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γδ T-cell lines isolated from intestinal epithelium respond to a B-cell lymphoma

Y. SANO,* E. DUDLEY,† S. CARDING,‡ R.-H. LIN,§ A. C. HAYDAY† & C. A. JANEWAY, JR *Section of Immunobiology Howard Hughes Medical Institute, Yale University School of Medicine, Connecticut and †Department of Biology, Yale University New Haven, Connecticut, U.S.A.*

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SUMMARY

γδ T-cell hybrid clones were obtained from intestinal intraepithelial lymphocytes (i-IEL) by fusion with the BW5147 thymoma line. Four clones which expressed different Vγ/Vδ genes were selected for further study. All of the γδ clones secreted interleukin-2 (IL-2) in the presence of the BALB/c-derived B-lymphoma line, A20. No αβ T-cell hybrid clones derived from spleen or i-IEL responded to A20. We obtained several pieces of evidence which strongly suggest that these responses are mediated by the γδ T-cell receptor (TcR). Class II major histocompatibility complex (MHC), FcR and surface Ig expressed on A20 are not involved in the response. Native i-IEL derived from BALB/c selectively survive in culture in the presence of A20 cells. The ligand may be a superantigen-like molecule because all our γδ T-cell clones responded to A20 in spite of their different combinations of Vγ/Vδ gene segments.

INTRODUCTION

Some γδ T cells occupy surface epithelial layers in the mouse.[1,2] The cells in these sites express T-cell receptors (TcR) characterized by very limited diversity of Vγ segments within each particular tissue.[3,4] These facts have led to speculation that such cells recognize alterations in the expression of self or of highly conserved pathogen molecules and provide a first-line defence against infection.[5] Although self proteins such as self major histocompatibility complex (MHC) class IB,[6] class II,[7] CD1 molecules[8] and heat-shock proteins[9,10] have been proposed as candidates for the specific ligands of γδ TcR, further progress in this area requires that the ligands recognized by γδ TcR be identified, and the native populations in the tissues be shown to respond to these ligands.

To address this question, we prepared αβ and γδ TcR-bearing hybridoma lines from intestinal intraepithelial lymphocytes (i-IEL). All the resulting γδ hybrids, but none of the αβ hybrids, responded to a murine B-cell lymphoma line. Furthermore, freshly isolated native i-IEL also responded to the B-cell line. The possible nature of the ligand on the stimulating B-cell line is discussed.

* Present address: Dept. of Dermatology, Faculty of Medicine, Tottori University, Yonago 683, Japan.

‡ Present address: Dept. of Microbiology, University of Pennsylvania School of Medicine, 209 Johnson Pavilion, Philadelphia, PA 19106, U.S.A.

§ Present address: National Taiwan University, College of Medicine, Institute of Microbiology & Immunology, Taipei, Taiwan, 10018.

Correspondence: Dr C. A. Janeway, Jr, Dept. of Immunobiology, Yale University School of Medicine, New Haven, CT 06510, U.S.A.

MATERIALS AND METHODS

Mice

Mice were obtained from Jackson Laboratories (Bar Harbor, ME). (C57BL/6 × AKR/J)F_1 and BALB/c mice were originally obtained from Jackson Laboratories and thereafter bred in our facility. Mice were used between 6 and 10 weeks of age.

Antibodies

Anti-murine CD3ε (YCD3-1) was developed at Yale University. For TcR cross-linking experiments, the antibody solution (4 μg/ml) was allowed to bind to culture wells for 24 hr at 37°. Unbound antibody was then washed out with phosphate-buffered saline (PBS). Anti-αβ TcR monoclonal antibody (mAb) (H57-597.2) was provided by Dr R. Kubo (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). Anti-TcR γδ mAb (3A10) was a kind gift from Dr S. Tonegawa (MIT, Cambridge, MA). Anti-Vδ4 antibody (GL2) was purchased from Pharmigen Co. (San Diego, CA). Anti-TcR Vδ 6.3 mAb (clone C504.17C) was developed at Yale.[11] The Fab fragment of anti-TcR Vδ 6.3 was prepared by the method described by Kaye *et al.*[12] Anti-FcR antibody (2.4.G2), anti-I-E antibody (14.4.4S) and anti-I-A antibody (212.A1) and goat anti-mouse IgG antisera were used for cytofluorometric analysis.

