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Immunology

Heat shock protein Hsp60-reactive γδ cells: A large, diversified T-lymphocyte subset with highly focused specificity

REBECCA L. O'BRIEN*†‡§, YANG-XIN FU*, ROBIN CRANFILL*, ANGELA DALLAS*, CHRISTINA ELLIS†, CHRISTOPHER REARDON*, JULIE LANG*, SIMON R. CARDING¶, RALPH KUBO*†, AND WILLI BORN*†

*Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206; Departments of †Microbiology, and ‡Biochemistry, University of Colorado Health Sciences Center, Denver, CO; and ¶Department of Microbiology, University of Pennsylvania School of Medicine, 209 Johnson Pavilion, Philadelphia, PA 19104

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ABSTRACT **Previously, we detected a subset of γδ T cells in the newborn mouse thymus that responded to the mycobacterial heat shock protein Hsp60, as well as with what seemed to be a self-antigen. All of these cells expressed Vγ1, most often in association with Vδ6$^+$. It was not clear, however, whether similar, mature γδ cells with Hsp60 reactivity are common outside of the thymus, or rather, whether they are largely eliminated during development. From the data presented here, we estimate that γδ cells responding to Hsp60 comprise 10–20% of normal splenic and lymph node γδ T cells. Such cells, derived from adult spleen, always express a Vγ1–Jγ4–Cγ4 γ chain, although not all cells with this γ chain show Hsp60 reactivity. Many of these Vγ1$^+$ cells also express Vδ6–Jδ1–Cδ, though fewer than in Vγ1$^+$ cells from the newborn thymus. Extensive diversity is evident in both the γ and δ chain junctional amino acids of the receptors of these cells, indicating that they may largely develop in the thymus of older animals or undergo peripheral expansion. Finally, we found that all such cells responding to both a putative self-antigen and to mycobacterial Hsp60 respond to a 17-amino acid synthetic peptide representing amino acids 180–196 of the *Mycobacterium leprae* Hsp60 sequence. This report demonstrates that a large subset of Hsp60-reactive peripheral lymphoid γδ T cells preexists in normal adult mice, all members of which respond to a single segment of this common heat shock protein.**

T cells bearing the γδ T-cell antigen receptor (TCR) have been detected as a distinct lymphocyte subset in many mammalian species and in birds (1–6), suggesting that γδ T cells play a functional role worthy of evolutionary conservation. We have previously reported on a subset of γδ T cells present in the newborn mouse thymus that responds to a mycobacterial heat shock protein (Hsp) Hsp60 (7) and, in particular, to a 17-amino acid peptide of Hsp60 (8). The vast majority of the cells of this subset, like other subsets of γδ T cells, bear closely related TCRs, in this case, TCRs with the variable region (V) chains Vγ1 and Vδ6 (9). These cells, when immortalized as hybridomas, produce interleukin 2 (IL-2) in the absence of any deliberate stimulation. This "spontaneous" IL-2 production may arise from stimulation of the cells by their own endogenous Hsp60 or a related protein because some of the cells also recognize a synthetic peptide representing the same region of mouse Hsp60, as well as this region of Hsp60 from other disparate species (8). The subset thus shows a propensity to recognize a certain protein and always the same segment of that protein, despite some degree of junctional variation in both the γ and δ chains of the TCR and even occasional use of a different δ chain. We considered whether such potentially autoreactive γδ T cells are normally eliminated during thymic differentiation. However, in the study reported here, we have characterized similar cells that appear to exist as a large circulating subset in the adult mouse. These cells, showing the same pattern of Hsp60 reactivity, express a Vγ1 chain, and many express a Vδ6 chain, as was seen in the newborn thymus γδ cells; moreover, extensive junctional diversity is evident. Unless these cells normally exist in an inactive state, a possibility that cannot be ruled out in an experiment involving hybridomas, our data suggest a functional role for Hsp60-reactive γδ T cells in the periphery.

