Attributes of $\gamma\delta$ intraepithelial lymphocytes as suggested by their transcriptional profile

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Contributed by Mark M. Davis, June 25, 2001

$\gamma\delta$ T lymphocytes in the intestinal intraepithelial layer ($\gamma\delta$ IELs) are thought to contribute to immune competence, but their actual function remains poorly understood. Here we used DNA microarrays to study the gene expression profile of $\gamma\delta$ IELs in a *Yersinia* infection system to better define their roles. To validate this approach, mesenteric lymph node CD8$^+$ $\alpha\beta$ T cells were similarly analyzed. The transcription profiles show that, whereas lymph node CD8$^+$ $\alpha\beta$ T cells must be activated to become cytotoxic effectors, $\gamma\delta$ IELs are constitutively activated and appear to use different signaling cascades. Our data suggest that $\gamma\delta$ IELs may respond efficiently to a broad range of pathological situations irrespective of their diverse T cell antigen receptor repertoire. $\gamma\delta$ IELs may modulate local immune responses and participate in intestinal lipid metabolism, cholesterol homeostasis, and physiology. This study provides a strong basis for further investigations of the roles of these cells as well as mucosal immune defense in general.

Despite intense efforts, the functional roles of $\gamma\delta$ T cells in maintaining host immune defense remain enigmatic. One unique feature of $\gamma\delta$ T cells that distinguishes them from $\alpha\beta$ T cells is their tissue distribution. Although $\gamma\delta$ T cells represent a small percentage (<5%) of the lymphocytes in the central immune system of humans and mice, they are a sizable population (10–50%) in the mucosal epithelia (1, 2). The murine $\gamma\delta$ T lymphocytes in the intestinal intraepithelial layer ($\gamma\delta$ IELs) exhibit a diverse T cell antigen receptor (TCR) repertoire (3, 4) and thus have the potential to recognize a variety of antigens. These cells have been implicated in regulating the development of epithelial cells (5) and in controlling intestinal $\alpha\beta$ T cell responses in an *Eimeria vermiformis* infection model (6). Recently, we found that mice lacking $\gamma\delta$ T cells (TCR$\delta^{-/-}$) are much less resistant than either normal mice or mice without $\alpha\beta$ T cells (TCR$\beta^{-/-}$) to the dissemination of the enteric pathogen *Yersinia pseudotuberculosis* to the liver and spleen 1–4 days after oral infection (7). To gain insight into the scope of $\gamma\delta$ IEL responses in this system, we compared the gene expression of $\gamma\delta$ IELs isolated from mice orally infected with *Yersinia* to that of $\gamma\delta$ IELs isolated from uninfected animals by using the Affymetrix (Santa Clara, CA) GENECHIP technology (8). This approach allowed us to examine a large number of transcripts including many not associated with lymphocyte functions and to gain insight into the cellular mechanisms operating in $\gamma\delta$ IELs. To validate the experimental approach, and to serve as a basis of comparison for the $\gamma\delta$ IEL data, we also analyzed the expression profiles of mesenteric lymph node (MLN) CD8$^+$ $\alpha\beta$ T cells at the peak of the peripheral responses to oral *Yersinia* infection (7).

We find that, whereas transcripts associated with cytotoxic functions and activation are significantly induced in CD8$^+$ $\alpha\beta$ T cells by the infection, $\gamma\delta$ IELs from infected and uninfected animals appear to be constitutively activated and to express very high levels of cytotoxic genes. Interestingly, $\gamma\delta$ IELs express several inhibitory receptors, which could keep their effector functions in check but allow them to be readily turned on with little or no *de novo* transcription. These properties could allow these cells to participate in both innate and acquired immune defense by responding quickly to a broad range of pathological situations irrespective of their diverse TCR repertoire. Our data also show that $\gamma\delta$ IELs may recruit other leukocytes and down-regulate immune responses by targeting cells such as macrophages. Surprisingly, $\gamma\delta$ IELs express genes associated with lipid and cholesterol metabolism that are complementary to those expressed by the intestinal epithelial cells, suggesting a new role for $\gamma\delta$ IELs in intestinal homeostasis and physiology. Overall, this approach has allowed us to evaluate many more potential attributes of $\gamma\delta$ IELs than previously possible and provides important insights into $\gamma\delta$ IEL regulation and function.

