THE SJL/J T CELL RESPONSE TO BOTH SPONTANEOUS AND TRANSPLANTABLE SYNGENEIC RETICULUM CELL SARCOMA IS MEDIATED PREDOMINANTLY BY THE Vβ17a$^+$ T CELL CLONOTYPE

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The Reticulum Cell Sarcoma (RCS)[1] is a spontaneous lymphatic tumor of the SJL/J (H-2^s) mouse, first observed by Murphy (1) in upwards of 90% of SJL/J mice of a mean age of 13.3 mo. RCS tumors are considered B cell lineage neoplasms that exhibit Ig H chain gene rearrangements, but no surface or cytoplasmic Ig (2). Additionally, RCS tumors express IA^s MHC-encoded polypeptides (3). RCS tumors require an obligate syngeneic host CD4$^+$ T cell response for growth (4, 5). The in vivo passive administration of Gk1.5 mAb (anti-CD4) or haplotype-specific anti-Ia antibody to SJL/J mice, before or shortly after transfer of the transplantable RCS line, RCS LA-12, resulted in the complete abrogation of tumor growth (5, 6).

The nature of the RCS tumor-associated antigen responsible for the stimulation of syngeneic T cell proliferation has remained elusive. However, Katz et al. (7) made the observation that both the in vivo growth of RCS and the in vitro RCS-specific T cell response correlated with the lack of IE expression. Studies by Wilbur et al. (8, 9) further implicated the role of IE in tumor growth. Furthermore, two of four RCS-specific T cell hybridomas when cocultured with IE^d- or IE^k-bearing allogeneic spleen cells were stimulated to release T cell growth factors, such as IL-2 (10).

Recently, Kappler et al. (11) have developed an mAb, termed KJ23a, which specifically interacts with TCR containing the Vβ17a variable segment gene products. Interestingly, only mice that lack thymic IE exhibit KJ23a$^+$ T cells in the periphery, since Vβ17a expression confers IE reactivity and are deleted by IE-expressing mice (12). SJL/J (IE$^-$) mice possess KJ23a$^+$ T cells in their periphery. Since the RCS tumors stimulate T cells that appear to recognize "IE-like" antigens and IE$^+$ F_1 mice failed to support RCS tumor growth, we undertook studies to examine the role that Vβ17a$^+$ (KJ23a$^+$) T cells may play in the RCS-specific T cell response.

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[1] *Abbreviations used in this paper:* MLTI, mixed lymphocyte tumor interaction; RCS, reticulum cell sarcoma.

Evidence is presented that supports a major role for KJ23a$^+$ T cells in the response to both spontaneous and transplantable RCS tumors.

Materials and Methods

Mice. Female SJL/J, DBA/2, and BALB/c mice (6–8 wk) were purchased from The Jackson Laboratory, Bar Harbor, ME. BALB/c *nu/nu* mice were bred in the nude mice facility at UCLA, Los Angeles, CA.

Medium. All cultures were performed in high glucose formula DMEM (Gibco Laboratories, Grand Island, NY) supplemented with 10% FCS (Gemini Bio-Products, Calabasas, CA), sodium pyruvate, nonessential amino acids, L-glutamine, penicillin, streptomycin (all from Gibco Laboratories), and 5×10^{-5} M 2-ME (Sigma Chemical Co., St. Louis, MO).

mAbs. The KJ23-588.1 (KJ23a), MIgG2a, hybridoma specific for Vβ17a TCR-bearing T cells, as described (11), was the generous gift of Drs. P. Marrack and J. Kappler (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). KJ23a mAb was purified from ascites fluid of BALB/c mice using protein A-agarose (Bio-Rad Laboratories, Richmond, CA). F23.1.R2 (F23.1), MIgG2a, hybridoma specific for Vβ8 TCR-bearing T cells, as described (13), was the generous gift of Dr. E. Sercarz (UCLA, Los Angeles, CA). F23.1 was purified from ascites fluid of BALB/c *nu/nu* mice using protein A chromatography as described (14). Gk1.5, Rat IgG2a, hybridoma specific for CD4 (L3T4), as described (15), was purchased from American Type Culture Collection, Rockville, MD (TIB 207). Gk1.5 was used as an ascites fluid obtained from BALB/c *nu/nu* mice. HO-13-4, MIgM, specific for Thy1.2 T cells, as described (16), was purchased from American Type Culture Collection (TIB 99) and used as an ascites fluid obtained from BALB/c *nu/nu* mice.

