ELSEVIER

The MHC Class I Related Fc Receptor, FcRn, is Expressed in the Epithelial Cells of the Human Mammary Gland

Petru Cianga, Corina Cianga, Laurette Cozma, E. Sally Ward, and Eugen Carasevici

ABSTRACT: The major histocompatibility complex (MHC) class I related neonatal Fc receptor (FcRn) plays multiple roles, being involved in transporting immunoglobulin G (IgG) and protecting this antibody class from catabolism. The presence of this receptor was previously demonstrated in the lactating murine mammary gland. In the current study we have investigated FcRn expression in various histologic types of human breast carcinoma and lymph node metastases. We used immunohistochemical methods to demonstrate the presence of FcRn in epithelial cells, whereas this Fc receptor could not be detected in mammary gland endothelial cells. The presence of the receptor was also found in the metastasizing epithelial cells within the lymph nodes, and this provides a useful marker for their identification. *Human Immunology 64, 1152–1159 (2003).* © American Society for Histocompatibility and Immunogenetics, 2003. Published by Elsevier Inc.

KEYWORDS: FcRn; mammary gland; epithelial cells; endothelial cells; metastasis

ABBREVIATIONS

FcRn	neonatal Fc receptor
PBS	phosphate buffered saline
MHC-I	major histocompatibility complex class I
HRP	horse radish peroxidase
DAB	diaminobenzidine
ECL	enhanced chemoluminescence
BSA	bovine serum albumine

INTRODUCTION

The neonatal Fc receptor (FcRn) is the only known Fc receptor with an major histocompatibility complex (MHC) class I-like structure [1]. The receptor binds to immunoglobulin G (IgG, reviewed in [2]) and it is involved in the transport of IgGs within and across cells. In addition, FcRn plays a role in salvaging IgG from degradation and, therefore, regulates the serum levels of IgG (reviewed in [3]). FcRn was initially identified in the rat neonatal intestine as the molecule responsible for the transport of maternal IgG from mother's milk across the brush border epithelial cells of neonates [4]. Further studies demonstrated that the receptor is expressed both neonatally or at the maternal–fetal barrier (brush border, yolk sac, some stromal placental cells, and syncytiotrophoblast [5–7]), and in adult tissues. Expression in mammalian adult endothelial cells [8–10], hepatocytes [11, 12], histiocytes [13], monocytes, intestinal macrophages, dendritic cells [14], intestinal epithelial cells [15, 16], bronchial epithelial cells [17], kidney epithelial cells [18, 19], at the blood–brain barrier (brain microvasculature and choroid plexus epithelium) [20], and adipose tissue [10] has been reported, indicating that FcRn functions in a wide array of tissues throughout the body. Heterogeneity in the distribution in endothelial cells of the muscle capillaries was observed, and this was attributed to the morphologic differences between the cells that line various types of capillaries [10, 21]. However,

From the Immunology Department (P.C., C.C., E.C.), University of Medicine and Pharmacy, "Gr. T. Popa," and Public Health Institute (L.C.), Iasi, Romania; and Center for Immunology and Cancer Immunobiology Center (S.E.W.), University of Texas Southwestern Medical Center, Dallas, TX, USA.

Address reprint requests to: Dr. Petru Cianga, Laboratorul de Imunologie Tumorala, Spital Universitar, "Sf. Spiridon", Blvd. Independentei 1, Iasi-6600, Romania; Tel/Fax: +40 (232) 213212; E-mail: petrucianga@hotmail.com.

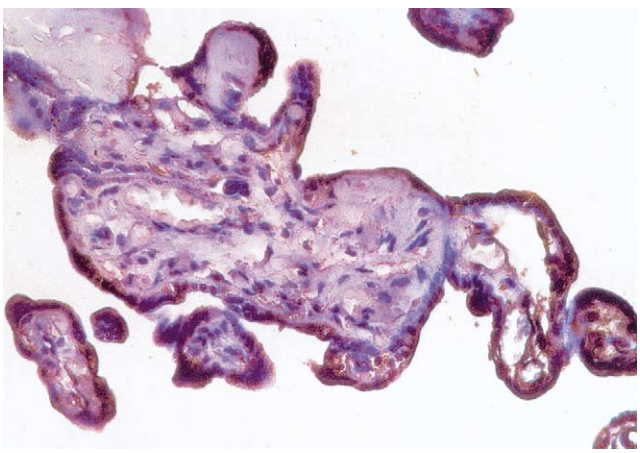
Received August 7, 2002; revised July 28, 2003; accepted August 1, 2003.

0198-8859/03/$–see front matter
doi:10.1016/j.humimm.2003.08.025

FIGURE 1 Immunoprecipitation of human endothelial cell (HME.C 1) lysates with anti-neonatal Fc receptor (anti-FcRn) sera made by immunizing rabbits with denatured, recombinant human FcRn α-chain [8] or FcRn derived peptide [LNGEEFMKFNPRIG]. The sizes of the α chain and β_2-microglobulin (β_2-m) are estimated to be 50 kDa and 16 kDa, respectively. The presence of β_2-m was also confirmed using an antihuman β_2-m antibody (not shown).