Methods

Sample Preparation. Seven- to twelve-week-old female C57BL/6 mice (The Jackson Laboratory and Stanford University) deprived of food overnight were infected by oral gavage with *Y. pseudotuberculosis* YPIII or with a YopE mutant bacterium (kindly provided by Stanley Falkow, Stanford University). $\gamma\delta$ IELs were isolated 5 days after mice were infected with 5×10^8 colony-forming units (cfu) of *Y. pseudotuberculosis* YPIII. $\alpha\beta$ T cells were isolated 10 days after mice were infected with 1×10^8 cfu of *Y. pseudotuberculosis* YPIII or with a YopE mutant. $\alpha\beta$ T cells isolated from YPIII- or YopE-mutant-infected animals showed virtually no difference in gene expression. Therefore, no distinction was made in the data analysis here.

Cells from the small intestine were isolated as described (9) but without the gradient step. $\gamma\delta$ IELs were isolated by positively selecting with biotin-conjugated GL3 (anti-δ chain) antibody and avidin-conjugated magnetic beads (Dynal, Oslo). CD8$^+$ $\alpha\beta$ T cells from mesenteric lymph nodes were isolated by negative depletion with FITC-conjugated antibodies against CD4, B220, CD11b, $\gamma\delta$TCR, and anti-FITC-conjugated magnetic beads (PerSeptive Biosystems, Framingham, MA) followed by FACS sorting with phycoerythrin-conjugated anti-CD8α antibody. CD8$^+$ FITC$^-$ cells were collected. Epithelial cells were obtained by sorting intestinal cell suspensions on the basis of forward and side scatter profiles and propidium iodide exclusion. The purity

Abbreviations: $\gamma\delta$ IELs, $\gamma\delta$ T lymphocytes in the intestinal intraepithelial layer; MLN, mesenteric lymph node; TCR, T cell antigen receptor; NK, natural killer; KGF, keratinocyte growth factor; TNF, tumor necrosis factor.

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Table 1. Expression levels of genes expressed preferentially by MLN CD8$^+$ $\alpha\beta$ T cells (*Left*) and by genes up-regulated in these cells after *Yersinia* infection (*Right*)

$\alpha\beta$ T	$\gamma\delta$ T	Description	Uninfected	Infected	Description
Preferentially expressed cell-surface molecules			Up-regulated after *Yersinia* infection		
Cytokine/chemokine/similar			Effector functions		
367	A	CCR7	139	779	RANTES
179	A	IL-7 receptor	A	173	Granzyme A
115	11	CXCR4	A	87	Granzyme B
72	A	Thromboxane A2 receptor	27	71	Flt3 ligand
64	A	IL-6 receptor	Activation, proliferation, cell cycle		
54	A	IL-17 receptor	153	325	Nucleolin
Other surface molecules			56	303	Ly-6E
1653	303	pB7	64	114	Proliferation-associated protein 1 (p38-2G4)
822	64	Thy-1	56	108	CDC47
454	A	Ly-6C	51	99	Pim-2
298	A	CD2	14	80	CDKN2A/INK4a/MTS1
264	A	L-selectin	22	73	Stathmin
226	16	CD97	21	55	γ-glutamyl transpeptidase
224	26	Lectin L-14	21	52	Mdm2
220	A	Ly-6E	Chromosomal structure		
164	A	Semaphorin B	144	431	HMG2
148	A	CD5	166	306	HMG14
113	A	CD6	55	141	HMG17
84	A	CD97	Protein synthesis/degradation/targeting		
63	A	LFA-1	126	214	Proteasome sub., α type 2
60	12	CD47	115	190	Cathepsin S
50	A	ICAM-2	86	169	Cytosolic chaperone containing TCP-1, θ
			59	158	Cathepsin D
			54	99	Splicing factor Srp20/X16
			54	91	Valosin containing protein (VCP)
			31	74	Ubiquitin-conjugating enzyme E2H (E20-20K)
Preferentially expressed signal and transcription factors			22	60	FXR1
Signal transduction			17	52	U2-snRNP b″ (pRNP31), homolog
9792	1944	Receptor for activated C kinase	A	51	Ac39/physophilin
199	38	GTPase-activating protein GapIII	Surface molecules		
134	A	Diacylglycerol kinase, α	68	301	Lectin L-14
117	15	Jak1, homolog	128	255	CD48
111	A	Diacylglycerol kinase, α	36	85	Galectin-3 (Mac-2)
83	A	Dual-specificity phosphatase PAC-1	43	76	Integrin β-1 subunit
51	A	Protein kinase C, θ	Signal transduction and transcription factors		
Transcription factors			50	98	NF-YB
554	A	Krüppel-like factor LKLF	34	81	Maid
407	18	SATB1	41	75	Protein phosphatase 2A, cat.sub.α, hmlg
148	27	RFLAT-1, homolog	36	73	Protein phosphatase 2A, B′α3 reg.sub
144	12	TCF-1	28	55	Protein phosphatase 2A, cat.sub.β, hmlg
140	A	LEF-1	39	75	FK506-binding protein (FKBP-12)
73	A	Krüppel-like factor 3	33	74	MyD88
60	A	HMG-I(Y)	39	68	NFATx/NFAT4/NFATc3