RCS Tumor and Tumor Purification. The transplantable RCS line, RCS LA-12, used in the present studies arose spontaneously in a female SJL/J mouse and was established as a transplantable line in our laboratory. This tumor was maintained in vivo by intraperitoneal passage of 10^7 viable cells in young SJL/J mice. Spontaneous primary tumors were obtained from mesenteric lymph nodes of 14–15-mo-old female SJL/J mice. RCS single cell suspensions were made from excised tumorous mesenteric lymph nodes (8–14 d after inoculation for RCS LA-12), and separated from B cells and macrophages on a nylon wool column. T cells were removed by the use of HO-13-4 and low-tox rabbit complement (Cedarlane Laboratories, Hornby, Ontario), 1:10, followed by isolation of RCS on Ficoll-Hypaque density-gradient centrifugation as previously described (17).

IL-2 Production by RCS-specific T Cell Hybridomas. RCS-specific T cell hybridomas and the IL-2 production assay were described previously in (10). Briefly, 2×10^5 hybridoma cells were cocultured with 10^5 glutaraldehyde-fixed (0.01%) RCS or 10^6 γ-irradiated (2,000 rad) SJL/J spleen cells in 200 μl of complete medium at 37°C for 24 h in a humidifying CO_2 incubator. 50 μl of supernatant from each well was transferred to microtiter plates containing 5×10^3 CTLL-2 and incubated at 37°C for 16–20 h. CTLL-2 cells were then pulsed with 1 μCi of [^{3}H]TdR for an additional 8 h, after which cells were MASH harvested onto glass fiber filters (W. A. Bioproducts, Walkersville, MD) and [^{3}H]TdR incorporation was assessed in the presence of 1.0 ml of Safety-sol II scintillation fluid (Research Products International, Mt. Prospect, IL) using a beta counter. [^{3}H]TdR incorporation is expressed in cpm ± SD.

CTLL-2 Line. The IL-2-dependent CTLL-2 cell line, as described (18), was purchased from American Type Culture Collection (TIB 214) and maintained in 9 parts complete medium and 1 part rat Con A supernatant as a source of IL-2.

SJL/J Spleen T Cells and the Mixed Lymphocyte Tumor Interaction (MLTI) and MLR Cultures. Splenic T cells from 8–12-wk-old SJL/J mice were purified as described (8). For MLTI cultures, 5×10^5 SJL/J T cells were incubated with 5×10^4 γ-irradiated RCS (10K R) in a total volume of 200 μl complete medium, in 96-well flat bottomed plates. The cultures were then incubated for 96 h at 37°C in a humidifying 10% CO_2 incubator. A 1.0 μCi pulse of [^{3}H]TdR was added to each well during the last 24 h, after which the plates were MASH harvested and [^{3}H]TdR incorporation was assessed. For MLR cultures, 5×10^5 SJL/J T cells were incubated with 5×10^5 γ-irradiated (2,000 rad) autologous SJL/J spleen cells. For fluorocytometric analysis parallel 0.8-ml MLTI cultures were performed in 24-well cluster

dishes (Costar, Cambridge, MA). The viable cells were collected on Ficoll-Hypaque gradients and analyzed after antibody staining by flow cytometer.

mAb Mediated Inhibition of IL-2 Production by T Cell Hybridomas and T Cell Proliferation. Inhibition assays using affinity-purified monomeric KJ23a were performed analogous to inhibition assays described (10). Briefly, IL-2 production and T cell proliferation assays were performed as described above, except 10 μg/ml filter-sterilized affinity-purified monomeric KJ23a mAb was added at the initiation of each culture. Monomeric KJ23a was obtained by ultracentrifugation of 200 μg/ml affinity-purified KJ23a at 35,000 rpm in an SW50.1 rotor at 4°C for 1.5 h. The resulting supernatant was collected and sterilized by filtration through 0.22 μM filter (Gelman Sciences, Inc., Ann Arbor, MI). The nonreactive mAb F23.1 was treated similarly and added in parallel as a negative control. Inhibition by KJ23a of IL-2 production was expressed relative to the IL-2 production by the hybridomas in the presence of F23.1 mAb.