FcRn expression could not be detected in endothelial cells of human placenta nor rodent mammary gland, indicating that there may be variations in expression in distinct endothelial cell types [5, 13]. However, in some studies [6, 7] placental endothelial expression was reported, indicating that this issue is controversial.

FIGURE 2 Immunohistochemical staining of chorial villi of human placenta, illustrating positive staining for neonatal Fc receptor (FcRn) in the surface trophoblast (original magnification ×200).



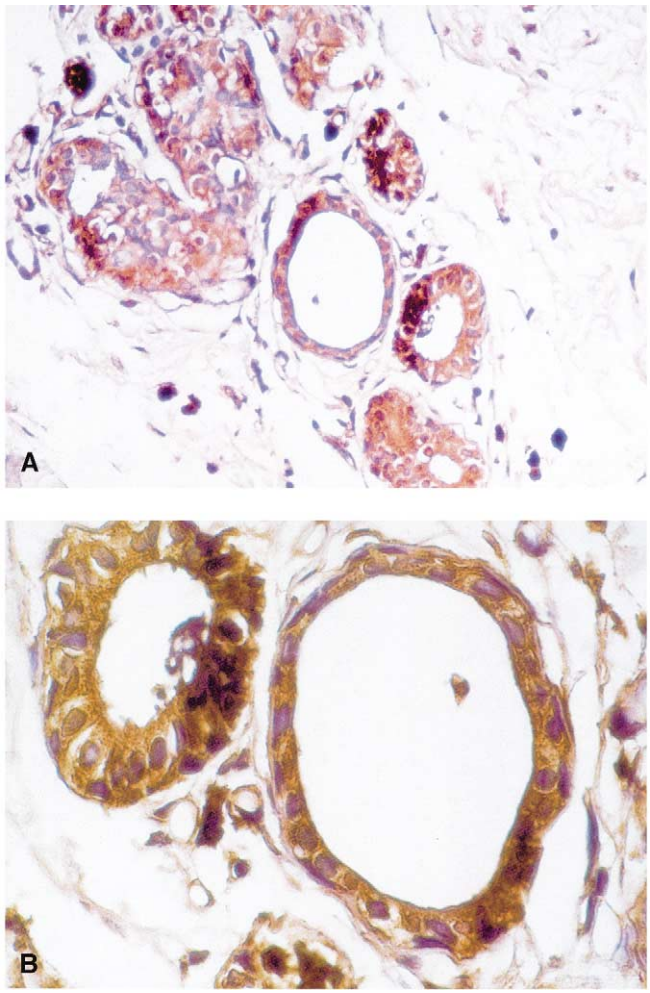


FIGURE 3 (A) Immunohistochemical staining of a benign tumor periphery. Normal acini and a dilated duct with no secretion. Positive staining for neonatal Fc receptor (FcRn) of the epithelial cells (original magnification ×200). (B) Cytoplasmic staining pattern of the epithelial cells for FcRn (original magnification ×1000).

The function of FcRn appears to be to transport IgG within and across (polarized) cells, and observations with FcRn transfected epithelial (MDCK or IMCD) cells provide support for this [22, 23]. In a previous report, we were able to demonstrate the presence of FcRn within the epithelial cells of the murine lactating mammary gland, whereas the endothelial cells were negative [13]. Furthermore, we were able to demonstrate that transport of IgG into mouse milk occurs in inverse correlation with affinity of the IgG for FcRn, suggesting that it acts at this site primarily as a recycling receptor [13]. This recycling most likely resembles the process by which IgG homeostasis has been proposed to be maintained by endothelial cells [2]. However, our observations in mouse mammary glands