Gene expression values are shown of (*Upper Left*) a partial list of cell surface molecules preferentially expressed by MLN CD8$^+$ $\alpha\beta$ T cells ($\alpha\beta$ T; averaged from all six samples) together with their averaged expression values in $\gamma\delta$ IELs ($\gamma\delta$ T; averaged from all four samples). Genes called absent are indicated by A. (*Lower Left*) signal transduction molecules and transcription factors preferentially expressed in MLN CD8$^+$ $\alpha\beta$ T cells. (*Right*) genes expressed at least 2-fold higher in cells isolated from *Yersinia*-infected (*Inf.*), as compared to uninfected (*Uninf.*), animals. These genes had to be called present with minimum expression values greater than 30 in at least one of the duplicate samples of $\alpha\beta$ T cells isolated from either *Yersinia*-infected mice or uninfected mice. A comprehensive list, including accession numbers, can be found in Tables 3, 6, 8. cat, catalytic; sub, subunit; reg, regulatory; hmlg, homolog.

of the cells was estimated to be greater than 95% for $\gamma\delta$ IELs and epithelial cells and greater than 99% for CD8$^+$ $\alpha\beta$ T cells. Analysis of RNA expression by using Affymetrix GENECHIP microarrays was carried out according to published procedures (10) with at least 1×10^7 cells for each sample, isolated from a minimum of three mice.

GENECHIP Data Analysis. Each gene is represented by $\approx$20 probe pairs on the array. Each probe pair consists of a 25-mer oligo complementary to the gene of interest [the perfect match (PM) oligo] and a second mismatched (MM) oligo, which contains a single base change at the middle nucleotide. Expression values represent the median of PM–MM values for each gene after normalization. Chips were normalized on the basis of the 75th percentile of PM–MM values for all probe pairs (PM–MM values for each chip were multiplied by the 42/75th percentile for that chip). GENECHIP 3.0 software (Affymetrix) was used to determine the presence or absence of mRNA for specific genes in each sample. Analysis specific for individual sets of data is described in the table legends.

Results

Gene Expression Correlates Well with Known Protein Expression and Cell Function. To determine the gene expression patterns of γδ IELs and MLN CD8$^+$ αβ T cells from uninfected and *Yersinia*-infected mice, duplicate samples of mRNA were prepared from isolated cell populations. In addition, one sample of mRNA isolated from intestinal epithelial cells was also analyzed. Of the 6,352 probe sets found on the microarrays, about 2,100 genes were expressed by the lymphocytes (both αβ and γδ T cells), and about 800 genes were expressed by the epithelial cells. This difference in the number of genes expressed presumably reflects a bias in the database toward mRNAs identified from hematopoietic cells.

Genes expected to be expressed in αβ, γδ T cells, and epithelial cells were identified in the array analysis. These include the T cell receptors, lymphocyte cell surface markers, and structural genes characteristic of each cell type. A partial list of cell surface molecules expressed in CD8$^+$ αβ T cells is shown in Tables 1 and 3 (which is published as supplemental data on the PNAS web site, www.pnas.org) (the complete data sets will be available from Y.-h.C. on request). As expected, there is no linear correlation between the expression levels of genes identified from epithelial cells and either T cell population. Interestingly, the differences in individual gene expression levels between mesenteric CD8$^+$ αβ T cells and γδ IELs are much greater than those observed between the same cell types isolated from infected and uninfected animals, as discussed in the next section (Fig. 2, which is published as supplemental data on the PNAS web site).

Effector Function Genes Are Constitutively Expressed in γδ IELs but Induced in MLN CD8$^+$ αβ T Cells. Many of the abundantly expressed genes in γδ IELs are associated with specialized functions. Genes coding for Granzymes A, B, and RANTES are among the 10 most abundantly expressed mRNAs (Table 4, which is published as supplemental data on the PNAS web site). Surprisingly, these genes are expressed at similarly high levels in cells isolated from both infected and uninfected mice. In fact, only 37 genes and ESTs are identified as having statistically significant, albeit small (less than 3-fold), differences between infected and uninfected γδ IEL samples (Table 5, which is published as supplemental data on the PNAS web site). None of these is thought to be associated with cell activation or effector function.