Fluorocytometric Analysis. For single-color analysis of T cells and T cell hybridomas, 10^6 cells were incubated with 20 μg of affinity-purified KJ23a or F23.1, or 25 μg of Gk1.5 ascites in 200 μl in 3% FCS/HBSS on ice for 30 min. Afterwards, the cells were washed twice in ice-cold 3% FCS/HBSS. Gk1.5-labeled cells were then incubated with a 1:100 dilution of FITC-conjugated goat anti-rat IgG, F-GAR, (Jackson ImmunoResearch, Avondale, PA) for 30 min on ice. KJ23a- or F23.1-labeled cells were incubated with 1:100 dilution of FITC-conjugated goat anti-mouse IgG, F-GAM, (Cappel Laboratories, Cochranville, PA) for 30 min on ice. Afterward, the cells were washed thrice in ice-cold 3% FCS/HBSS and resuspended in 1.0 ml of 1% paraformaldehyde (Sigma Chemical Co.), 0.1% NaN_3 in PBS (fix solution). Cells were then analyzed on an EPIC-C flow cytometer. For two-color analysis, 10^6 T cells were incubated with one or more of the above-mentioned primary antibodies for 30 min on ice. The cells were then incubated for 30 min on ice with 200 μl of a two-color fluorochrome cocktail containing, 1:100 dilution of F-GAR and 1:50 dilution of phycoerythrin-conjugated goat anti-mouse IgG, PE-GAM (Tago Inc., Burlingame, CA), which had been previously adsorbed to rat IgG to remove crossreactivity. The cells were then washed and fixed as above. The cells were analyzed by dual-color parameters on an EPIC-C flow cytometer. In all experiments, cells were incubated with only the fluorochrome-coupled secondary antibody(s) as a control for background fluorescence and to establish size gates. Two-color analysis is displayed at 2, 8, and 16% map levels in Figs. 1 and 2.

Results

RCS Tumor-specific T Cell Hybridomas Express Vβ17a-bearing TCR. In a previous study, we have characterized four RCS-specific T cell hybridoma clones (10). Two hybridomas, IF8-11-2 and 2D5-3-3, were RCS specific and released IL-2 only when cocultured with RCS tumors. The other two hybridomas, 45-2-4 and 1A4-6, released IL-2 when cocultured with RCS or IE^+ allogeneic spleen cells. Because of this response to IE-bearing cells, we examined whether the hybridomas were expressing Vβ17a-bearing TCR. Fluorocytometric analysis revealed that all four T cell hybridomas stained positively with KJ23a (anti-Vβ17a) and were negative for the control mAb F23.1 (anti-Vβ8) (data not shown). To corroborate these findings, we tested whether monomeric KJ23a mAb would inhibit the RCS-stimulated release of IL-2 by the hybridomas. As seen in Table I (Exp. 1), KJ23a inhibited by >90% the release of IL-2 by the hybridomas in the presence of RCS; F23.1 had no effect. Furthermore, KJ23a inhibited the allo-stimulatory response of 45-2-4 to DBA/2 spleen cells (Table I, Exp. 2). These results demonstrate that all four RCS-specific T cell hybridomas bear Vβ17a-containing TCR. Furthermore, these results indicated that the same β chain of the TCR is involved in both the RCS and allogeneic response by 45-2-4.

The Syngeneic Proliferative Response, In Vitro, to RCS Is Predominantly Mediated by $CD4^+$ $Vβ17a^+$ T Cells. To address the role of $KJ23a^+$ T cells in the generation of the syn-

TABLE I

Inhibition of RCS-specific T Cell Hybridoma Secretion of IL-2 in the Presence of KJ23a mAb

Exp.	Hybridoma (2×10^5)	Stimulator (10^5)	mAb (10 μg/ml)	[^{3}H]TdR incorporation*	
				cpm ± SD	
1	45-2-4	RCS (LA-12)‡	-	36,666 ± 639	
			KJ23a	1,322 ± 146	(96.4)
			F23.1	34,175 ± 4,225	
		Con A (10 μg/ml)	-	45,280 ± 1,464	
		-	-	315 ± 1	
	2D5-3-3	RCS	-	35,825 ± 6,351	
			KJ23a	1,701 ± 393	(95.2)
			F23.1	32,007 ± 7,229	
		Con A	-	29,219 ± 3,136	
		-	-	503 ± 85	
	1A4-6	RCS	-	26,571 ± 4,885	
			KJ23a	1,957 ± 778	(92.6)
			F23.1	20,519 ± 684	
		Con A	-	53,538 ± 3,536	
		-	-	220 ± 165	
	IF8-11-2	RCS	-	78,032 ± 1,019	
			KJ23a	3,056 ± 918	(96.0)
			F23.1	67,239 ± 906	
		Con A	-	80,250 ± 643	
		-	-	1,661 ± 461	
2	45-2-4	DBA/2 spleen§	23,542 ± 1,946		
			KJ23a	1,300 ± 766	(94.9)
			F23.1	26,804 ± 1,688	
			Gk1.5	2,876 ± 425	
			14-4-4s	5,583 ± 1,384	

