgA-producing cells (Nickerson and Heald, 1982), although others (Doymaz *et al.*, 1988) have not found an increase in mammary plasma cell numbers during infection.

Mammary plasma cells arise from migration of lymphocytes from the intestinal mucosal immune system (Husband, 1985; Hunziker and Kraehenbuhl, 1998). Maternal exposure to antigens *via* the gastrointestinal tract results in activation of B lymphocytes found in gut-associated lymphoid tissue (GALT) which includes Peyer's patches, lymphoid and myeloid cells in the *lamina propria* and IELs (Kelsall and Strober, 1999). Some of these lymphocytes migrate to the mammary gland to become mammary IgA-secreting plasma cells, thereby providing a direct link between intestinal and mammary immune systems (Telemo and Hanson, 1996; Hunziker and Kraehenbuhl, 1998). The local mammary production and secretion of IgA specific for intestinal antigens, and possibly for respiratory antigens (Telemo and Hanson, 1996), means that milk contains antibodies against potential pathogens encountered by the neonatal mucosal tissues. Differences in Ig distribution between milk and other external secretions is dependent upon the antigen specificity of the migrating B lymphocytes (Dahlgren *et al.*, 1987).

9.3.3 Absorption in the neonate

Intestinal uptake of macromolecules, including Ig, occurs by an endocytic pathway (Staley and Bush, 1985). For a period after birth, this pathway results in transport of macromolecules across the enterocyte, followed by release into the *lamina propria* from which the macromolecules can be absorbed into the lymphatic or portal circulation. Intestinal closure occurs when this macromolecular transport is terminated even though uptake of macromolecules into enterocytes may continue (Staley and Bush, 1985). Transport of macromolecules occurs primarily in the small intestine (Table 9.3) and particularly in the jejunum (Staley and Bush, 1985). Selectivity of transport of macromolecules by the neonate intestine varies with species. In the newborn human, guinea pig and rabbit, little Ig is transported across the enterocytes and the intestine is selective to the point of exclusion of all proteins (Table 9.3). In contrast, ungulates exhibit little selectivity toward proteins which are absorbed prior to closure. Rodents form an intermediate group in which there is high selectivity in transport of IgG across the intestinal barrier which occurs *via* the FcRn (see Section 9.3.1). Selective transport of IgG by the rat intestine continues for about 3 weeks.

Intestinal closure generally is considered to be completed in ruminants by about 24 h after birth and in about 36 h in pigs and horses (Table 9.3). Delaying feeding of colostrum to calves after birth reduces the amount of Ig absorbed by the intestine. Loss of absorptive capacity of the intestine begins soon after birth and progresses continuously until closure is complete. The process of closure is affected by environmental stress, by severe dystocia and possibly by the nutritional status of the calf (discussed by Davis and Drackley, 1998). Failure to transfer passive immunity results in significant risk of the neonate to disease. Serum Ig concentration in calves is associated with calf mortality, disease and growth, and with milk production when the calf matures (Nocek *et al.*, 1984; Donovan *et al.*, 1986; Robison *et al.*, 1988; DeNise *et al.*, 1989; Selim *et al.*, 1995; Wells *et al.*, 1996). Failure of the transfer of passive immunity is generally considered to have occurred when a calf's blood IgG concentration at 48 h after birth is less than 10 mg/mL (Bovine Alliance on Management and Nutrition, 1995). Over 40% of calves have a blood IgG concentration of less than 10 mg/ml (National Animal Health Monitoring System, 1993).