That almost all of the genes associated with effector function and activation are expressed at comparable levels in γδ IELs from infected and uninfected mice is clearly not a failure of the detection system. A large increase in the expression of genes encoding cytotoxic functions (e.g., Granzymes A and B), the chemokine RANTES, and activation markers (e.g., Ly-6E) was readily observed in mesenteric CD8$^+$ αβ T cells from infected mice compared with those from uninfected mice (Tables 1 and 6, which is published as supplemental data on the PNAS web site). This difference was seen despite the fact that only 8% of the lymph node CD8$^+$ αβ T cell population from infected animals showed an activated phenotype (CD44^{hi}, L-selectin^{low}).

It is also unlikely that the γδ IEL transcription program is activated by the isolation procedure, which includes a 30-min 37°C incubation to detach the IELs (all subsequent steps in the isolation procedure are carried out at 0–4°C, precluding significant mRNA synthesis). RNA samples were obtained from a whole intestine manipulated only to remove Peyer's patches and from a whole intestine processed to detach, but not remove, epithelial cells and IELs. Similar levels of Granzyme A and RANTES were found in both samples by Northern analysis (data not shown). Consistent with this finding, all γδ IELs, but not most splenic γδ T cells that underwent the same isolation procedures, showed surface CD69 expression—a marker associated with activated natural killer (NK) cells, αβ T cells, and B cells (the gene is not represented on the chips) (Fig. 1*A* and data not shown).

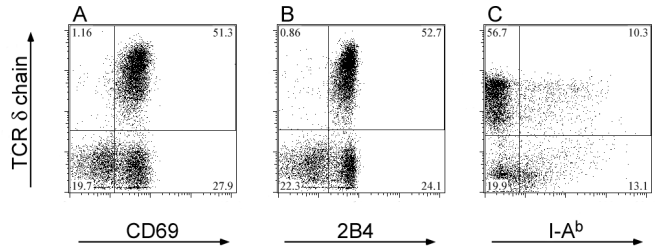

Fig. 1. γδ IELs express (*A*) CD69, (*B*) 2B4, and (*C*) I-A. IELs were isolated and stained with FITC conjugates of anti-CD69, -2B4, and -I-A (PharMingen) and phycoerythrin (PE)-conjugated GL3 (anti-δ-TCR) (purified and conjugated to PE according to standard protocols). Cy-chrome-conjugated G235–2356 (anti-2,4,6,-trinitrophenol, hamster isotype control) and propidium iodide-positive cells were excluded from analysis.

In addition, all γδ IEL samples express genes implicated in sustaining specialized cell functions. These include genes associated with growth arrest (e.g., *c-fes*, *gadd45*, *gadd153*, and *gas3*) and differentiation (e.g., *atf-4*, *blimp-1*, *cdc25*, *mad*, *tis21*, and *agp/ebp*). Several proteases and enzymes (e.g., Furin, Mep-1, CD73), which have been implicated in the maturation of molecules associated with growth and differentiation, are also expressed (Tables 2 and 7, which is published as supplemental data on the PNAS web site). Taken together, these results indicate that γδ IELs in uninfected animals are constitutively activated to transcribe genes associated with effector functions.

γδ IELs May Be Activated Through Signaling Pathways Distinct from Those in αβ T Cells. Despite γδ IELs having an activated phenotype, they do not appear to express transcripts for certain key signaling proteins used by αβ T cells. These include protein kinase C θ (PKC θ), an important component of TCR-mediated NF-κB activation in mature αβ T cells (11), and diacylglycerol kinase α, a protein responsible for the removal of diacylglycerol, which normally activates PKC θ. Conversely, PI3-kinase levels are increased when compared with αβ T cells. Whereas the signal transduction genes preferentially expressed by MLN CD8$^+$ αβ T cells (Tables 1 and 8, which is published as supplemental data on the PNAS web site) largely fit into known lymphocyte signaling pathways, those preferentially expressed by γδ IELs do not (Tables 2 and 7).

In this context, it is interesting to note that although γδ IELs express high levels of RANTES, RFLAT-1, which is important in the later stages of RANTES expression after CD8$^+$ αβ T cell activation (12), is not expressed. Because the RANTES promoter contains NF-κB- and interferon regulatory factor-binding sites, the expression of RANTES could result from signals from tumor necrosis factor (TNF)α and IFNγ, respectively (13). In fact, both the TNFα and IFNγ receptor genes are expressed in γδ IELs. Thus, some of the effector genes expressed by both γδ and peripheral αβ T cells appear to be triggered by different signaling cascades in the two cell types.