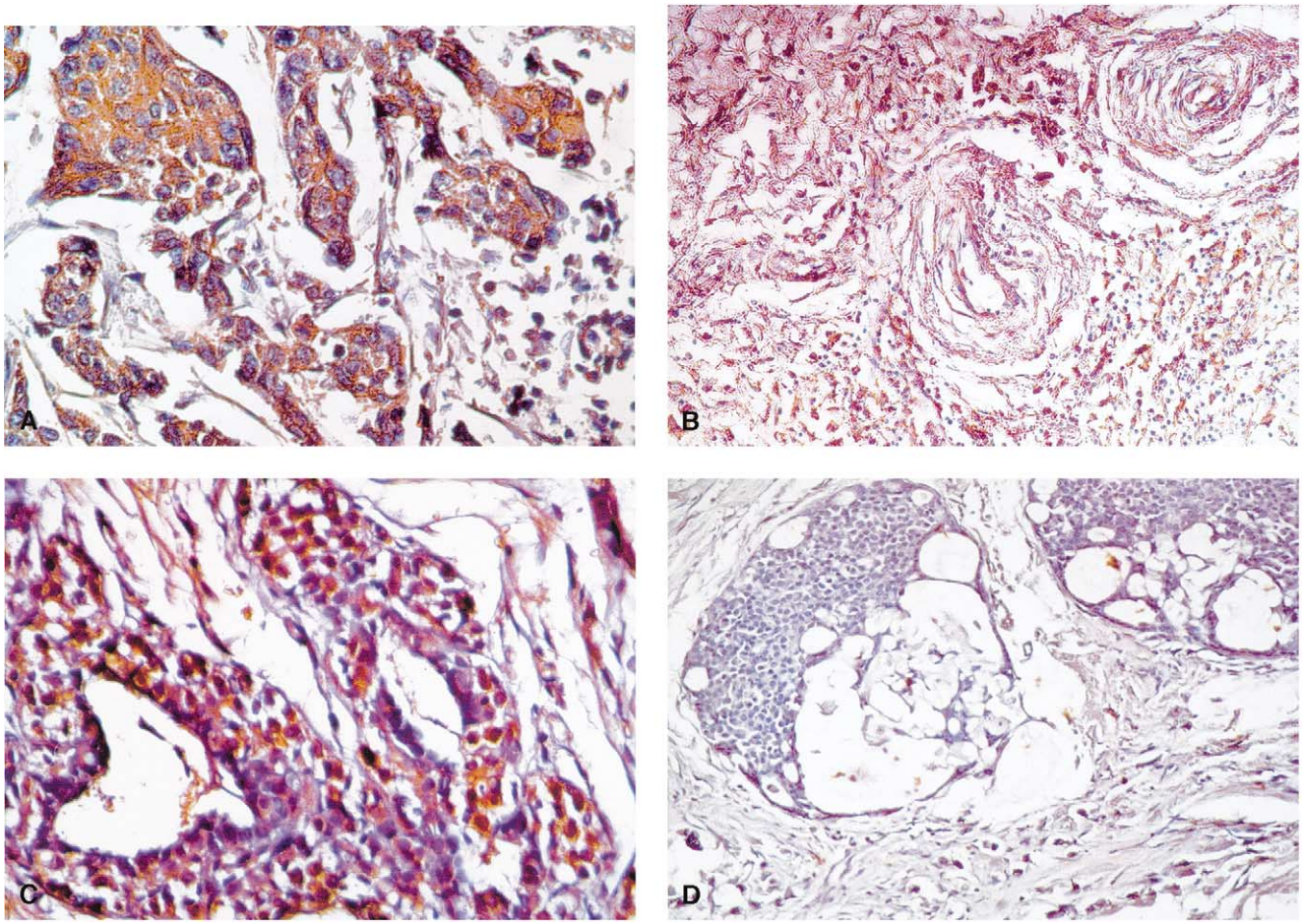


FIGURE 4 (A) Ductal invasive carcinoma, revealing positive staining for neonatal Fc receptor (FcRn; original magnification ×400). (B) Medulary carcinoma, illustrating positive staining for FcRn (original magnification ×200). Residual tumor cells after radiotherapy. (C) In situ lobular carcinoma developed on a proliferative fibrocystic mastopathy with an atypic ductal and lobular hyperplasia, demonstrating positive staining for FcRn within the epithelial cells (original magnification ×400). (D) Ductal invasive carcinoma labeled with rabbit IgG polyclonal antibodies as a negative control (original magnification ×200).

do not exclude the possibility that FcRn also acts to transport IgG into mother's milk by transcytosis, but indicate that recycling predominates. In this context, a mutated IgG that does not bind to FcRn is transported into milk [13], suggesting that in addition to FcRn-mediated transcytosis, other pathways are involved in transport.

In humans, the transmission of maternal IgG to mediate passive immunity occurs primarily *in utero* rather than during the neonatal period [24]. Consistent with this, the amount of IgG in human milk secretion is 50 mg/l and 1 g/l in the colostrum [25]. The aim of this study was to investigate if FcRn is present in the human mammary gland. We were also interested to see if the receptor is present in various types of breast carcinomas and if the malignant transformation of the mammary gland inhibits FcRn expression.

MATERIALS AND METHODS

Immunoprecipitation

Dermally derived human endothelial cells (HMEC.1; available from the Centers for Disease Control [Atlanta, GA, USA], and generously provided by Mr. Francisco Candal) were treated with EZ-link-Sulpho-NHS-biotin (Pierce, Radisson, IL, USA) for 30 minutes at room temperature and lysed as previously described [10]. Lysates from $\sim 6 \times 10^6$ cells were incubated with preimmune rabbit serum and protein G-Sepharose, and the unbound fraction incubated with anti-FcRn peptide serum (1:16 diluted; isolated from rabbit serum following immunization with the YCLNGEEFMKFNPRIG peptide coupled to BSA, obtained as previously described [8]), and protein G-sepharose. This peptide is derived from murine FcRn and the corresponding region in human FcRn is highly homologous [26, 27].