MATERIALS AND METHODS

Hybridoma Generation. For each fusion experiment, adult spleen cells from ≈10 C57BL/10 mice were enriched for γδ T cells by using a "panning" technique, as described (10), to remove B cells and αβ T cells. For anti-αβ TCR panning, B cells were first removed on goat anti-mouse immunoglobulin-coated dishes or with nylon wool. Monoclonal antibody (mAb) H57-597 (11) was applied at ≈100 μg/ml in balanced salts solution, the blocking step was allowed to proceed for not more than 30 min at 37°C, and the cells were allowed to bind during an incubation at 37°C. These cell preparations were 20- to 60-fold enriched for γδ T cells and were thus suitable for fusion experiments.

Enriched splenic γδ T-cell preparations were then fused either directly or after culture in Iscove's modified Dulbecco's medium (supplemented with essential and nonessential amino acids, glucose, pyruvate, 2-mercaptoethanol, antibiotics, and 10% fetal bovine serum) plus Con A at 5 μg/ml by using 4×10^6 cells per well in 1.5-ml 24-well plate cultures, for 3 or 4 days. Although in most experiments, the γδ cells for fusion were prepared from unprimed mice, in one case (69BAS), the mice had received ≈50 μg of *Mycobacterium tuberculosis* purified protein derivative (PPD) intravenously about 1 week prior to removal of the spleens. There was no evidence that the immunization attempt had affected the composition or numbers of resulting γδ hybridomas, however, so that fusion is included here. Hybridomas arising from most of the fusions averaged about 15% γδ$^+$ of the total TCR$^+$ hybridomas, regardless of the purification technique. We obtained 108 γδ$^+$ hybridomas from Con A-expanded enriched cells, as well as 18 hybridomas from enriched cells fused directly without *in vitro* expansion.

Cells were fused to a T-cell α and β gene negative variant of the AKR thymoma BW5147, BW/α$^-$β$^-$ (12), as described (13). In cases where clonality was uncertain, the arising hybridomas were subcloned by limiting dilution.

Hybridoma Screening. Cells were stained to identify the type of TCR expressed by using anti-αβ TCR (H57-597) (11) or anti-γδ TCR (403A10) (14) mAb-containing culture super-

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Abbreviations: Hsp, heat shock protein; TCR, T-cell antigen receptor; V, variable region; C, constant region; J, joining region; D, diversity region; PPD, purified protein derivative from *Mycobacterium tuberculosis*; IL-2, interleukin 2; mAb, monoclonal antibody.

§To whom reprint requests should be addressed.

natants, followed by a fluorescein isothiocyanate-conjugated rabbit anti-hamster immunoglobulin polyclonal antibody (Jackson ImmunoResearch).

IL-2 Assays. Hybridomas were screened initially for spontaneous reactivity (unstimulated IL-2 production) by using the IL-2/IL-4-dependent cell line HT-2 as described (13). The response to cross-linking anti-CD3 mAb (145-2C11) (15) was tested at the same time, using wells precoated for about 1 hr with anti-CD3 at 100 μg/ml. Hybridomas failing to produce at least 50 units of IL-2 per ml when stimulated by anti-CD3 were not included in further analyses. Positive cells showing some spontaneous IL-2 production, as well as some negative cells as controls, were then retested in triplicate for spontaneous reactivity, mycobacterial PPD reactivity (200 μg/ml), and response to the synthetic peptide representing amino acids 180–196 of the *Mycobacterium leprae* Hsp60 sequence (40–50 μg/ml) (8), all in the absence of exogenous presenter cells. IL-2 produced in units per ml was determined by a colorimetric assay that detects live cells (16).