γδ IEL Function. Because the gene expression pattern of γδ IELs is characteristic of that of effector cells, whereas the gene expression pattern of αβ T cells is typical of that of naïve cells, we analyzed the genes preferentially expressed in γδ IELs to identify potential γδ IEL functions. This analysis was complemented by a search through all of the data sets for the expression of particular genes. Some of these genes, subdivided by function, are shown in Tables 2 and 7.

Table 2. Expression levels of genes preferentially expressed by $\gamma\delta$ IELs

$\gamma\delta$ T	$\alpha\beta$ T	Epi.	Description
Immune defense mediators			
Cytokines/chemokines			
3065	566	A	RANTES
215	49	A	Lymphotactin
58	25	A	TIS7/PC4, homolog
39	A	A	MIP-1-α
38	A	A	MIP-1-β
36	A	A	Eta-1
30	A	A	Transforming growth factor, β 3
22	8	A	TIS7/PC4/IFN-related developmental regulator 1
Cytotoxic proteins/related			
2630	116	19	Granzyme A
2117	59	8	Granzyme B
583	258	22	Serglycin
275	A	A	Fas ligand
222	8	153	Cryptdin
Enzymes, inflammation			
174	97	19	Leukotriene A-4 hydrolase
73	35	40	p47phox
Cholesterol/lipid biosynthesis and metabolism			
110	A	A	Apolipoprotein E
66	27	A	Farnesyl diphosphate synthase, homolog
65	16	A	Squalene synthase
61	11	A	Plasma phospholipid-binding protein
54	29	A	Acetyl CoA dehydrogenase, long-chain
51	A	A	LDL receptor
50	A	A	Squalene epoxidase
44	23	A	Stearoyl-coenzyme A desaturase 2
38	A	A	Adipose differentiation related protein
Intestinal function and homeostasis			
289	A	A	Carbonic anhydrase isozyme II
226	A	A	Fibrinogen-like protein
152	8	A	Spi2/EB1 proteinase inhibitor
116	A	A	Furin
116	59	A	Cystatin 7 (Cst7/leukocystatin)
91	A	A	p6-5 (preproelastase, homolog)
21	A	A	Platelet-activating factor acetylhydrolase, 1b, a1
45	A	91	Monocyte/neutrophil elastase inhibitor, homolog
108	A	111	Serine protease inhibitor, Kazal type 3 (Spink3)
165	14	150	Alcohol dehydrogenase class I (ADH-A-2)
104	A	297	Meprin 1 β
Cell-surface molecules			
TCR associated			
726	394	A	CD3-γ
597	16	17	Fc-epsilon-RI γ subunit
NK activating/inhibitory receptors			
182	A	A	NK cell receptor 2B4
138	A	A	LAG-3
87	A	A	Ly-49E-GE (Klra5)
58	A	A	NK cell receptor gp49B
55	A	13	PD-1/programmed cell death 1
49	A	A	CTLA-4
38	A	A	NK cell receptor NKR-P1A
Cytokine/chemokine/similar			
262	46	A	TNF receptor 2
125	49	100	TNF receptor 1
225	91	30	Interferon γ receptor
209	70	A	IL-2 receptor, β chain
31	A	A	IL-12 receptor, β 1
40	A	A	L-CCR chemokine receptor
62	A	A	Prostaglandin E receptor, EP4 subtype
Signal transduction			
1020	A	A	Regulator of G-protein signalling 1 (RGS1)
620	101	77	MAPK phosphatase 1 (3CH134)
337	195	A	Stat3/APRF
271	94	113	Pim-1
185	41	17	A1
155	62	A	SH2 containing inositol-5-phosphatase (Ship)
123	77	A	ASM-like phosphodiesterase 3a
122	14	A	PI-3-kinase, regulatory subunit p85α
22	A	A	PI-3-kinase, catalytic subunit, β, homolog
109	55	A	Rb2/p130
36	A	A	Rb1/p105Rb
105	36	A	TNF receptor-associated factor 5 (Traf5)
98	59	A	Serine/threonine kinase (MAP4K1, homolog)
95	28	A	cAMP-dependent protein kinase, β-cat. sub.
39	27	A	cAMP-dependent protein kinase, α subunit
88	A	A	cAMP-responsive element modulator
86	50	A	MAP kinase kinase kinase 1 (Mekk1)
86	41	A	Guanine nucleotide-binding protein, α 13
66	26	91	Mitogen-activated protein kinase (erk-1)
61	27	51	Early growth response 1 (Egr1/zif/268)
61	17	A	Cytokine-inducible SH2-containing protein
57	A	A	Protein tyrosine phosphatase STEP61
56	14	A	MyD118
23	A	A	Ddit1/Gadd45
56	A	A	c-Fes (tyrosine kinase)
54	26	A	Lithium-sensitive myo-inositol monop'tase A1
52	A	A	Caspase-3/CPP32
52	25	A	Fyn proto-oncogene (Fyn/p59fyn)
48	25	A	Protein tyrosine kinase, tec type I
38	17	A	G protein γ-2 subunit, homolog
35	A	45	Phospholipase C β3
34	A	27	Lyn-B protein tyrosine kinase
Transcription factors			
1251	353	271	ATF-4/CREB2
478	167	A	Id-2
59	11	188	Id
454	233	58	Jun-B
299	171	26	H3 histone, family 3B (H3f3b)
298	125	100	c-Fos
281	80	50	Max/Myn (Myc-associated factor X)
157	49	A	Nur77/N10/NGFI-B
152	99	A	Butyrate response factor 1/TIS11
92	26	A	A20/TNF induced protein 3
86	14	A	TG interacting factor
76	24	A	Gfi-1
70	24	61	Ddit3/Chop-10/Gadd153
68	33	A	General transcription factor IIB (GTF2B), hmlg.
64	31	A	MafK
59	A	104	Kruppel-like factor 4 (gut) (Klf4/Ezf/Zie)
58	A	A	PEBP2a1/PEBP2αA/CBFA1
57	30	A	TSC-22-like protein, homolog
51	15	A	X box-binding protein-1 (Xbp1)
51	21	36	p45 NF-E2 related factor 2
49	A	A	Son of Sevenless 2
48	A	A	C/EBP β
48	A	A	Interferon Consensus sequence-binding protein
47	A	A	Arylhydrocarbon receptor
44	A	73	LRG-21
36	A	41	TSC-22
35	18	A	c-Jun
33	A	A	Blimp1