Reagents

Purified protein derivative (PPD) was obtained from Connaught Laboratories Limited (Ontario, Canada).

Molecular analysis of i-IEL-derived γδ hybridomas

Total cell RNA was made from the hybridomas and checked for quality by electrophoresis as previously described.[13] cDNA was

prepared using random hexamers, and the capacity for β tubulin to be amplified quantitatively by polymerase chain reaction (PCR) was demonstrated.[14] Amplification of various TcR combinations was then attempted on equivalent amounts of cDNA, using the primers listed below:

Vγ1: AAGAATTCAGTCGACCTTGGGATGCTGCTCCTGAGA;
Cγ4: CCATCCATTATGAACGTTGATCATCAC
Vγ4: CTTGCAACCCCTACCCATAT;
Vγ5: GAGGATCCCGCTTGGAAATTGATGAGA;
Vγ6; GATCCAAGAGGAAAGGAAATACGGC;
Vγ7: GATCCAACTTCGTCAGTTCCACAAC;
Cγ1: CCACCACTCGTTTCTTTAGG
Vδ1: TTAAGTATACAAGCTAAATAGC;
Vδ2: AAAGTTCCCTGCAGATCCAA;
Vδ3: GAGACCACGGTCATCAAC;
Vδ4: CTGGAGATCTCCGACTCGCAGCTGGG;
Vδ5: TGCACGTACAATGCGGATTCTCCAA;
Vδ6: AAGCGGCCGCGTCGACATGAGTCACATGCCTCCTCA;
Vδ7: TATTACTGTGCTATGG;
Cδ: CTCATGTCAGCCCACCTTAA.

Reaction products were electrophoresed, and bona fide TcR amplifications judged by three criteria: ethidium bromide-visible fragments of appropriate size; hybridization to a specific internal probe; and sequencing of cloned products.

DNA sequencing

PCR products were gel purified and the appropriate size fragments cloned into the *Sma*I site of pUC18. DNA sequencing was performed on double-stranded plasmid DNA by the didoxy method using Sequenase (US Biochemical Co.).[15]

Cell lines

A20/2J is a B-lymphoma cell line derived from BALB/c mice. It expresses IgG, FcR and Ia molecules on its surface. LIg18, Ld18, and IIA1.4.1 are A20 variants which lack IgG, Ia and FcR, respectively. I-A^b-reactive T-cell hybrid clone A11.6 and I-E^b-reactive T-cell hybrid clone F3.24 are autoreactive clones generated from B10.A(5R) mice at Yale (R.-H. Lin, S. Rath, C. A. Janeway, unpublished data). P3X63.Ag8 is a BALB/c-derived myeloma line.

i-IEL

Intestinal IEL were prepared according to the method described by Davis[16] with some modification. Briefly, the full length of the small intestines of mice were put into complete Click's medium (Gibco, Grand Island, NY). Mesenteric membrane, vessels around the outer wall and Payer's patches were removed to prevent contamination by peripheral blood lymphocytes. The small intestines were then cut into approximately 3 cm lengths, opened longitudinally and cut into approximately 0·5 cm length pieces. These pieces were collected in a beaker and stirred in 60 ml of 1 mM EDTA–PBS with a magnetic stirrer for 20 min at 37°. The first supernatant was collected, then mixed with 30 ml of complete medium to inhibit EDTA activity. PBS containing 1 mM EDTA was added again to the intestinal fragments and stirred for another 20 min at 37°. The second supernatant was then mixed with the first. Dithioerythritol (DTE; Sigma Chemical Co., St Louis, MO) was added at 0·5 mM to the cell suspension to dissolve residual mucous. The cell suspension was passed through a glass wool column to remove aggregate, then spun at 200 *g* for 5 min. The cells were washed again in plain Click's EHAA media. The cell pellet was then suspended in Percoll (Pharmacia, Uppsala, Sweden) which was diluted to 40% with plain Click's EHAA medium and spun at 600 *g* at 20° for 20 min. The pellet was washed twice with medium. This preparation usually contains 20–40% lymphocytes and 60–80% epithelial cells.

