Brief Definitive Report

Expression of γ/δ T Cell Receptors on Lymphocytes from the Lactating Mammary Gland

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Summary

γ/δ cells were at least four times more frequent in lactating mouse mammary glands than among T cells of the most proximal lymph nodes. Two-color staining of freshly isolated T cells and a study of clonally expressed γ/δ receptors on hybridomas further revealed that the mammary γ/δ population is heterogeneous, including at least three different subsets, among them cells expressing Vγ5, Vγ4 together with Vδ4, or none of these V regions.

Antigen receptors on T lymphocytes are heterodimeric molecules, composed of either α and β or γ and δ chains. Lymphocytes expressing γ/δ antigen receptors constitute a minor population in lymphoid tissue but are a major population within epidermal, gastrointestinal, and vaginouterine epithelia (1–3). Based on the limited diversity of their antigen receptors and the localization of γ/δ cells in these epithelia, it has been proposed that γ/δ T cells are involved in immunological surveillance of epithelia separating the external environment from the internal milieu (4). Here, we have examined mammary glands, another epithelial organ in contact with the external environment, in terms of γ/δ T cell content and TCR-γ/δ expression. Lactating mammary glands were selected for study because local immunity and epithelial surface area are both increased in the mammary gland during lactation (5). TCR-γ/δ-bearing cells are present in lactating murine mammary tissue at a frequency at least four times greater than in lymph nodes draining that tissue and can be classified into three subsets based on TCR expression.

Materials and Methods

mAbs. H57-597.2 (anti-pan TCR-α/β), GL2 (anti-Vδ4), and GL3 (anti-pan TCR-γ/δ) were generated in the laboratories of R. Kubo (6) and L. Lefrancois (2), respectively. 145-2C11 (anti-CD3 ε chain), 403A.10 (anti-pan TCR-γ/δ) and 536 (anti-Vγ5) were gifts of J. Bluestone (7), O. Kanagawa (8), and J. Allison (9), respectively.

Cell Preparation. Mammary gland tissue from lactating C57Bl/10, C57Bl/6, or C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME), 1–7 d post-partum, was dissected from the subcutaneous tissue plane of the abdominal fat pads. Axillary, brachial, and epigastric lymph nodes were removed from the mammary tissue. Cell suspensions were nylon wool purified. Nylon wool–nonadherent cells were activated with con A (5 μg/ml) or surface-bound, crosslinking mAb 145-2C11.

T Cell Hybridoma Production and Analysis. 1–3 × 10^6 nylon wool–purified mammary cells or epigastric lymph node cells were fused with 2 × 10^7 BW5147 α^-/β^- cells (10). The hybridomas as well as the purified fresh mammary and lymph node cells were stained with the above mAbs as previously described (6), followed by one- or two-color cytofluorimetric analysis.

SDS-PAGE Gel Analysis of Hybridoma TCR. Hybridomas were cell surface labeled with I^{125}, solubilized with digitonin, and their TCR protein chains were separated as previously described (11).

Amplification and Sequencing of Hybridoma TCR cDNAs. cDNA fragments were prepared and amplified as described (12). In-frame transcripts were found with the following primers: Cγ1 (internal), GGGGAAATGTCTGCATCAAG; Cγ1 (external), GAAGGAGGGAAAATAGTGGG; Vγ4 (external), GCAACCTGAAATATCAATTT; Cδ (internal), TGGTTTGGCCGGAGGCTGGC; Cδ (external), TGTTCCATTTTTCATGATGA; Cδ4 (external), CAGACAGTGTCTCAGCCTCAG. Single-stranded cDNA was sequenced using dideoxy-nucleotides (13).

Results and Discussion

Mammary TCR-γ/δ-bearing Lymphocytes. As shown in two of five replicate experiments with lactating C57Bl/10 and C3H/HeJ mice, γ/δ cells in mammary T lymphocyte preparations were at least four times more frequent than in T cell preparations from mammary-associated lymph nodes (Table 1). Notably, relative frequencies of mammary γ/δ cells were more variable (range, 8.4–31%) than frequencies of lymph node γ/δ cells (range, 1.4–3.0% among epigastric and

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Table 1. *Cytofluorometric Characterization of TCR-γ/δ-bearing Mammary Cell Populations*