Identification of V Genes Contributing to γδ Hybridoma TCRs. We prepared total cellular RNA (17) from the γδ hybridomas that responded well to cross-linking anti-CD3 antibody (≥50 units IL-2 per ml). Hybridization was carried out on RNAs dotted onto nitrocellulose, at ≈1 μg of RNA per dot, by using a Minifold apparatus and the method described by White and Bancroft (18). Hybridization probes indicated below were used to detect specific γ and δ mRNAs as preliminary evidence of gene usage: Vδ1 and Vδ2, ref. 19; Vδ4, Vδ5, and Vγ6, ref. 20; Vγ7, ref. 21; Vδ6 (Vα7.1), ref. 22; Vγ1, -2, and -3, gift of D. Raulet (Massachusetts Institute of Technology); Vδ3 (Vα6.1), Vδ7 (Vα9,10), Vδ8 (Vα2), Vδ-G8 (Vα11), and Vδ-2.3 (Vα4), gifts of E. Palmer (National Jewish Center for Immunology and Respiratory Medicine); and Vδ4, unpublished results of A.D. Nomenclature for γ and δ genes is from the following references: Vγ1–Vγ7, ref. 23; Vδ1–Vδ6, ref. 24; Vδ7, ref. 23; Vδ8, ref. 9; Vδ-G8, ref. 26; and Vδ-2.3, ref. 25. Blots were hybridized by using 50% formamide at 42°C and processed as described (27), using a final wash in 0.1× standard saline citrate/0.1% SDS at 56°C for 30 min. The dot blots were exposed to x-ray film (Kodak X-Omat AR) for 1–4 days.

Sequence Analysis. Transcripts containing specific Vγ and Vδ genes were amplified, using PCR, from hybridoma RNAs by using the following oligonucleotide primers: Cγ4(−), GAAGGAAGGAAAATAGTAGG; Vγ1(+), GGGCTTGGGCAGCTGGAGCA; Cδ(−), TGTTCCATTTTTCATGATGA; Vδ6(+), GGATCTAATGTGGCCGAGA; Vδ4(+), CAGACAGTGTCTCAGCCTCAG; Vδ5(+), GTGCATCACGCTGACCCAGA; Vδ3(+), CAACCAGATTCAATGGAAAG; Vδ8(+), AGCAGCAGGTGAGACAAAGT; Vδ7(+), ATGAAGAGGCTGCTGTGCTC; Vδ-G8(+), GGAGATCAGGTGGAGCAGAG; Vδ-2.3(+), TCAGTGACTCAGACGGAAGG. Reaction conditions were as described (28). The Sequenase system (Stratagene) was used for sequencing with dideoxynucleotides by using [^{35}S]dATP and 1 μl of a 1 μM solution of the appropriate primer per reaction: Cγ4(−), GGAGAAAAGTCTGAGTCAGT; Vγ1(+), AGTATCTAATATATGTCTCA; Cδ(−), TGGTTTGGCCGGAGGCTGGC.

Immunoprecipitation and SDS/PAGE. This analysis was carried out as described (11) by using cell surface radioiodination and a digitonin lysis buffer (29). TCRs were immunoprecipitated by using the anti-CD3 mAb (15) and protein A-Sepharose and were separated on nonreducing SDS/PAGE gels as described (11).

RESULTS

Characteristics of Splenic γδ Hybridomas. We examined adult spleen as a potential peripheral site for Hsp60-reactive γδ T cells. C57BL/10 adult spleen cells depleted of B cells and αβ T cells were hybridized to the T-cell fusion line BW/$\alpha^-\beta^-$ (12). γδ TCR$^+$ hybridomas arising were then tested for spontaneous IL-2 production (which may stem from self Hsp60 reactivity; see ref. 8) and reactivity to mycobacterial PPD, an impure source of Hsp60 (7). We found that 41 out of 73 γδ hybridomas derived from adult spleen showed these responses, designated as the Hsp60 reactivity pattern. As with newborn thymus-derived hybridomas, all cells that showed a strong PPD response were also spontaneously reactive; however, weakly PPD-reactive hybridomas that gave extremely weak spontaneous responses (within the background) have also been classed as showing the Hsp60 reactivity pattern.

We next examined the receptors of these hybridomas, using PCR technology, to test for transcription of the Vγ1–Cγ4 and Vδ6–Cδ (C = constant region) genes. This pair is functionally expressed by ≈90% of the Hsp60-reactive hybridomas derived from newborn thymus (9). PCR-amplified cDNA from each hybridoma was sequenced to identify in-frame (functionally rearranged) V–J or V–D–J (J = joining region; D = diversity region) junctions. The results of this study are summarized in Table 1.