γδ T-cell hybrid clones

T-cell hybrid clones were made by fusion to BW514/TcR$\alpha^-\beta^-$[17] with polyethyleneglycol as described by Harwell *et al.*[18] Fused cells were selected with HAT medium (Boehringer-Mannheim Co., Mannheim, Germany). αβ and γδ T-cell clones were screened by cytofluorometric analysis using anti-TcRαβ and TcRγδ antibodies.

Culture of γδ T-cell clones for IL-2 production

γδ T-cell hybrid clones (2×10^4/well) and A20 cells (5×10^5/well) were co-cultured in Click's EHAA medium plus 5% fetal calf serum (FCS) in 96-well microplates for 24 hr. The supernatants were collected and frozen in a −70° freezer before IL-2 assay.

IL-2 assay

IL-2 activity was determined by a modified microassay method with the use of the IL-2-dependent murine cloned cytotoxic T-cell line (CTLL) according to the method described by Stadler *et al.*[19] IL-2 activities are described as the incorporation of [^{3}H]-thymidine (c.p.m.) by IL-2-dependent cells; geometric means ±SE of three replicate wells are shown.

Cytofluorometric analysis

Analyses were performed with FACScan (Becton Dickinson, Mountain View, CA). Antibodies used for the analyses are described where indicated. Phycoerythrin (PE)-conjugated streptavidin was used for two-colour analysis. Cells were fixed with 1% paraformaldehyde after being stained, then analysed within 48 hr.

Statistical analysis

Student's *t*-test was employed to determine statistical differences.

RESULTS

Four γδ T-cell hybrid clones expressing different TcR Vγ/Vδ genes were obtained from i-IEL

To study the antigen specificity of i-IEL, it was necessary to propagate clonal populations *in vitro*; despite extensive effort, all our attempts to grow non-transformed i-IEL failed. MacDonald and colleagues have experienced similar difficulties which they attribute to rapid induction of apoptosis of γδ i-IEL in culture.[20] To circumvent this difficulty, we fused i-IEL to the T-cell thymoma line, BW5147$\alpha^-\beta^-$. The resulting hybrids contained both γδ T cells and αβ T cells. The latter were used only as controls for the γδ T cells. Of 140 hybrids obtained, 17 γδ T-cell lines were identified. The results of TcR V gene analysis of the four cloned hybrids used in these studies are summarized in Table 1. Vγ usage was determined by PCR of mRNA. Vδ usage was checked by cytofluorometric analysis using mAb to Vδ4 and Vδ6.3 as well as by PCR. The four γδ T-cell clones express

Table 1. Sequences of rearranged TcR genes in IEL hybrids

GD13 sequences		
δ chain	V6	D2
	TGC GCT CTC TCG GAA CTC TC C TTC TCC C GG GAT ACG TGC TGG CT C GAC	
γ chain	V1	J
	TAC TGT GCA GTC TG G GG T CGA TCA GGC ACA TCA TGG GTC	
	V7	J
	GCT CTG TAC TGT GCC TCC TGG GCT GGA CG C AGC TCA GGT	
GD31 sequences		
δ chain	V6 D1 D2 J	
	TGC GCT CTC TCG GAA CT G A GG C G T CGG AGG G TC GTC TTT	
γ chain	V1	J
	TAC TGT GCA GTC TG G GG T CGA TCA GGC ACA TCA TGG GTC	
	V7	J
	GCT CTG TAC TAC TGT GCC TCC TGG GCT GGA AGC TCA GGT	

different TcR Vγ/Vδ gene segments. This is consistent with the structural diversity of TcR of i-IEL.[2] Two clones, GD13.1 and GD31.20, expressed Vγ7 mRNA which is one of the major Vγ in i-IEL;[2] however, both of the clones expressed Vγ1 as well (Table 1). The rearranged TcR V genes of these two clones were sequenced (Fig. 1), revealing that both Vγ1 and Vγ7 were in-frame. Which of these two Vγ genes encode surface γδ TcR is not known. Both of the clones express a Vδ6 family member which differed in inserted N and D fragments (Fig. 1). None of the i-IEL T-cell hybrid clones express Vδ4. Thus, each of these four clones express different combinations of Vγ/Vδ genes. All of these clones originally expressed CD8 on the surface, as do most i-IEL γδ T cells, but they lost CD8 fairly quickly during culture.