(*Legend appears at the bottom of the opposite page.*)

γδ IELs Have the Potential to Kill a Variety of Targets by Using Different Mechanisms. In addition to Granzymes A and B, γδ IELs were found to express other cytotoxic mediators. These include the antimicrobial peptide cryptdin, an α-IFN homolog, lymphotoxin β, Fas ligand, and genes implicated in generating or enhancing the lytic response of NK cells such as NKR-P1A, NKR-P1C, LAG-3, and 2B4. LAG-3 has been reported to bind class II MHC (14), suggesting that macrophages, B cells, and epithelial cells may interact with γδ IELs. 2B4 is related to CD2 and binds to the same ligand, CD48 (15). The expression of 2B4, but not CD2, on virtually all γδ IELs can be demonstrated by FACS (Fig. 1*B* and data not shown).

Intriguingly, mRNAs coding for a variety of inhibitory receptors (CTLA-4, gp49, PD-1, and the NK inhibitory receptors Ly49-E, F, and G) are found in the γδ IEL samples. Because our data suggest that γδ IELs are actively transcribing genes related to effector functions, it seems likely that these cells, although ready to act, are being held back by inhibitory receptors *in situ*. Most functions could thus be kept in check and yet be readily turned on, with little or no *de novo* transcription. This interpretation would also be consistent with the very few differences in gene expression seen between γδ IELs from infected versus uninfected mice (Table 5).

γδ IELs Can Recruit Other Leukocytes, Down-Regulate Immune Responses, and Present Antigens on Class II MHC Molecules. γδ IELs express genes that are known to down-regulate immune responses. These include TGFβ and TJ6/J6B7. Perhaps the most intriguing one is Eta-1 (osteopontin), which has been postulated to be part of a surprisingly rapid T cell-dependent response to infection preceding classical forms of T cell-dependent immunity. Recent experiments indicate that Eta-1 can differentially regulate macrophage IL-10 and -12 expression and thereby play a key role in the establishment of cell-mediated immune responses to viral and bacterial infections (16). After *Listeria* infection, mice deficient in Eta-1 fail to form granulomas—a phenotype that is also observed in mice deficient in γδ T cells (17, 18).

Transcripts corresponding to chemokines such as RANTES, lymphotactin, macrophage inflammatory protein (MIP)-1α, and MIP-1β are present in γδ IEL populations. It is worth noting that other than very low levels of IL-2, none of the other cytokines on the arrays, including IL-1–5, -7, -10–12, -15, and -17, and IFNγ, were expressed. This expression pattern suggests that, whereas γδ IELs may play a role in recruiting other leukocytes, their ability to modify immune responses may be more focused.