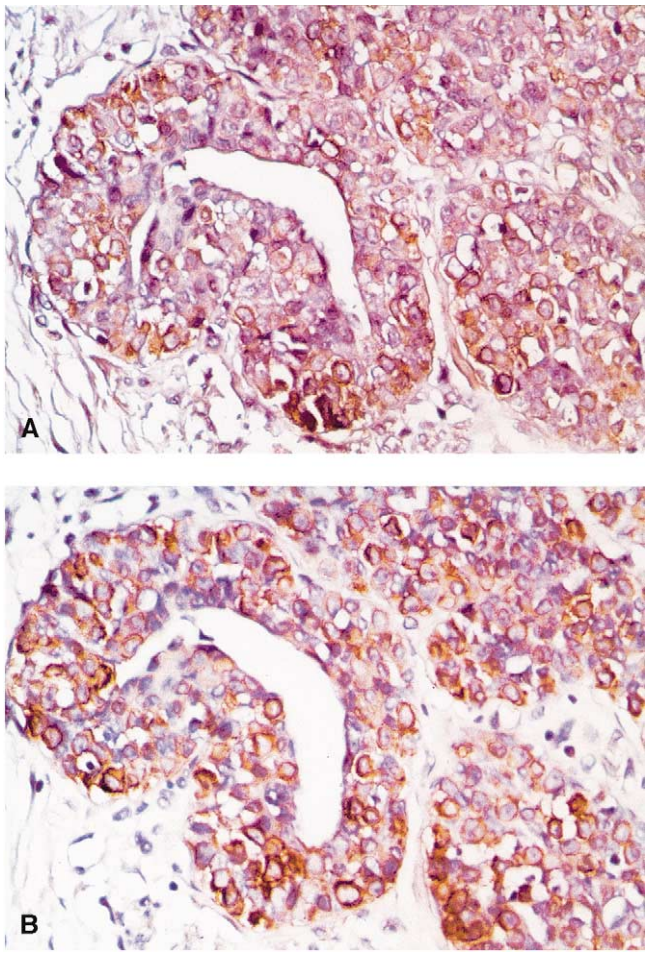


FIGURE 5 (A) In situ multifocal lobular carcinoma revealing positive staining for neonatal Fc receptor (FcRn; original magnification ×200). (B) Positive staining for cytokeratin (original magnification ×200).

In addition, sera isolated from rabbits immunized with gel purified, denatured recombinant human FcRn α chain [28] were also used at a 1:16 dilution. Sepharose beads were extensively washed and run on 15% polyacrylamide gels. Following transfer of electrophoresed proteins to PVDF membranes, membranes were incubated with extravidin-horse radish peroxidase conjugate. Bound conjugate was detected with the enhanced chemoluminescence (ECL) detection kit (Amersham, Braunschweig, Germany).

Source of Tissue

The samples were harvested in the third Surgical Clinic of the "Sf. Spiridon" Hospital (Iasi, Romania) either during diagnostic biopsies or tumor removal. The placental tissue was provided by the "Cuza-Voda" Clinic of Obstetrics and Gynecology (Iasi, Romania).

Immunohistochemistry

FcRn expression was investigated by immunohistochemistry, using a three step approach. All sections were formalin-fixed, paraffin-embedded, and sectioned at 5 μm. The samples were both primary tumors and the corresponding axillary lymph node metastases. Sections were deparaffinized in xylene and rehydrated in a series of graded ethanol solutions. Antigen retrieval was performed by heating the sections for 20 minutes in antigen retrieval solution (Dako, Glostrup, Denmark), pH 6.1, at 98° C, in a water bath. Nonspecific binding was blocked by incubation with 1.5% normal human AB serum/PBS for 15 minutes, at room temperature (RT) followed by 3% hydrogen peroxide/distilled H_2O for 5 minutes to quench endogenous peroxidase.

We have used a polyclonal IgG preparation as the primary antibody, isolated from rabbit serum immunized with an FcRn-derived peptide and obtained as previously described [8]. Sections were incubated with the antibody at a final concentration of 7.5 μg/ml, at room temperature, for 3 hours. For detection, a commercially available biotinylated anti-mouse/anti-rabbit antibody (LSAB; Dako) was used, for 10 minutes, followed by a 10-minute incubation at room temperature with streptavidin-HRP (LSAB; Dako), according to the vendor's indications. HRP activity was detected by adding DAB/H_2O_2 (LSAB; Dako), for 10 minutes at room temperature. Sections were counterstained with hematoxylin (Harris; Sigma, Taufkirchen, Germany), rinsed in water, dehydrated through ethanols, and coverslipped with Permount.

As negative controls, the primary antibody was replaced by a monoclonal mouse IgG1 antibody (clone DAK-GO1; Dako) or rabbit IgG (a gift from Dr. Victor Ghetie), followed by detection as above. All the staining experiments (labelings and washings) were performed at pH 7.2. Endothelial cells were detected by staining with anti-human CD34 II, clone QBEnd 10, mouse IgG1 (Dako), at a 1:50 dilution of the 50 μg/ml stock concentration [29].

Epithelial cells were detected by staining with a monoclonal mouse IgG1 anti-human cytokeratin antibody, clone MNF116 (Dako), at a 1:100 dilution of the 200 μg/ml stock concentration. This antibody reacts with an epitope that is present in a wide range of cytokeratins: 5, 6, 8, 17, and probably 19 [30]. The labeling protocol for the commercially available antibodies was the same as for the anti-FcRn antibody.