9.3.4 Other roles in protection of the neonate

The value of colostral and milk Ig, particularly IgA, for protection of the gastrointestinal tract is well established (Rejnek *et al.*, 1968; Renegar and Small, 1999). The effect of breast feeding in humans extends beyond

protection of the infant against gastrointestinal disorders to include respiratory infections, urinary infections and others (Hanson and Telemo, 1999). Furthermore, ingestion of products of the mammary gland immune system by the neonate may affect the development of the neonate's own immune responses (Hanson and Telemo, 1999). Studies on calves and pigs indicate that maternal IgG can reduce *de novo* antibody synthesis (Husband and Lascelles, 1975; Klobasa *et al.*, 1981); however, other studies on mice indicate that maternal IgG does not affect *de novo* Ig synthesis (Delassus *et al.*, 1997). In addition to a direct role of milk IgA in the protection of the gastrointestinal tract, breast feeding by humans promotes the development of the local intestinal immune response and production of IgA (Prentice, 1987; Koutras and Vigorita, 1989). Transfer of maternal milk leucocytes to the neonate (Archambault *et al.*, 1988; Tuboly *et al.*, 1988; Riedel-Caspari, 1993; Williams, 1993; Hanson and Telemo, 1999) and consumption of anti-idiotypic antibodies contained in human milk (Hanson and Telemo, 1999) also may affect the development of neonatal immunity.

9.4 Control of transport and mammary gland immunity

Transepithelial transport of Ig in the mammary gland occurs in relation to the physiological state of the mammary tissue. Expression of the pIgR in rabbit mammary tissue is inhibited by elevated progesterone and estrogen concentrations, but is stimulated by prolactin (Rosato *et al.*, 1995). This is consistent with the pre-partum increase in mammary tissue IgA transport and plgR expression (Rosato *et al.*, 1995). Expression of pIgR also is regulated by cytokines (Hunziker and Kraehenbuhl, 1998).

A role for ovarian steroid hormones in stimulating selective transport of IgG in the bovine mammary gland was demonstrated originally when treatment of non-lactating cows with estrogen and progesterone resulted in the formation of colostrum (Smith *et al.*, 1971). This observation has provided the basis for many subsequent efforts to hormonally induce lactation in cattle. Administration of glucocorticoid to estrogen- and progesterone-treated cows results in a reduced IgG_1 concentration in mammary secretions (Winger *et al.*, 1995). Recent studies suggest that prolactin may decrease the expression of IgG_1 receptor in bovine mammary tissue (Barrington *et al.*, 1997b). Both glucocorticoid and prolactin are part of the lactogenic complex of hormones responsible for inducing lactation. Pre-partum removal of mammary secretions in cattle can alter the concentration of IgG_1 in secretions (Guy *et al.*, 1994). Unilateral removal of secretions suggests that local mammary gland factors also affect IgG_1 transport (Guy *et al.*, 1994). Both hormonal and local factors contribute to the control of IgG_1 transport in the ruminant mammary gland (McFadden

et al., 1997). Further research is needed to define the mechanisms which control the bovine IgG_1 receptor and IgG transport in the mammary gland.

Enhanced selective transport of IgG_1 during involution of the ruminant mammary gland occurs transiently during the initial days after milk stasis (Watson *et al.*, 1972; Zou *et al.*, 1988). In contrast, transport of IgA may persist longer after milk stasis in the bovine mammary gland (Zou *et al.*, 1988). Selective transfer of IgG_1 into milk occurs during mammary gland inflammation (Darton and McDowell, 1980), resulting in acute increases in the concentration of Ig in milk during mastitis (Harmon *et al.*, 1976; Guidry and Miller, 1986; Caffin and Poutrel, 1988).

9.5 Interactions with other proteins in milk

Physical and functional properties of Ig in mammary secretions can be affected by other proteins found in milk. Secretory IgA is bound to SC, which is part of the plgR (see Section 9.2.2). Free SC is also present in colostrum and milk (Pringnitz *et al.*, 1985a,b). β_2-Microglobulin is part of the FcRn. β_2-Microglobulin was originally isolated from bovine milk and called lactollin (Groves *et al.*, 1963; Groves and Greenberg, 1982). Lactollin was subsequently found to be the MHC gene product, β_2-microglobulin. It has a monomeric molecular mass of approximately 12 kDa, but exists as a tetramer in milk (Whitney, 1988). Free bovine milk β_2-microglobulin may arise from milk monocytes (Pringnitz *et al.*, 1985a,b). A specific role for the FcRn, or for the β-microglobulin subunit, in IgG transport in the mammary gland has not been demonstrated (see Section 9.3.1).