With respect to cytokine receptor genes, γδ IELs express the β and γ chains of the IL-2R, the IL-4R, the β chain of the IL-12R, receptors for IFNs α, β, and γ, tumor necrosis factor receptors 1 and 2, and also 114/A10, a responsive element of IL-3. Cytokine receptor gene transcripts not found in γδ IELs include the receptors for IL-3, -5–11, -15, and -17. Additionally, γδ IELs transcribe the gene encoding the (bacterial) lipopolysaccharide-induced chemokine receptor (L-CCR), but lack transcripts for CXCR4 and CCR7 that are associated with peripheral homing. In fact, the transcripts for L-selectin, α-actinin-1, and lymphocyte function-associated antigen-1 are also absent in γδ IELs. Thus, γδ IELs lack both the extracellular and intracellular components required for peripheral homing.

γδ IELs expressed both the invariant chain and MHC class II molecules. FACS analysis showed that 15% of the γδ IELs express I-A on the surface (Fig. 1*C*). This finding indicates that γδ IELs may serve as antigen-presenting cells for $CD4^+$ αβ T cells, possibly presenting peptides from intestinal lumen- or epithelial cell-derived antigens.

γδ IELs May Contribute to Intestinal Lipid Metabolism, Cholesterol Homeostasis, and Physiology. The most surprising result is the expression of many genes relating to lipid and cholesterol metabolism in the γδ IEL samples (Tables 2 and 7). Cholesterol is a major structural component of the plasma membrane lipid rafts where many signaling proteins are found in activated T and B cells (reviewed in ref. 19). Thus, the enhanced expression of genes involved in the cholesterol biosynthetic pathway is compatible with our proposal that γδ IELs are constitutively activated. In addition, the expression of these genes may also be important in intestinal physiology.

Although it is well known that the small intestine plays a major role in the metabolism of dietary lipids, it is commonly assumed that these functions are carried out by enterocytes, a major population of epithelial cells. Indeed, high levels of transcripts for fatty acid-binding protein and apolipoproteins A-I, A-IV, and C-III were detected in epithelial cell mRNA. Unexpectedly, mRNAs for apolipoprotein E, phospholipid-binding protein, low-density lipoprotein receptor, and some enzymes involved in fatty acid, lipid, and cholesterol biosynthesis were expressed only in the γδ IEL samples. The expression of these transcripts raises the possibility that lipid metabolism is carried out through the collaboration of epithelial cells and γδ IELs. These results also suggest that γδ IELs may play a role in the generation of lipoprotein particles including chylomicrons, very low density lipoprotein, and high-density lipoprotein. The lipoprotein particles may promote the efflux of cholesterol from the membranes of dying cells and provide cholesterol to the rapidly dividing epithelial cells, thereby maintaining homeostasis. Consistent with the supposition that γδ IELs are involved in lipid/cholesterol metabolism, two of the six genes that show an increase in cells isolated from infected versus uninfected animals are involved in glycolipid metabolism (Table 5).

The protein encoded by *adh*-1, ADH-A2, is the only known murine class I alcohol dehydrogenase that is capable of oxidizing retinol (vitamin A) to retinaldehyde. This process is the first enzymatic step in the conversion of retinol into its biologically active metabolite, retinoic acid, a potent inducer of cellular differentiation and morphogenesis. The expression of ADH-A2 is absent in the αβ T cell samples and is expressed at a lower level in epithelial cells. Carbonic anhydrase II may regulate the acid-base balance within IELs. The abundance of transcripts encoding this protein suggests that IELs may exist in an environment where the pH may reach drastically different levels compared with the normal blood circulation.

The production of keratinocyte growth factor (KGF) by *in vitro* culturing of γδ IELs with TCR crosslinking (20) has led to

(*On the opposite page.*) A partial list of genes identified as being more abundantly-expressed in γδ IELs than in mesenteric lymph node $CD8^+$ αβ T cells by ANOVA (10). The complete list can be found in Table 7. Average gene expression values are shown for γδ IELs (γδ T), MLN $CD8^+$ αβ T cells (αβ T), and epithelial cells (*Epi.*). Because the differences in gene expression between the infected and uninfected samples of either the αβ or the γδ T cells were very small, all four γδ IEL samples were compared against all six $CD8^+$ αβ T cell samples. In all, 235 genes fit the following criteria. These genes (*i*) have a *P* value $<7.87 \times 10^{-6}$ (1,206/6,352 genes), (*ii*) are more highly expressed in γδ than in the αβ T cells (449/1,206 genes), (*iii*) are called ''present'' or ''moderate'' in at least two γδ IEL samples (344/449 genes), (*iv*) have a difference in median expression of at least 15 between the γδ and αβ T cell samples (246/344 genes), and (*v*) show at least a 1.5-fold difference in median expression between the γδ and αβ T cell samples (235/246 genes). To avoid large differences in expression because of negative or very small values in αβ T cell gene expression, all αβ T cell expression values <1 were made equal to 1 before calculating the absolute and fold differences in steps *iv* and *v*.

a proposed role for these cells to maintain intestinal homeostasis. We failed to detect KGF expression in any γδ IEL samples.