RESULTS

FcRn expression was assessed using immunohistochemistry of tissue sections and a polyclonal antibody made by immunizing rabbits with a peptide derived from murine

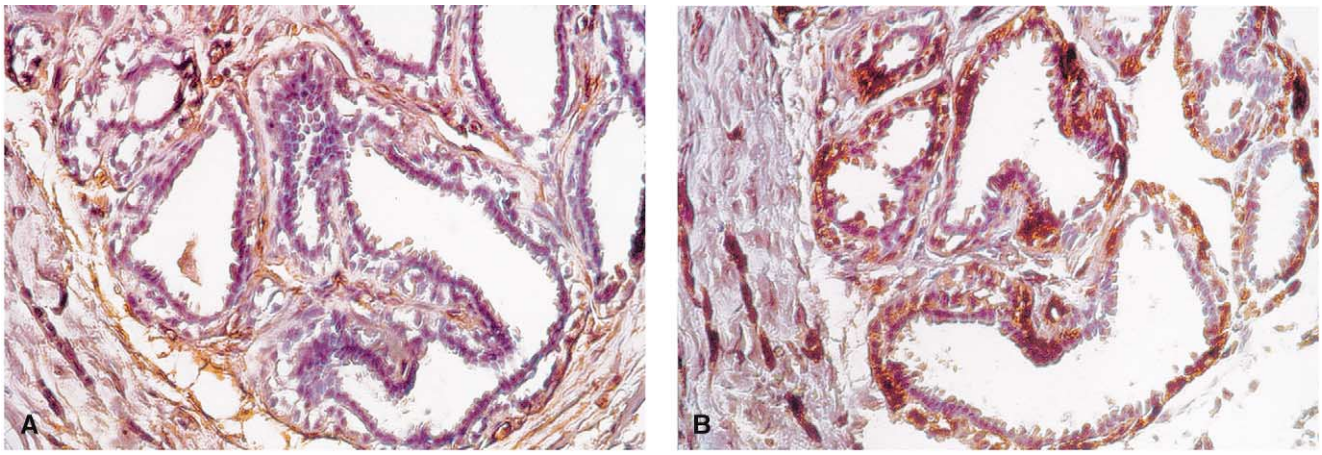


FIGURE 6 (A) Ductal invasive carcinoma with an insular and alveolar pattern, revealing positive staining for FcRn within the epithelial cells (original magnification ×400); section 2 is illustrated. (B) Positive staining of the endothelial cells with an anti-CD34 II antibody (original magnification ×400); section 7 is illustrated.

FcRn. This antibody immunoprecipitates endogenous FcRn from human endothelial cells (Figure 1), and has been demonstrated in a previous study to stain human endothelial cells by immunohistochemistry [8]. Initially we analyzed FcRn expression in syncytiotrophoblast, a human tissue for which the FcRn expression has been well documented [5]. Sections that have chorial villi are illustrated in Figure 2. The selective labeling of the trophoblast demonstrated that the anti-FcRn antibody is specific for human FcRn. High expression of FcRn in placenta can be seen, consistent with the data of others [5].

We next analyzed the expression of FcRn in the human mammary gland. For this, a number of breast carcinomas and normal tissue harvested from the periphery of benign tumors were analyzed. Staining of the normal mammary gland sections with the anti-FcRn antibody demonstrated that the expression of the molecule is restricted to the epithelial cells (Figure 3A).

FcRn expression was investigated in several histologic types of breast carcinomas. The most frequent malignancy available for our study was the ductal carcinoma, but we were able to investigate other variants such as lobular and medullary carcinomas. Sections from mammary gland of each tumor type were positive for FcRn expression. Furthermore, examination at a high magnification (× 1000) demonstrated that the labeling is primarily intracellular (Figure 3B). The receptor is consistently present within the epithelial cells of the gland and its expression does not appear to be inhibited by malignant transformation (Figures 4A–4C). When replacing the primary antibody with a monoclonal mouse IgG1 antibody or rabbit IgG, no staining could be noticed (Figure 4D). The immunohistochemical method we have used does not allow precise quantitation of the labeling intensity, yet, given the fact that all the slides were labeled under standard conditions, we can speculate that the expression levels appear to be similar in tissues of distinct histologic type. Also, as previously noted for murine mammary gland [13], the histiocytes scattered within the interstitium reveal an intense positive staining (data not shown). To further analyze whether FcRn expression is confined to the epithelial cells, sequential sections were labeled with anti-FcRn antibody and anti-cytokeratin antibody. The staining pattern demonstrates the labeling of the epithelial cells (Figure 5).