Overall, the complexity of the γδ receptor associated with the Hsp60 reactivity pattern is greater in cells derived from adult spleen than in cells derived from newborn thymus. However, as in the newborn thymus hybridomas, all cells showing the Hsp60 reactivity pattern express a functionally rearranged Vγ1–Cγ4 gene (41 out of 41), indicating that this γ chain is necessary for Hsp60 reactivity. Whereas Vγ1 chains were rare among non-Hsp60-reactive γδ T cells from the newborn thymus (3 out of 25 nonreactive hybridomas), nearly all (66 out of a total of 73) of the adult spleen-derived hybridomas express an in-frame Vγ1 transcript, whether or not they show the Hsp60 reactivity pattern. This tendency to express a Vγ1 chain is evident in cells derived from several fusions (see *Discussion*).

A strong correlation between cells expressing Vδ6 and those having the Hsp60 reactivity pattern is evident in the adult spleen-derived hybridomas, as was previously found in the newborn thymus-derived hybridomas. Among cells derived from either tissue, non-Hsp60 reactive Vδ6$^+$ γδ hybridomas are rare. In both cases, among cells expressing a Vδ6 family member, Vδ6.3$^+$ cells are the most common (9), with a lesser percentage of cells expressing Vδ6-λ12. As in the newborn thymus hybridomas, only these two Vδ6 family members were detected in Hsp60-reactive cells, except for one adult spleen hybridoma, which expressed Vδ6.1. Vδ6.2$^+$ (30) cells were not found in either group despite the fact that Vδ6.2 is more closely related to Vδ6.3 than any other Vδ6 family member that has been published (96% identical on the amino acid level). Additionally, as was found in hybridomas

Table 1. Adult spleen γδ hybridomas: Overview of cell types

Receptor type	No. expressing receptor type*	No. showing Hsp60 reactivity pattern	(No. of Hsp60 reactive/total no. of Hsp60 reactive) × 100%
Vγ1$^+$	66	41	100
Vδ6.3$^+$	18	17	41.5
Vδ6-λ12$^+$	7	6	15
Vδ6.1$^+$	1	1	2
Vγ1$^+$, other Vδ	40	17	41.5

Cells reproducibly producing ≥10 units of IL-2 per ml either spontaneously or when incubated with PPD are classified as showing the Hsp60 reactivity pattern.

*Number of hybridomas (out of a total of 73 hybridomas) expressing the given receptor type.

from the newborn thymus, all adult spleen hybridomas with the Hsp60 reactivity pattern have so far revealed δ chains containing Jδ1 rather than Jδ2, regardless of the Vδ gene expressed.

Whereas in the newborn thymus hybridomas, $V\gamma1^+V\delta6^+$ cells accounted for almost 90% of the total Hsp60 reactive γδ cells, only 58% of hybridomas with the Hsp60 reactivity pattern derived from adult spleen use this γδ pair (see Table 2). Of the identified Vδ genes used by non-Vδ6 adult spleen hybridomas with the Hsp60 reactivity pattern, 6 are $V\delta4^+$ and 2 are $V\delta5^+$, with 11 as yet uncharacterized (also see Fig. 1). It is interesting to note that the Vδ4 family is of all the Vδ genes the most closely related to the Vδ6 family (over 60% on the nucleotide level) (24); we found approximately as many cells with the Hsp60 reactivity pattern that were $V\delta4^+$ as we did of those that were $V\delta6\text{-}\lambda12^+$. In summary, expression of both Vγ1 and Vδ6 by any γδ T cell strongly predicts that it will show the Hsp60 reactivity pattern, although many cells with this reactivity express other Vδ genes.

All Spleen γδ Hybridomas with the Hsp60 Reactivity Pattern Respond to the Same Portion of Mycobacterial Hsp60. We (8) previously reported on responses by newborn thymus-derived Hsp60-reactive hybridomas to a 17-amino acid synthetic peptide representing residues 180–196 of the *M. leprae* Hsp60 sequence. We have now tested spontaneously reactive adult spleen hybridomas, as well as some that are not spontaneously reactive as negative controls, for response to this peptide. Titrations with the peptide on both newborn thymus and adult spleen hybridomas revealed that responses usually began to plateau only around a peptide concentration of 50 μg/ml. We therefore tested the adult spleen hybridomas with 40–50 μg of this peptide per ml. As shown in Fig. 1, for every spontaneously reactive cell, we were able to detect a response, sometimes very strong, to the mycobacteria Hsp60 peptide. The degree of response varied considerably, although most of the strongly responsive cells were $V\delta6^+$ hybridomas. This variation suggests that some of the $V\gamma1^+V\delta6^-$ hybridoma lines classified as nonreactive could actually be very weakly Hsp60 reactive. These data indicate that the entire subset of γδ cells showing the Hsp60 reactivity pattern focuses on a single region of the Hsp60 protein.