Mouse strain	TCR components stained (two-color staining)	Percent and no. of stained cells					
		Mammary gland		Epigastric nodes		Axillary/ brachial nodes	
		%		%		%	
C3H/HeJ	CD3	84	(15,333)*	87	(86,584)	89	(177,120)
	α/β‡	91	(13,993)	99	(85,389)	99	(174,939)
	CD3 and γ/δ	8.7	(1,340)	1.4	(1,195)	1.2	(2,181)
	CD3 and Vδ4	0.8	(106)	0.3	(252)	0.1	(165)
	γ/δ and Vδ4	8.9	(152)	13	(375)	15	(430)
C57B1/10	CD3	80	(7,285)	97	(6,416)	ND	
	α/β‡	92	(6,676)	98	(6,287)		
	CD3 and γ/δ	8.4	(609)	2.0	(129)		
	CD3 and Vγ5	1.7	(136)	1.0	(70)		
	γ/δ and Vδ4	9.8	(156)	25	(45)		

Two-color analysis was done using nylon wool–enriched T cells from C57B1/10 or C3H/HeJ mice. The above staining patterns were detected with the following mAbs: 145-2C11 for CD3, GL2 for Vδ4, and 536 for Vγ5. TCR-γ/δ staining of cells from C3H/HeJ and C57B1/10 was done with mAbs GL3 and 403A.10, respectively.

* Numbers in parentheses represent total numbers of stained cells counted for each mAb staining pattern and are independent of other parenthesized numbers.

‡ Percent α/β^+ T cells are approximated as the number of $CD3^+$ cells that are not γ/δ cells.

1.2–3.7% among brachial/axillary node T cells) (not shown). This variation could be due to the smaller sample sizes of mammary T cells or might actually reflect physiological changes in the dynamic mammary tissues.

To characterize mammary γ/δ T cells further, two V region–specific mAbs (mAb 536, anti-Vγ5; and mAb GL2, anti-Vδ4) were used for two-color staining in combination with anti-CD3 (mAb 145-2C11) or panspecific anti-mouse γ/δ (mAbs GL3 and 403A.10) antibodies. Unlike TCR-γ/δ-bearing lymphocytes in mouse epidermis (1), only ~20% of mammary γ/δ cells expressed Vγ5. In addition, ~10% of mammary γ/δ cells expressed Vδ4, a V gene that also is expressed by gut epithelium–associated lymphocytes (2). Thus, γ/δ populations in the lactating mammary gland are heterogeneous and can be divided into three subsets expressing different TCRs based on the staining experiments and further evidence outlined below. Whether the composition of mammary γ/δ cells differs from γ/δ cells in the nearest lymph nodes, which presumably drain the mammary tissues, is not clear. Nevertheless, the higher frequency of γ/δ cells among mammary T lymphocytes suggests that γ/δ cells specifically home to or expand in this organ.

$V\delta4^+$ γ/δ Cells. Because of the availability of anti-Vδ4 mAb, further experiments focused on the $V\delta4^+$ subset in lactating mammary tissue. An attempt was made to generate mammary γ/δ T cell hybridomas, since $V\delta4^+$ cells could be selected for clonal receptor characterization. In the first fusion experiment with freshly prepared cells from lactating C57Bl/10 mammary glands, 41 hybridomas were generated, but in three repetitive fusions, no further hybridomas were obtained. As mammary fusions were usually carried out in parallel with lymph node cell fusions where no such variation was observed, experimental conditions were not likely

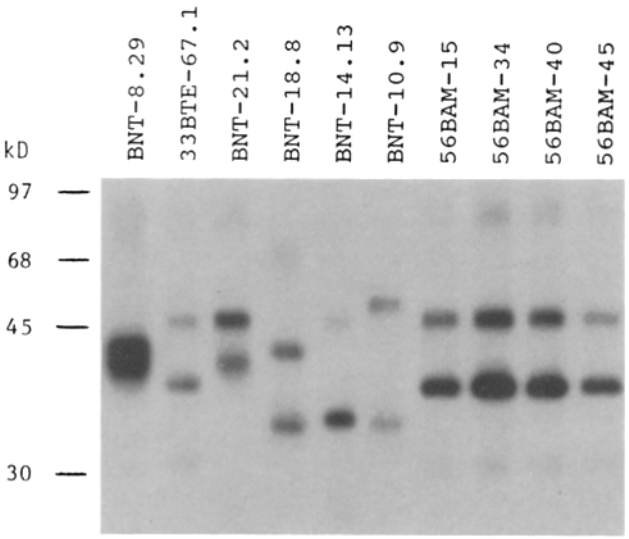

Figure 1. Analysis of TCR γ and δ chains from mammary-derived T cell hybridomas. Mammary-derived hybridomas, 56BAM-15, -34, -40, and -45, along with control fetal and newborn thymus–derived hybridomas, were I^{125} labeled and lysed with digitonin. 145-2C11-precipitated membrane proteins were separated by a two-step nonreduced–reduced SDS-PAGE procedure (11). Apparent molecular masses of mammary γ and δ chains were ~37 and ~47 kD, respectively.