A B-cell lymphoma line stimulates the γδ i-IEL but not αβ T-cell hybrid clones

In earlier studies of αβ and γδ T-cell hybrids, we have used the B-cell lymphoma line A20/2J as a source of Fc receptors to cross-link anti-TcR antibodies.[21] In carrying out similar experiments

GD13.1		
	V6	D2 J
δ chain	TGC GCT CTC TCG GAA CTC TC C TTC TCC C GG GAT ACG TGC TGG CT C GAC	
	V1	J
γ chain	TAC TGT GCA GTC TG GGG T CGA TCA GGC ACA TCA TGG GTC	
	V7	J
	GCT CTG TAC TGT GCC TCC TGG GCT GGA CG C AGC TCA GGT	
GD31.20		
	V6 D1 D2 J	
δ chain	TGC GCT CTC TCG GAA CT GA GG C GT CGG AGG G TC GTC TTT	
	1 V1 J	
γ chain	TAC TGT GCA GTC TG GGG T CGA TCA GGC ACA TCA TGG GTC	
	V7 J	
	GCT CTG TAC TAC TGT GCC TCC TGG GCT GGA AGC TCA GGT	

Figure 1. Sequences of rearranged TcR Vγ/Vδ genes in Vγ7$^+$ T-cell hybrid clones. PCR products were sequenced. N nucleotides are underlined.

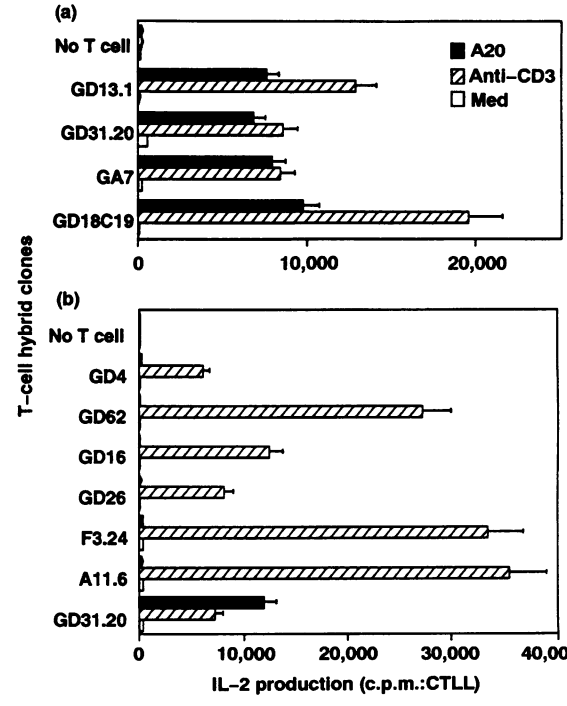

Figure 2. γδ T-cell hybrid clones respond to A20 (a), while αβ T-cell hybrid clones do not (b). The protocol for this culture and IL-2 assay are described in Materials and Methods. Four of the αβ T-cell hybrid clones (GD4, GD62, GD16, GD26) were derived from i-IEL, and two αβ T-cell hybrid clones (F3.24, A11.6) are MHC class II specific and derived from lymph node cells. IL-2 activities in the supernatants are shown as [^{3}H]thymidine uptake (c.p.m.) of CTLL and are geometric means of triplicate culture cells (±SEM). Responses of γδ T-cell clones with A20 (■), without A20 (medium) (□) and with anti-CD3 antibody precoated in the plates (■). GD31.20 was added in experiment (b) as a positive control.

on γδ T-cell hybrids derived from i-IEL, we made the surprising observation that γδ i-IEL T-cell hybrids secrete IL-2 in the presence of A20 cells without added antibodies (Fig. 2a). A20 cells stimulate all of the γδ i-IEL T-cell hybrid clones. In this report, we study the nature of this response. This response appears to be specific to the γδ TcR-bearing i-IEL hybrids, because none of the hybrids bearing αβ TcR responded to A20 cells, even if they were derived from i-IEL (Fig. 2b).