Vγ1 Usage by Non-Hsp60 Reactive γδ Hybridomas. We were concerned that an in-frame Vγ1-Jγ4 junction might be an insufficient predictor of actual surface expression of a functional Vγ1 chain. Therefore, we decided to analyze, by SDS/PAGE, the TCRs actually present on a selection of adult spleen hybridomas expressing in-frame Vγ1 transcripts. The Vγ1–Cγ4 chain, because of extra amino acids in the C region (31), has the highest molecular weight of all known mouse γ chains, and it can thus be easily identified on a sizing gel. All hybridomas predicted to be $V\gamma1^+$ by cDNA junctional sequence analysis, whether reactive with Hsp60 or not, showed a band the size of Vγ1 (39–41 kDa) on an SDS/PAGE gel of anti-CD3 immunoprecipitates (data not shown). Thus, Vγ1 cDNA sequencing indeed seems to reliably predict a functional Vγ1 chain.

Table 2. Summary of comparisons between adult spleen- and newborn thymus-derived hybridomas

Parameter	Newborn thymus	Adult spleen
Total γδ hybridomas		
Hsp60-reactive cells, %	30	56
Hsp60-reactive cells that are $V\gamma1^+$, %	100	100
Hsp60-reactive cells that are $V\gamma1^+V\delta6^+$, %	89	58
Hsp60-reactive hybridomas		
$V\delta6^+$ cells with Hsp60 reactivity, %	90	92
Amino acids between invariant portions of Vγ1 and Jγ4, mean ± S.E.	1.1 ± 0.7	2.0 ± 0.8
Amino acids between invariant portions of Vδ6 and Jδ1, mean ± S.E.	8.3 ± 1.3	9.6 ± 2.3
$V\delta6^+$ Hsp60-reactive hybridomas		
Dδ1 usage, %	29	35
Dδ2 usage		
Reading frame 1 (I G G I R), %	64	39
Reading frame 2 (R R D T), %	14	26
Reading frame 3 (S E G Y E), %	7	26
Dδ2 not used, %	0	9

Junctional Sequences of Hsp60 Reactive Hybridomas. Greater variety in the TCRs of adult spleen Hsp60-reactive hybridomas, as compared to those from newborn thymus, was evident because many of the adult spleen cells express Vδ genes other than Vδ6 (see Table 1). More variability was also evident in the junctional nucleotides encoding both γ and δ chains (see Fig. 2 and Table 2). In our previous report of sequences of Vγ1–Jγ4 and Vδ6–Jδ1 junctions from the newborn thymus hybridomas (9), we may have assigned as N region additions some nucleotides that could as well have originated from the 3′ position of the V gene. In the adult spleen-derived hybridomas, most of the nucleotides assigned as encoding N regions are not of this type (Fig. 2). Another difference between the two groups is seen in the reading frame of Dδ2 segments (Table 2). Whereas newborn thymus Vδ6 chains showed a marked preference for one reading frame (9), we noticed a more equal distribution of all three possible reading frames in the Dδ2 segments of adult spleen-derived $V\delta6^+$ hybridomas.

DISCUSSION

In this study, we have characterized the receptors of an Hsp60-reactive γδ T-cell subset in the spleen of adult mice. These cells exist at least in part as a lymphoid γδ subset, but might sometimes infiltrate diverse tissues and organs. Infrequently, cells with TCRs typical for this subset have in fact been isolated from murine skin (ref. 30; C.R., unpublished work) and lactating mammary gland (C.R., unpublished results). Moreover, three such skin-derived cells were shown to spontaneously produce a lymphokine (32). However, $V\gamma1^+V\delta6^+$ cells are usually rare among skin γδ T cells (refs. 33 and 34; C.R., unpublished observations). Indeed, in non-lymphoid tissues, we have thus far found only trace amounts of Vδ6 and Cγ4 (to which Vγ1 rearranges) RNA transcripts (35).