Immunoglobulins interact with another milk antimicrobial protein, lactoferrin (Chapter 10). Lactoferrin is an 80 kDa glycoprotein which is generally known for its iron-binding properties. Lactoferrin also binds a variety of other macromolecules, including Ig (Hurley, 1993). A large proportion of lactoferrin in bovine mammary secretions can exist in complexes with other proteins, including Ig (Wang and Hurley, 1998). Whether Ig-lactoferrin complexes affect the antimicrobial activity of either protein is not known. However, a clear cooperativity of antimicrobial activity between lactoferrin and IgA from human milk has been noted (see Hurley, 1993).

9.6 Manipulation of mammary gland immunity

Milk from various species has been recommended for treating a number of human diseases and health problems, even in ancient times (see Mestecky *et al.*, 1999a). Manipulation of mammary gland immunity holds significant promise for control and treatment of disease in the neonate and adult human and in other species, as well as for controlling mastitis in the

mammary gland. Immunization of cattle during the dry period between successive lactations has been used successfully for control of coliform mastitis. Immunization of dairy cattle with a mutant strain of *Escherichia coli* 0111:B4 (J5) during the dry period, while generally not preventing intramammary infection, does result in reduced severity and lower rates of clinical coliform mastitis compared with unimmunized controls (Gonzalez *et al.*, 1989; Hogan *et al.*, 1995). Immunization with other coliform antigens and approaches for controlling other mastitis pathogens in cattle by immunization are under active investigation (Amorena *et al.*, 1994; Scott *et al.*, 1998; Lin *et al.*, 1999).

Manipulation of mammary gland immunity to provide specific passive immunization against gastrointestinal diseases for the neonate or for adults also has shown substantial promise (Mestecky and Russell, 1998). Immunoglobulins isolated from colostrum of cows immunized against human intestinal pathogens are effective in protecting young gnotobiotic pigs against challenge infections of those pathogens (Cordle *et al.*, 1991). Immunization of sows against transmissible gastroenteritis virus enhances the protective value of the sow colostrum and milk for the suckling pig (Aynaud *et al.*, 1991; Salmon, 1995).

Intragastric gavage of rabbit pups with human secretory IgA protects against challenge with *Escherichia coli* K100 (Maxson *et al.*, 1996). Successful prophylaxis of bacterial and viral diarrhea in humans and treatment of human rotaviral infections has been achieved in children and adults with bovine Ig (Tacket *et al.*, 1988; Davidson *et al.*, 1989; Levine, 1991; Mitra *et al.*, 1995; Bogstedt *et al.*, 1996). Other examples of the use of bovine colostrum and colostral Ig in human clinical applications are discussed by McFadden *et al.* (1997).

9.7 Conclusion

Current interest in Ig of mammary secretions extends well beyond the transfer of passive immunity from mother to neonate. Substantial progress has been made in characterizing the biochemical and molecular structure of Ig, including those in mammary secretions of several species. Furthermore, the impact of a high concentration of Ig in colostrum on neonate passive immunity is generally appreciated. However, considerable opportunity still exists for enhancing our understanding in several important areas. Characterization of the transport mechanism for IgG in the mammary gland remains a prime area for investigation. The specific role of Ig in cell-mediated immunity and immunobiology of the mammary gland is an area of great opportunity, as is the role of Ig and other mammary-derived components in the development of the neonatal immune system. Success in manipulating mammary immunity for protection against mastitis has been

built upon an increasing understanding of mammary immunobiology. Capitalizing upon this knowledge for the ultimate purpose of further enhancing mammary gland disease resistance and of using bovine colostral Ig to provide passive immunity for humans requires continued investigation into all aspects of mammary immunobiology.

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