Responsiveness to A20 requires cell-to-cell contact via the γδ T-cell receptor

We have obtained several pieces of evidence that the response of γδ i-IEL T-cell hybrids to A20 cells is mediated by cell-to-cell contact via the γδ TcR. First, the response of T-cell hybrids bearing a γδ TcR to the B-lymphoma cells requires mixing of cells; supernatants of A20 cells are inactive (data not shown). Second, fixed A20 cells are nearly as active as viable A20 cells in the stimulation of γδ T-cell hybrids, as shown in Table 2. Third, whenever TcR negative variants of the γδ T-cell hybrids arose, they uniformly lost the ability to respond to A20 cells (data not shown). Fourth, a mAb specific for Vδ6.3 inhibits the response only of those γδ T-cell hybrids bearing Vδ6.3, as shown in Table 3. There appears to be some non-specific activity at higher antibody concentrations, causing inhibition higher than 100% in Vδ6.3$^+$ clones and slight inhibition in the Vδ6.3$^-$ clone. Those data, together with the association of this response with γδ and not αβ T-cell hybrids, argue strongly for a role of the γδ TcR in this response to A20 B-lymphoma cells.

The activation of γδ T-cell hybrids by A20 cells does not require MHC class II molecules, Fc receptors, or surface immunoglobulin

To explore the nature of the ligand presented to γδ T cells by A20 cells, we used a panel of mutant A20 lines generated in the laboratory. These lines lack surface MHC class II molecules, Fc receptors, or surface IgG (Fig. 3). All variants stimulate γδ T-cell hybrids as shown in Table 4. This result suggests that the response to A20 does not require MHC class II, FcR, or surface IgG. MHC class I-deficient A20 variants were not available.

γδ T-cell hybrids do not respond to purified protein derivative (PPD)

The 65,000 MW heat-shock protein (hsp) which is contained in PPD has been reported to be a potent stimulator for γδ T cells.[9]

Table 2. Live and fixed A20 cells are able to stimulate γδ T cells

	IL-2 production in the presence of*		
T-cell hybrid clones	Medium	A20	Fixed A20
GD13.1	1600±1·24	37,566±1·17	26,522±1·09
GD31.20	1245±1·14	38,621±1·28	13,486±1·16
GC18C19	2122±1·22	21,444±1·20	17,529±1·26
GA7	458±1·30	14,447±1·19	8477±1·08

A20 cells were added to the culture before or after fixation with 0·4% paraformaldehyde–PBS at room temperature for 2 min.

* IL-2 production was assayed by the proliferation of CTLL as determined by incorporation of [^{3}H]thymidine (c.p.m.).

Data are expressed as geometric means±SE of triplicate wells.

Table 3. IL-2 production by γδ6.3$^+$ T-cell hybrids can be inhibited in the presence of anti-Vδ6.3 antibody

T-cell hybrid clones		% inhibition by anti-Vδ6.3		
Clones	Vδ6.3	Undigested Ab (5 μg/ml)	Fab (5 μg/ml)	Fab (0·5 μg/ml)
GD18C19	+	101·6	83·7	67·5
GA7	+	113·9	111·1	77·8
GD31.20	−	27·8	13·5	10·8

Either an undigested anti-Vδ6.3 antibody or a Fab fragment of the antibody was added at the start of the culture. The supernatants were collected and assayed for IL-2. IL-2 production is presented as the proliferation of CTLL (incorporation of [^{3}H]thymidine). Anti-Vδ6.3 had no influence upon the proliferation of CTLL. % blockade was calculated as:

$$\frac{\text{Response with A20} - \text{response with A20 and anti-V}\delta6.3}{\text{Response with A20} - \text{background}}$$