To determine the actual frequency of $V\gamma1^+V\delta6^+$ cells in spleen and lymph node, we stained enriched preparations of γδ T cells with a fluorescently labeled antibody specific for Vδ6.3 chains (S.R.C. and R.L.O., unpublished results); ≈10% of the γδ T cells in adult spleen and lymph node were found to be $V\delta6.3^+$ (data not shown). As noted above and previously (9), we have found that virtually all $V\delta6.3^+$ cells are associated with Vγ1 and are nearly always Hsp60 reactive, so detection of Vδ6.3 alone is probably sufficient to identify this subset. If this assumption is valid, because about half of the Hsp60-reactive adult spleen hybridomas are $V\delta6.3^+$, we estimate that the Hsp60-reactive subset comprises about 20% of splenic and lymph node γδ T cells. Thus, the Hsp60-reactive subset is likely to be a large one in the periphery, although the hybridomas growing out of the adult spleen fusions seem to overrepresent it by ≈3-fold.

Another laboratory has also detected a high frequency of $V\gamma1^+$ cells, including a large component of $V\gamma1^+V\delta6^+$ cells, in adult mouse spleen (36), but the question of Hsp60 reactivity in these cells was not addressed. The results of Ezquerra *et al.* (36) differed somewhat from ours in that, of 15 adult spleen-derived hybridomas, about half expressed a Vγ1–Cγ4 chain, as opposed to nearly 90% of our C57BL/10