A previous study [13] demonstrated that the presence of FcRn is not ubiquitous in the endothelial cells because expression cannot be detected in the endothelial cells of murine mammary gland or the lamina propria of the neonatal intestine. Also, whether FcRn is expressed in human placental endothelial cells appears to be controversial, with some studies reporting its presence [6–8], and others not [5]. Therefore, we labeled sequential sections of human breast carcinomas with anti-FcRn and anti-human CD34 II (specific for capillary endothelial cells). The labeling pattern illustrates that the CD34 II positive cells express FcRn at undetectable levels. This feature is consistent for all of the sections, regardless of the histologic type of the tumor (Figure 6).

The consistent expression of the receptor within the glandular epithelial cells prompted us to investigate if metastatic epithelial cells retain FcRn expression (Figure 7). Cytokeratin expression (a routine marker used for detecting breast cancer micrometastases [31]) in some cells present in the regional lymph nodes was coincident with the expression of FcRn (data not shown). Therefore, FcRn expression appears to be stably maintained during metastasis to the lymph nodes, and the receptor might serve as a metastatic marker for mammary carcinomas.

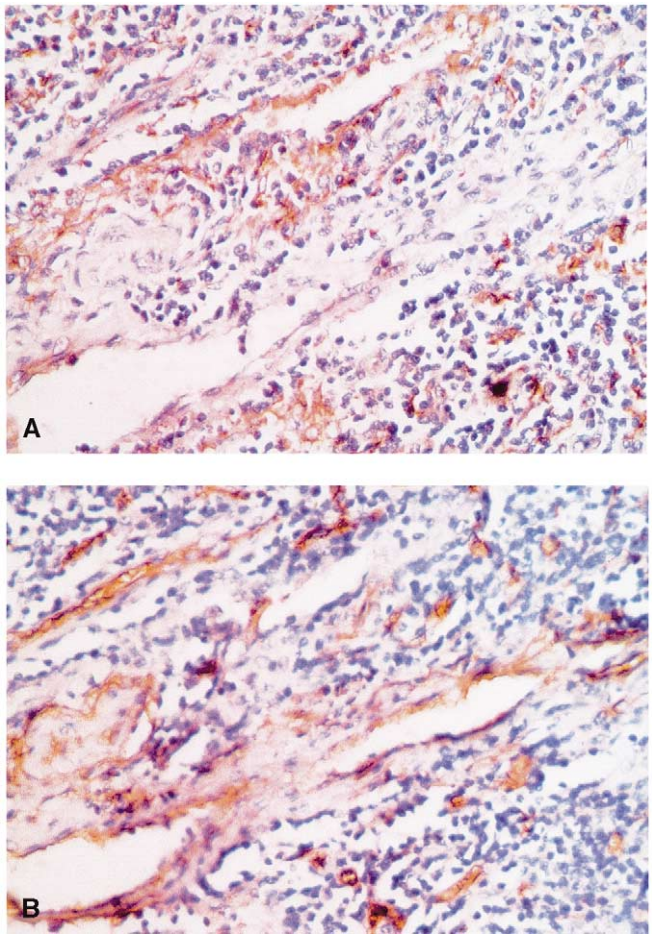


FIGURE 7 (A) Ductal invasive carcinoma metastasized ipsilatheral lymph node, revealing positive staining for FcRn (original magnification ×400). (B) Positive staining for CD34 II (original magnification ×400).

DISCUSSION

The mammary gland is one of the key organs in the transmission of passive immunity from mother to fetus. The presence of FcRn was initially identified in the mouse lactating mammary gland [13], but subsequent studies demonstrated the presence of FcRn orthologs in the mammary glands of other species [32–35]. In this context, the expression of FcRn in the human mammary gland is not surprising, despite the knowledge that the majority, if not all, of the transfer of maternal IgG occurs prenatally [24]. The molecule is present both in normal glandular epithelial cells and in malignant cells; the neoplastic change does not appear to inhibit the expression of this receptor. FcRn could be identified in all of the examined breast carcinomas regardless of the histologic type of the tumor. Unlike the neonatal intestinal brush border, where this receptor could be detected on the surface of the cells [36], the pattern of FcRn expression in the mammary epithelial cells appears to be primarily intracellular. Intracellular expression of FcRn has been reported to be the dominant site of expression for other cell types [6, 8, 10, 14, 17, 19, 34], in addition to transfectants expressing FcRn [37, 38]. When examining the regional nodes, we were able to clearly identify the presence of micrometastases due to the exclusive staining of the epithelial cells for FcRn. This suggests that FcRn could be used as a marker for the detection of micrometastases.

The placental transfer of human immunoglobulin G subclasses has been reported to fall in the order IgG4>IgG1>IgG3>IgG2, consistent with differences in their half-lives [39]. However, the amount of IgG found in the human milk is very low (50 mg/l in the milk secretion, and 1 g/l in the colostrum) in comparison with IgA, which represents approximately 90% of the total milk immunoglobulins (32 g/l) [25]. In mice, we have suggested that FcRn functions in a manner that results in a balance of IgG isotypes in mother's milk, which is in inverse correlation with affinity of the isotypes for FcRn [13]. Ultimately this could result in a balance of IgG isotypes in the neonatal circulation resembling that of the mother, as IgGs are transported across the neonatal gut in direct correlation with their affinity for FcRn [40–42]. Thus, the function of FcRn in the human mammary gland might be to act primarily as a recycling receptor. Ethical limitations preclude the direct testing of our hypothesis *in vivo*, but it could be tested *in vitro* with appropriately transfected cells.