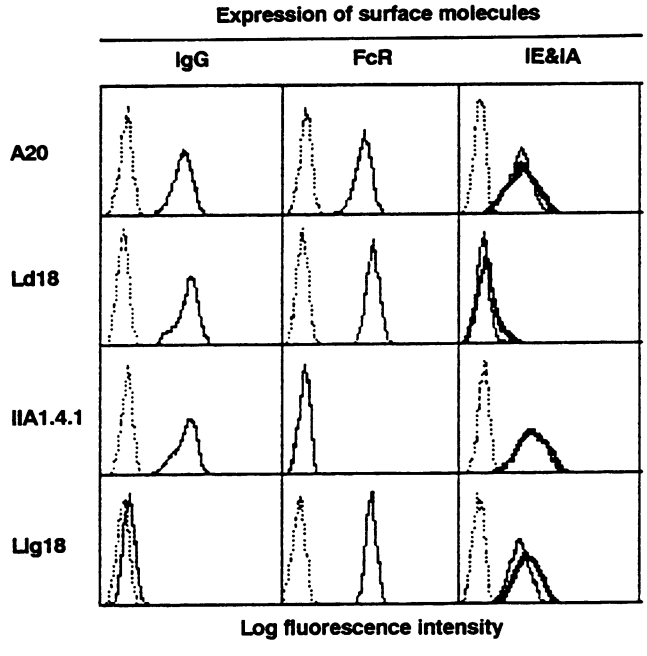

Figure 3. Expression of surface molecules on A20 and its variants. Ld18, IIA1.4.1 and LIg18 were Ia$^-$, FcR$^-$ and surface IgG$^-$ variants, respectively. They were analysed by FACScan. Cells were stained with antibodies specific for the indicated molecules (—); unstained control cells (····); expression of I-A (—). The responsiveness of γδ T-cell clones to these variants is shown in Table 4.

However, none of the i-IEL-derived γδ T-cell hybrids were detected of responding to PPD, while control γδ T-cell hybrids from fetal thymus did respond to PPD in the same assay (data not shown).

Normal intestinal intraepithelial γδ T cells respond to A20 cells

Fresh i-IEL prepared from BALB/c mice were tested for responsiveness to A20 by co-culture (Table 5). As reported

Table 4. Several variants of A20 stimulate γδ T cells

Stimulating cells	Deficiency	IL-2 production by γδ T-cell hybrid clones				
		No T cells	GD13.1	GD31.20	GA7	GD18C19
A20	None	150±1·21	25,147±1·19	43,219±1·22	62,471±1·24	32,582±1·10
Ld18	Ia	213±1·11	21,056±1·29	57,206±1·03	48,426±1·11	41,248±1·20
IIA1.4.1	FcR	234±1·28	21,596±1·20	31,652±1·31	51,351±1·24	29,427±1·16
LIg18	sIg	126±1·06	21,352±1·15	40,286±1·14	49,352±1·29	33,527±1·11
No cells	—	214±1·30	2568±1·17	1529±1·16	1210±1·16	854±1·07

γδ T-cell clones were co-cultured with A20 cells or variants for 24 hr. The supernatants were introduced to the IL-2 assay after culture. IL-2 production is presented as the proliferation of CTLL (incorporation of [^{3}H]thymidine). The data are expressed as geometric means ± SE of triplicate wells.

Table 5. Yield of live cells from 2-day culture of fresh BALB/c i-IEL with A20

Stimuli	No. of viable cells ($\times 10^{-4}$)*		
	Total cells	γδ TcR$^+$ CD8$^-$	γδ TcR$^+$ CD8$^+$
(before culture)	(90)	(9·9)	(38)
Control	4·5	0·5	1·7
Immobilized anti-CD3	6·7	0·6	1·7
MMC-treated A20	23	6·1	4·1
MMC-treated P3X63.Ag8	9·0	0·9	2·5

Freshly prepared i-IEL from BALB/c mice were cultured with mitomycin C (MMC)-treated A20 cells, P3X63.Ag8 cells, or immobilized anti-CD3 in the plates for 48 hr. Live cells were enriched by lymphocyte separation medium (LSM: Organ Teknika Corp., Durham, NC). These cells were stained with FITC-anti-Lyt-2 and biotinylated 3A10 anti-TcR plus PE-conjugated avidin and analysed by FACScan. Ten thousand viable lymphocytes were analysed in each group.

* The no. of live cells was counted with trypan blue staining, and the number in each subset calculated from this on the basis of FACScan analysis.

earlier by MacDonald *et al.*,[20] i-IEL die rapidly in culture, only 5% surviving for 2 days. By contrast, 25% of i-IEL survive for 2 days when cultured in the presence of A20 cells. Moreover, CD8$^-$γδ T cells, similar in phenotype to our γδ i-IEL T-cell hybrid clones, showed a markedly increased survival of >60% in the presence of mitomycin C-treated A20 cells, suggesting that A20 cells are recognized selectively by normal γδ T cells in i-IEL. It is unlikely that the specific ligand on A20 is a polymorphic molecule, because the responder and stimulator cells are both BALB/c. No increase in i-IEL γδ cells was observed when they were cultured in the presence of the BALB/c-derived myeloma line, P3X63.Ag8, indicating that the survival of i-IEL γδ T cells is specific for A20.