Discussion

In this report, we have characterized the gene expression pattern of γδ IELs in an effort to understand why these cells are important in the initial protection against oral *Yersinia* infection. Surprisingly, we found hardly any transcriptional changes in γδ IELs as the result of infection. Instead, γδ IELs from uninfected as well as infected animals appeared to be activated and transcribed high levels of cytotoxic and other genes associated with specialized functions. Although these results preclude us from estimating the magnitude of the γδ IEL response to *Yersinia* infection, they provide an important and unexpected clue for understanding how γδ IELs may function as a "first line of defense" against pathogens entering the digestive system.

It is commonly assumed that γδ IELs require antigen recognition to induce effector functions. This is because almost all of the well-defined functions attributed to freshly isolated γδ IELs were observed in *in vitro* assays with anti-CD3 antibody to crosslink the TCR. These include the spontaneous lytic response of γδ IELs as assayed in anti-CD3 redirected lysis (21) and the transcription of KGF (20) and lymphotactin (22) mRNAs in γδ IELs after *in vitro* activation with plate bound anti-CD3 antibody. Our data show that freshly isolated γδ IELs constitutively express very high levels of Granzymes A and B and RANTES transcripts, that these cells express NK-activating and inhibitory receptors, and that the activation of γδ IELs and peripheral αβ T cells appears to be triggered by different signaling cascades. These features raise the interesting possibility that the lytic activity of γδ IELs may not be induced exclusively through the antigen receptors. Instead, it could be induced through activating receptors such as those expressed by NK cells. This method of activation would allow γδ IELs to deal with a broad range of pathological situations very quickly, despite the diversity of γδ TCR expressed by these cells, and with little or no requirement for new gene expression—an effective way to participate in innate immune responses.

If γδ IELs are able to function in this fashion, what is the role of the γδ T cell receptor? One possibility is that it gives these cells an alternative route to induce cytotoxicity, free from the constraints imposed by the use of activating receptors. As γδ TCRs recognize intact antigens directly (reviewed in ref. 23), this ability could allow γδ IELs to detect infected or diseased cells through the recognition of activation- (24) or stress- (25) induced self antigens. γδ IELs could also recognize and kill pathogens directly [a herpesvirus-specific γδ T cell has been described (26)]. It is also possible that a different set of effector programs is triggered by TCR engagement. The induction of KGF mRNA may be such an example.

Although we do not have any evidence for such new gene expression here in the γδ IELs of *Yersinia*-infected mice, it may be that such genes are below the limits of detection here or are not represented on the arrays. In the case of the MLN $CD8^+$ αβ T cells, where 8% of them are activated in response to the infection, the most clearly up-regulated effector genes are the very abundant ones, which encode components of the cytotoxic granules. Cytokines, which are known to be expressed in activated $CD8^+$ αβ T cells and can be detected by sensitive reverse transcription–PCR in this infection system (7), are not detectable above background. In addition, as mentioned above, we do not know what fraction of the γδ IELs are responding to *Yersinia* antigens or to antigens induced by the infection.

Our earlier studies showed that mice lacking γδ T cells ($TCR\delta^{-/-}$) are much less resistant than either normal mice or mice without αβ T cells ($TCR\beta^{-/-}$) to the early dissemination of *Yersinia.* These findings suggest that γδ IELs are important and functionally distinct from αβ IELs in this infection model. We have tested some of the effector genes that are preferentially expressed in γδ IELs and found they are similarly expressed in αβ IELs. Similar observations were made from a more extensive serial analysis of gene expression (SAGE) analysis carried out by A. Hayday and colleagues on αβ and γδ IELs isolated from uninfected animals (A. Hayday, personal communication). This similarity between αβ and γδ IEL gene expression suggests that the differences between γδ and αβ IELs in contributing to immune competence are most likely because of their differences in antigen recognition (reviewed in ref. 24) and the functional consequences of such recognition.

In any event, this approach has allowed us to evaluate many more potential attributes of γδ IELs than previously possible and has provided important insights into their regulation and function.

This work was supported by grants from the National Institutes of Health (Y.-h.C.) and the Howard Hughes Medical Institute (M.M.D.). A.M.F. was supported by a Stanford Dean's fellowship and a Katherine McCormick fellowship. We thank Mamatha Mahatir and Suzanne Ybarra for expert technical advice and Drs. Richard Glynne and Jen-Tsan (Ashley) Chi for helpful discussions.

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