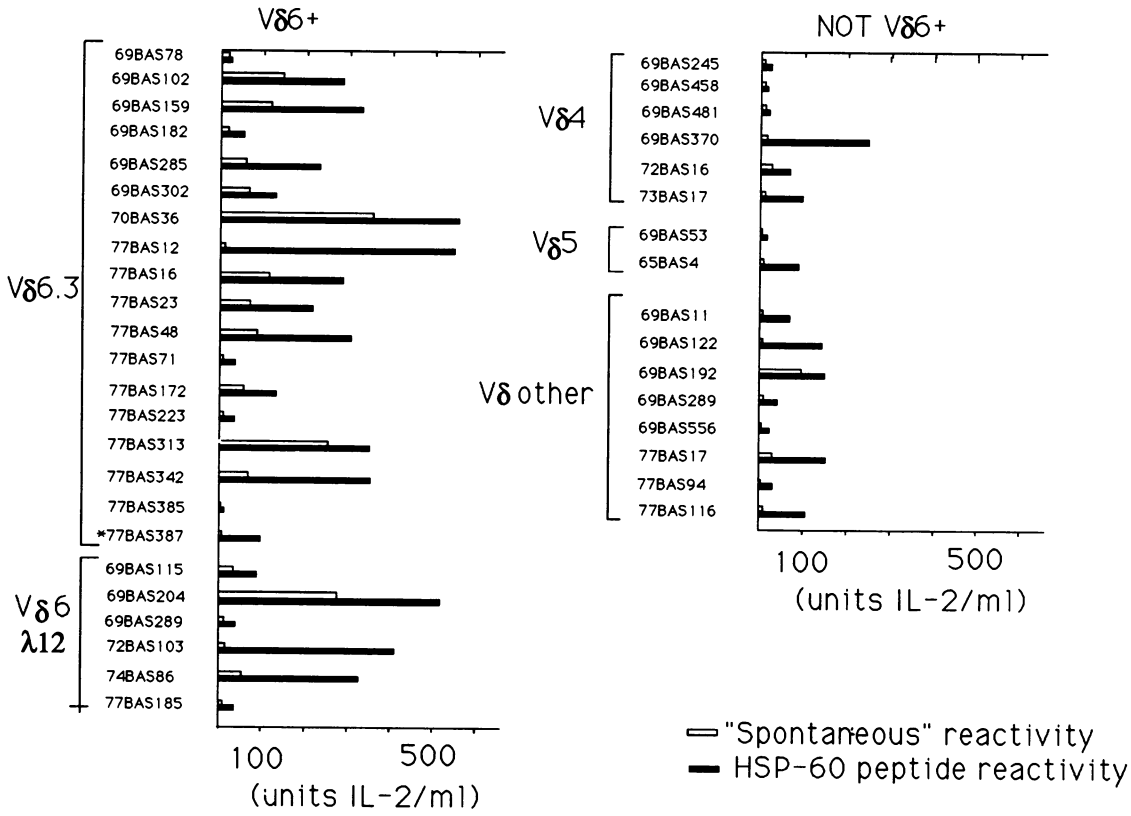

FIG. 1. IL-2 production by adult spleen hybridomas showing the Hsp60 reactivity pattern, incubated in medium only (open bars) or in medium plus a synthetic peptide representing amino acids 180–196 of *M. leprae* Hsp60 at 40 μg/ml (black bars). Values shown are the averages of triplicate cultures; SE were ≈15%. Although some hybridomas shown have insignificant spontaneous IL-2 production, all were classified as having the Hsp60 reactivity pattern, because they produced 10 units or more of IL-2 per ml, either spontaneously or when stimulated with PPD, in other experiments. The hybridoma marked with an asterisk expresses a Vδ6.1 gene, which is closely related to Vδ6.3.

spleen hybridomas. Several differences in protocol could account for this. The general prevalence of apparent Vγ1$^+$ cells in our fusions cannot, however, simply be explained as a PCR amplification artifact, because the junctional sequences determined are usually unique (see Fig. 2; R.L.O. and R.C., unpublished results).

All of the adult spleen hybridomas with the typical Hsp60 response pattern to self and PPD also responded to a syn-

A

	γ Chains				δ chains						
	Vγ1	N	Jγ4		Vδ6	N	Dδ1	N	Dδ2	N	Jδ1
69BAS78	AVW	IM	GT		ALW			QVV	GGIR	PY	KL
69BAS102	AVW	INL	SGT		ALWE	LEGYG	H	R	R	GSA	DKL
69BAS159	AVW	INW	GT		ALWE					LA	TDKL
69BAS182	AVW	VP	GT		ALWE	PQ	WH	H		IS	TDKL
69BAS285	AVW	IA	GT		ALWE	RYM	A	MV	GGIRA		DKL
69BAS302	AVW	TR	SGT		ALWE	LT	Y	A	GGIR	AG	TDKL
70BAS36	AVW	GRP	GT		ALWE	PY	I		GGIRA	GA	TDKL
77BAS12	AVW	R	SGT		ALWE			RI	SEGYE	LG	DKL
77BAS16	AVW	M	GT		ALWE	LI	WL		SEG	SA	TDKL
77BAS23	AVW	IG	GT		ALWE			PI	RRDT	SS	DKL
77BAS48	AVW	IV	GT		ALWE			LGY	RRDT	RGS	DKL
77BAS71	AVW	S	SGT		ALWE			LE	GG	SG	TDKL
77BAS172	AVW	I	GT		ALWE			PY	RRDT	SSG	DKL
77BAS223	AVW	IG	SGT		ALWE	LV	GIY	W	GY	K	TDKL
77BAS313	AVW	IW	GT		ALWE			RY	RRDT	T	DKL
77BAS342	AVW	A	GT		ALW			KG	GYE	A	DKL
77BAS385	AVW	ST	GT		ALWE			RI	SEG	P	TDKL
77BAS387	AVW	IT	GT		ALWE			LL	IGGIR	GA	DKL
69BAS115	AVW	S	GT		ALSE			LEGY	RRDT	T	TDKL
69BAS204	AVW	II	GT		ALSE	L	GIS		GGIR	AT	TDKL
69BAS289	AVW	INLG	GT		ALSE			PG	GGIR	S	TDKL
72BAS103	AVW	R	GT		ALSE			LRP	EG	FS	TDKL
74BAS86	AVW	ISA	GT		ALSE			PLSYMR	IGGI	P	DKL
77BAS185	AVW	V	SGT		ALSE					LS	DKL