DISCUSSION

The experiments in this paper provide the first evidence that a specific stimulus is able to activate the γδ T cells of i-IEL. Previous studies have either observed rapid apoptosis of these cells (20; Y. Sano and C. A. Janeway, unpublished observations) or have used anti-CD3 as a signal to activate cells immediately after isolation. We believe that the activation of γδ T cells by A20 cells is mediated through the γδ TcR. Although all our data are consistent with this idea, it could be argued that the TcR recognizes a ligand on the T cell itself, and that the response of the hybrids is enhanced by a co-stimulator carried by A20. We believe this is unlikely because A20 cells are very weakly co-stimulatory in other studies[22] and because fixed A20 cells are nearly as active as viable A20 cells although fixation is believed to inactivate or reduce co-stimulatory activity.[23]

If these γδ TcR i-IEL T-cell hybrid clones recognize a ligand or ligands expressed on A20 B-lymphoma cells with their TcR, what is the nature of that ligand? It is clearly not MHC class II molecules because an MHC class II-deficient A20 variant was as active as A20. The ligand could be MHC class I, including MHC class IB, which has not been addressed in these experiments. MHC class I has been suggested to be a specific ligand or restriction molecule for γδ T cells in several studies.[21,24] However, as the development of γδ TcR$^+$ i-IEL is not influenced in β_2-microglobulin-deficient mice in which CD8$^+$ αβ T cells are lost,[25] this seems unlikely.

The ligand could be some kind of heat-shock protein (hsp). There are several reports demonstrating that γδ T cells recognize a mycobacterial 65,000 MW hsp[9] which is contained in purified protein derivative (PPD). However, our γδ T-cell hybrids do not recognize this antigen. Certain sets of γδ TcR-bearing cells respond to hsp58 (groEL hsp) which was expressed on a B-lymphoma line.[10] It has also been found that dendritic epidermal

T cells which have very restricted structural diversity of their γδ TcR respond to a ligand expressed on self keratinocytes, although the ligand has not been identified.[26] Recently, it has been reported that the peptide-binding domain of hsp70 could be structurally quite similar to that of class I MHC molecules.[27] This suggests that hsp70 could present a peptide to T cells directly if hsp70 were expressed on the cell surface. CD1 molecules, which also have a structural resemblance to MHC, have antigen-presenting function to $CD4^-CD8^-$ T cells.[28] Heat-shock protein 70 and CD1 may represent families of antigen-presenting molecules separate from those encoded in the MHC. γδ T cells may recognize these molecules in an MHC-unrestricted manner.

A possible candidate for the specific ligand recognized by the γδ TcR on i-IEL is a superantigen. T cells bearing some sets of Vβ TcR responsd to superantigens in the context of MHC class II molecules.[29,30] However, there are very few reports describing γδ T-cell activation by superantigens. Fleischer and Schrezenmeier have reported that a $CD4^-$ $CD8^-$ T-cell close responded to SEB in the presence of MHC class II positive tumour cells,[31] while Koenig *et al.* found that a subset of human γδ T cells could be triggered to kill but not to proliferate by SEA.[32] Moreover, it has been proposed that human $V\gamma9^+$ T cells are expanded *in vivo* by a superantigen.[33,34] In this study, we found all the γδ T-cell hybrid clones derived from i-IEL responded to A20 in spite of their different combination of Vγ/Vδ TcR segments. We have also found that A20 can stimulate dendritic epidermal T cells (DEC) which express a very restricted Vγ5/Vδ1 TcR (Y. Sano, manuscript in preparation). The variety of different TcR able to respond to A20 cells suggests a superantigen-like molecule. If this were the case, the ligand would belong to a kind of superantigen different from SEB or *Mls* because the γδ T-cell hybrids used in this study respond to MHC class II^- A20 variants. While it has been reported that *Mycoplasma arthritidis* provides a superantigen to αβ T cells,[35] it is unlikely that the specific ligand on A20 is mycoplasma derived, because the A20 we used in this study was not infected with mycoplasma (data not shown). Recently, *Mls* -1^a was found to be a product of the Mouse Mammary Tumour Virus (MMTV).[36] The ligand on A20 could also be a viral product that might work as a superantigen. However, the nature of the stimulating structure for γδ T cells found on A20 cells is not known. Determining its nature may help to elucidate the role of γδ T cells in intestinal and other epithelia.

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