The absence of detectable levels of the receptor in endothelial cells of the mammary gland raises another intriguing question: how can IgGs be transported to and from the blood into the tissues in which endothelial expression of FcRn cannot be detected? It is therefore possible that FcRn-independent mechanisms operate to transport IgGs into these tissues, and understanding this will form the basis of future studies.

ACKNOWLEDGMENTS

We are particularly indebted to Dr. Victor Ghetie for the critical reading of this manuscript, for his continous support, and for providing expert assistance with the preparation of antisera. We also thank Dr. Corneliu Diaconu for the human breast cancer samples, Dr. Iolanda Blidaru for the placenta samples, Prof. Dr. Lorica Gavrilita for the interpretation of some slides and valuable advice, and Carlos Vaccaro for his excellent technical assistance. This work was supported in part by the National Institutes of Health (E.S.W., grant R01 39167).

REFERENCES

1. Simister NE, Mostov KE: An Fc receptor structurally related to MHC class I antigens. Nature 337:184, 1989.

2. Ghetie V, Ward ES: Multiple roles for the major histocompatibility complex class I-related receptor FcRn. Annu Rev Immunol 18:739, 2000.
3. Ghetie V, Ward ES: Transcytosis and catabolism of antibody. Immunol Res 25:97, 2002.
4. Jones EA, Waldmann TA: The mechanism of intestinal uptake and transcellular transport of IgG in the neonatal rat. J Clin Invest 51:2916, 1972.
5. Simister NE, Story CM, Chen HL, Hunt JS: An IgG-transporting Fc receptor expressed in the syncytiotrophoblast of human placenta. Eur J Immunol 26:1527, 1996.
6. Kristoffersen EK, Matre R: Co-localization of the neonatal Fc gamma receptor and IgG in human placental term syncitiotrophoblasts. Eur J Immunol 26:1668, 1996.
7. Leach JL, Sedmack DD, Osborne JM, Rahill B, Lairmore MD, Anderson CL: Isolation from human placenta of the IgG transporter, FcRn, and localization to the syncitiotrophoblast: implications for maternal-fetal antibody transport. J Immunol 157:3317, 1996.
8. Antohe F, Radulescu L, Gafencu A, Ghetie V, Simionescu M: Expression of functionally active FcRn and the differentiated bidirectional transport of IgG in human placental endothelial cells. Hum Immunol 62:93, 2001.
9. Junghans RP: Finally! The Brambell receptor (FcRB). Mediator of transmission of immunity and protection from catabolism for IgG. Immunol Res 16:29, 1997.
10. Borvak J, Richardson J, Medesan C, Antohe F, Radu C, Simionescu M, Ghetie V, Ward ES: Functional expression of the MHC class I-related receptor, FcRn, in endothelial cells of mice. Int Immunol 10:1289, 1998.
11. Blumberg RS, Koss T, Story CM, Barisani D, Polischuk J, Lipin A, Pablo L, Green R, Simister NE: A major histocompatibility complex class I-related Fc receptor for IgG on rat hepatocytes. J Clin Invest 95:2397, 1995.
12. Telleman P, Junghans RP: The role of the Brambell receptor (FcRB) in liver: protection of endocytosed immunoglobulin G (IgG) from catabolism in hepatocytes rather than transport of IgG to bile. Immunology 100: 254, 2000.
13. Cianga P, Medesan C, Richardson J, Ghetie V, Ward ES: Identification and function of neonatal Fc receptor in mammary gland of lactating mice. Eur J Immunol 29: 2515, 1999.
14. Zhu X, Meng G, Dickinson BL, Li X, Mizoguchi E, Miao L, Wang Y, Robert C, Wu B, Smith PD, Lencer WI, Blumberg RS: MHC Class I-related neonatal Fc receptor for IgG is functionally expressed in monocytes, intestinal macrophages and dendritic cells. J Immunol 166:3266, 2001.
15. Israel EJ, Taylor S, Wu Z, Mizoguchi E, Blumberg RS, Bhan A, Simister NE: Expression of the neonatal Fc receptor, FcRn, on human intestinal epithelial cells. Immunol 92:69, 1997.
16. Shah U, Dickinson BL, Blumberg RS, Simister NE, Lencer WI, Walker WA: Distribution of the IgG Fc receptor, FcRn, in the human fetal intestine. Pediatr Res 53:295, 2003.
17. Spiekerman GM, Finn PW, Ward ES, Dumont J, Dickson BL, Blumberg RS, Lencer WI: Receptor-mediated Immunoglobulin G transport across mucosal barriers in adult life: functional expression of FcRn in the mammalian lung. J Exp Med 196:303, 2002.
18. Haymann J-P, Levraud J-P, Bouet S, Kappes V, Hagege J, Nguyen G, Xu Y, Rondeau E, Sraer J-D: Characterization and localization of the neonatal Fc receptor in adult human kidney. J Am Soc Nephrol 11:632, 2000.
19. Kobayashi N, Suzuki Y, Tsuge T, Okomura K, Ra C, Tomino Y: FcRn-mediated transcytosis of immunoglobulin G in human renal proximal tubular epithelial cells. Am J Physiol Renal Physiol 282:F358, 2002.
20. Schlachetzki F, Zhu C, Pardridge M: Expression of the neonatal Fc receptor (FcRn) at the blood-brain barrier. J Neurochem 81:203, 2002.
21. Simionescu M, Simionescu N: Endothelial transport of macromolecules: transcytosis and endocytosis. Cell Biol Rev 25:1, 1991.
22. McCarthy KM, Yoong Y, Simister NE: Bidirectional transcytosis of IgG by the rat neonatal Fc receptor expressed in a rat kidney cell line: a system to study protein transport across epithelia. J Cell Sci 113:1277, 2000.
23. Claypool SM, Dickinson BL, Yoshida M, Lencer WI, Blumberg RS: Functional reconstitution of human FcRn in Madin-Darby canine kidney cells requires co-expressed human β_2-microglobulin. J Biol Chem 277:28038, 2002.
24. Mellbye OJ, Natvig JB: Presence and origin of human IgG subclass proteins in newborns. Vox Sang 24:206, 1973.
25. Mehta PD, Isaacs CE, Coyle PK: Immunoglobulin G subclasses in human colostrum and milk. In Mestecki J, Blair C, Ogra PL (eds): Immunology of Milk and the Neonate. New York: Plenum Press, 1991.
26. Ahouse JJ, Hagerman CL, Mittal P, Gilbert D, Copeland NG, Jenkins NA, Simister NE: Mouse MHC class I-like Fc receptor encoded outside the MHC. J Immunol 151: 6076, 1993.
27. Story CM, Mikulska JE., Simister NE: A major histocompatibility complex class I-like Fc receptor cloned from human placenta: possible role in transfer of immunoglobulin G from mother to fetus. J Exp Med 180:2377, 1994.
28. Firan M, Bawdon R, Radu C, Ober RJ, Eaken D, Antohe F, Ghetie V, Ward ES: The MHC class I-related receptor, FcRn, plays an essential role in the maternofetal transfer of γ-globulin in humans. Int Immunol 13:993, 2001.
29. Dercksen MW, Daams GM, de Haas M, von dem Borne AEKG, Van der Schoot CE: M10.2. Characterization of the CD34 cluster. In Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T (eds): Leucocyte Typing V: White Cell Differentiation Antigens. Oxford: Oxford University Press, 1995.
30. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R:

The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11, 1982.

31. Cote RJ: Role of immunohistochemical detection of lymph-node metastases in management of breast cancer. International Breast Cancer Study Group. Lancet 354: 896, 1999.
32. Adamski FM, King AT, Demmer J: Expression of the Fc receptor in the mammary gland during lactation in the marsupial Trichosorus vulpecula (brushtail possum). Mol Immunol 37:435, 2000.
33. Kacskovics I, Wu Z, Simister NE, Frenyo LV, Hammarstrom L: Cloning and characterization of the bovine MHC class I-like Fc receptor. J Immunol 164:1889, 2000.
34. Mayer B, Zolnai A, Frenyo LV, Jancsik V, Szentirmay A, Hammarstrom L, Kacskovics I: Localization of the sheep FcRn in the mammary gland. Vet Immunol Immunopat 87:327, 2002.
35. Schnulle PM, Hurley WL: Sequence and expression of the FcRn in the porcine mammary gland. Vet Immunol Immunopat 91:227, 2003.
36. Rodewald R: Distribution of immunoglobulin G receptors in the small intestine of the young rat. J Cell Biol 85:18, 1980.
37. Hunziker W, Kraehenbuhl JP: Epithelial transcytosis of immunoglobulins. J Mammary Gland Biol Neoplasia 3:287, 1998.
38. Ober RJ, Radu CG, Ghetie V, Ward ES: Differences in promiscuity for antibody −FcRn interactions across species: implications for therapeutic antibodies. Int Immunol 13:1551, 2001.
39. Malek A, Sager R, Zakher A, Schneider H: Transport of immunoglobulin G and its subclasses across the in vitro perfused human placenta. Am J Obstet Gynecol 173:760, 1995.
40. Kim J-K, Tsen MF, Ghetie V, Ward ES: Identifying the amino acid residues that influence plasma clearance of murine IgG1 fragments by site-directed mutagenesis. Eur J Immunol 24:542, 1994.
41. Medesan C, Matesoi D, Radu C, Ghetie V, Ward ES: Delineation of the amino acid residues involved in transcytosis and metabolism of mouse IgG1. J Immunol 158: 2211, 1997.
42. Medesan C, Cianga P, Mummert M, Stanescu D, Ghetie V, Ward ES: Comparative studies to further delineate the Fc: FcRn interaction site. Eur J Immunol 28:2092, 1998.