B

69BAS245	AVW	R	SGT	(Vδ4)	ALME			P	IGGIR	A	TDKL
69BAS370	AVW	KA	GT	(Vδ4)	ALME			RG	IGGIR	SL	TDKL
69BAS458	AVW	G	SGT	(Vδ4)	ALME			RA	IGGIR	P	DKL
69BAS481	AVW	RP	GT	(Vδ4)	ALME			RAKWRHR	IGGIR	AP	DKL
72BAS16	AV	L	GT	(Vδ4)	ALME			RAGG	RRD	S	TDKL
73BAS17	AVW	IP	GT	(Vδ4)	ALME			RAH	IGGIRA		TDKL
69BAS53	AVW	IRG	GT	(Vδ5)	ASGY						TDKL

FIG. 2. Deduced junctional amino acid sequences of γ and δ chains of Hsp60-reactive adult spleen hybridomas. Protein sequences (given in the single-letter code) were deduced by DNA sequencing of PCR-amplified RNA transcripts. Assignment to V, N, D, and J regions was made according to published sequences for the gene elements (24–31). (*A*) Vδ6$^+$ hybridomas are shown in three clusters; the top one consists of Vδ6.3$^+$ cells, the middle one consists of a Vδ6.1$^+$ cell, and the bottom one consists of Vδ6-λ12$^+$ cells. (*B*) Deduced amino acid sequences of γ and δ chains from non-Vδ6-expressing Hsp60-reactive hybridomas. Results from 7 hybridomas are shown; 10 have not yet been completely determined. Vδ chains are indicated in parentheses.

thetic 17-amino acid peptide representing a region of mycobacterial Hsp60 (8). We conclude that the entire subset of Hsp60-reactive γδ cells, despite Vδ gene diversity and extensive γ and δ chain junctional diversity, responds to a single region of the Hsp60 molecule. This large subset, therefore, shows somewhat surprising characteristics: all of its members bear related TCRs and preexist in the lymphoid tissues of normal, uninfected mice, a situation that contrasts sharply with the distribution patterns and specificities expected of unexpanded populations of normal peripheral αβ T cells.

Judging from the increase in junctional sequence diversity of adult spleen γ and δ sequences as opposed to those derived from newborn thymus, most of the adult spleen Hsp60-reactive γδ T cells do not appear to be descendants of similar cells that develop in the newborn thymus (9). They may rather develop in the thymus of older mice or reflect peripheral selection. Because continuing high levels of $C\gamma4^+$ and $V\delta6^+$ mRNA can be detected in the thymus of weanling and adult mice, comparable to or exceeding that in newborn thymus (ref. 35; R.L.O., unpublished results), we suspect that production of these cells is ongoing throughout the life of a mouse.

Large numbers of $V\gamma1^+V\delta6^+$ γδ cells have been found in the lungs of mice recovering from influenza infection, following an increase in Hsp60 expression by lung macrophages (ref. 37; S.R.C., unpublished results). Appearance on the cell surface of Hsp60-derived peptides from any source, self-derived or foreign, may indicate a potentially harmful situation that dictates immediate response; hence, a circulating subset of Hsp60-reactive lymphocytes may have evolved to patrol all peripheral sites for signs of excess production of this protein (35). Speculations as to the consequences of activation of this subset await further studies of the functional capabilities of these cells.

The reactivity of a large subset of γδ T cells with a single antigenic protein is reminiscent of superantigen responses of αβ T cells bearing particular Vβ genes. A number of details detract from this resemblance, however. First, the γδ T cells in question do not require class II molecules for this reactivity, whereas all known αβ superantigen responses require class II-presenting cells (38). Second, it is apparent from this work that one V gene (Vγ1) alone, though necessary, is not sufficient to confer this reactivity, because many $V\gamma1^+$ cells fail to respond to the antigen. Third, because the antigen still stimulates when supplied in the form of a small 17-amino acid peptide, unlike superantigens for αβ T cells (38), it need not be administered as an intact protein with its native globular structure. In addition to Vγ1, as we have seen, certain Vδ chains are also frequently associated with Hsp60 reactivity. Whether these γ and δ chains tend to be coupled for developmental reasons peculiar to γδ T cells, or whether cells with these TCRs are specifically selected for survival because they have antigen reactivity, still remains to be determined.

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