Table 2. *γ and δ Junctional Sequences from Vγ4⁺/Vδ4⁺ Mammary Hybridomas*

DNA source	Vδ4	P?	Dδ2	N	Jδ1
Germline	. . .ATG GAG CG. . .		. . .ATCGGAGGGATACGAG. . .		. . .CT ACC GA. . .
56BAM-15, -34, -40, -45	. . .ATG GAG CG	C	GGGATACGAG		CT ACC GA. . .
33BTE-67.1	. . .ATG GAG CG	C	GGGATACGAG		CT ACC GA. . .

	Vγ4	N	Jγ1
Germline	. . .GT TCC TAC GGC TAA AG. . .		. . .AT AGC TCA G. . .
BW5147*	. . .GT TCC TAC GGC T	AAAGGAGAGAGG	AGC TCA G. . .
56BAM-15, -34, -40, -45	. . .GT TCC TAC GGC T		AT AGC TCA G. . .
33BTE-67.1	. . .GT TCC TAC GGC T		AT AGC TCA G. . .

Mammary hybridomas have junctional sequences identical to those of the fetal thymocyte hybridoma, 33BTE-67.1. As in the fetal thymus–derived hybridoma, and in contrast to the AKR thymoma, BW5147 (13), N-region nucleotides are missing. The cytosine in the Vδ4-Dδ2 junctions appears to represent a palindromic nucleotide (15).

* Published sequence (13).

to be the reason for the variable outcome of the mammary fusions. Two further mammary cell fusions were carried out after normal T cells had been activated (see Materials and Methods). Again, the number of generated hybridomas varied greatly. Of 82 TCR-bearing hybridomas from six experiments, 40 were TCR-γ/δ^+. 39 of these (all derived from the first fusion, designated 56BAM) were reactive with the Vδ4-specific mAb but not with the Vγ5-specific reagent, and one cell did not stain with either antibody.

I^{125}-labeled receptors of four randomly chosen $V\delta4^+$ mammary hybridomas were further analyzed by two-step SDS-PAGE and compared with receptors of previously characterized TCR-γ/δ-bearing hybridomas (Fig. 1). γ and δ chains of all mammary hybridomas were found to have identical apparent molecular masses and to comigrate with the receptor proteins of a $V\delta4/V\gamma4^+$, C57Bl/6 day 16 fetal thymus–derived hybridoma, 33BTE-67.1 (9). Thus, gel electrophoresis confirmed the results of the staining experiments and suggested Vγ4-JCγ1 as part of the surface-expressed γ/δ heterodimer.

The sequence of the PCR-amplified single-stranded cDNA from the same four mammary hybridomas revealed functional rearrangements of both Vδ4 and Vγ4 genes (Table 2). Junctional sequences of these hybridomas were found to be identical to each other and to those of 33BTE-67.1. The Vγ4 sequence also appears to be shared by some vaginouterine cells (3). In contrast to the nonfunctional expressed Vγ4 gene of the fusion line, BW5147 (13), N-region nucleotides were absent. That the original mammary fusion was contaminated with the fetal thymus hybridoma is excluded by a molecular mass polymorphism of γ chains between C57Bl/6 (the source for 33BTE-67.1) and C57Bl/10 mice (the source for the mammary hybridomas), which has been observed by others (14) and is evident in Fig. 1. Because we were unable to detect differences between the mammary hybridomas by preliminary Southern blot analyses of TCR β gene loci (not shown), and since all $V\delta4/V\gamma4^+$ hybridomas were generated in the same fusion experiment, the possibility remains that the γ/δ receptors analyzed were all derived from one clonally expanded cell.

The data in this report document the existence of a TCR-γ/δ-bearing lymphocyte population in the lactating murine mammary gland. Unlike epidermal and vaginouterine γ/δ cells (1, 3), this population is heterogeneous, as shown by analyses of both mixed cell populations and individual clones. The heterogeneity is likely to correspond to the greater complexity of mammary tissues.

The $V\delta4^+$ subset of mammary γ/δ cells appears noteworthy both because of its anatomical location and because of the possibility that these cells are selectively activated and/or clonally expanded during lactation. Whether the $V\delta4/V\gamma4^+$ hybridomas that were generated in one fusion are fully representative of this subset remains uncertain. An alternative possibility is that the hybridomas represent a pathological process in one mouse, e.g., clonal expansion after an immune response within mammary tissues or neoplastic transformation of a $V\delta4^+$ clone. Nevertheless, both V gene usage and lack of N-region additions in the $V\delta4/V\gamma4^+$ hybridomas suggest that they are, like epidermal γ/δ cells, derived from developmentally early γ/δ precursors. $V\delta4^+$ lymphocytes in the mammary gland could be yet another example of a uniform and perhaps functionally specialized peripheral γ/δ population.

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