* [^{3}H]TdR incorporation as measured with the IL-2-dependent CTLL-2 cell line. CTLL-2 proliferation in the absence of IL-2 was <500 cpm, while in the presence of IL-2 (rat Con A supernatant 1:10) it was 71,500 cpm. Percent inhibition (in parentheses) by KJ23a of IL-2 production was expressed relative to the IL-2 production by the hybridomas in the presence of F23.1 mAb.

‡ RCS LA-12 stimulator cells were fixed with 0.01% glutaraldehyde and did not release measurable IL-2.

§ Assay was performed as above, except 10^6 γ-irradiated DBA/2 (IE^d) spleen cells were used as stimulator cells. DBA/2 cells alone did not release measurable amounts of IL-2.

geneic proliferative response to RCS, we performed MLTI cultures in the presence and absence of KJ23a mAb. Syngeneic MLR cultures were maintained in parallel, using autologous spleen cells, as a control for nonspecific proliferation. In the presence of 10 μg/ml of KJ23a mAb, the SJL/J T cell proliferative response was markedly inhibited from 25 to 76% (Table II). The control F23.1 mAb had no effect. The effective blocking by KJ23a mAb was not restricted to the particular RCS line used, in that the proliferative response of SJL/J T cells to spontaneous primary mesenteric RCS tumors, RCS Sp-3, RCS Sp-4, and RCS Sp-7, was also significantly inhibited by KJ23a (Table II, Exp. 2 and 3). The inhibition of T cell proliferation by KJ23a to both spontaneous and transplantable tumor was similar, suggesting that both the spontaneous tumors and the transplantable RCS LA-12 stimulate predominantly the same T cell subset, namely Vβ17a$^+$ T cells.

Since mAb directed to Vβ17a TCR significantly inhibited the proliferative re-

TABLE II
Inhibition of RCS-specific T Cell Proliferation by KJ23a mAb

Exp.	Stimulator cell§	[^{3}H]TdR incorporation* None	KJ23a mAb‡	F23.1 mAb
			cpm ± SD	
1	RCS LA-12	30,967 ± 2,920	8,877 ± 350	27,418 ± 1,579
2	RCS Sp-4	-	17,889 ± 1,514	67,233 ± 9,609
	RCS Sp-7	-	11,556 ± 1,539	46,811 ± 6,017
	SJL/J spleen	502 ± 206	-	-
3	RCS LA-12	49,099 ± 4,261	39,345 ± 400	52,823 ± 1,687
	RCS Sp-3	32,836 ± 1,689	24,267 ± 5,033	43,360 ± 721
	None	-	566 ± 215	86 ± 2

* Cultures are incubated at 37° for 72 h, whereupon the cultures are pulsed with 1 μCi of [^{3}H]TdR for 24 h and then harvested by MASH. Proliferation is expressed in cpm ± SD of triplicate cultures.
‡ Monomeric affinity-purified mAbs were added at the initiation of each culture at 10 μg/ml.
§ 5×10^4 γ-irradiated RCS or 5×10^5 γ-irradiated SJL/J spleen cells were cocultured with 5×10^5 SJL/J T cells in complete medium.

sponse of T cells to both spontaneous and transplantable RCS, we determined quantitatively the frequency of the responding Vβ17a$^+$ T cells in the T cell blast population. TCR-specific mAb staining was enumerated using PE-coupled goat anti-mouse IgG (red), while the CD4-specific staining was monitored by FITC-conjugated goat anti-rat IgG (green). As seen in Fig. 1 *A*, the initial T cell population on day 0 was 56% CD4$^+$ T cells (the sum of quadrants 2 and 4) of which 9% were double positive for KJ23a and Gk1.5 (quadrant 2 only). Furthermore, the T cells were of uniform size as determined by forward angle and 90° angle light scatter (data not shown). However, after 4 d of culture with the transplantable RCS LA-12 line, the T cells were 66% double positive for KJ23a and Gk1.5, when analyzed as a whole population (Fig. 1 *B*). Further, if only the blast population was analyzed, the resultant double-labeled T cells represented >80% of the total blast cells (Fig. 1 *C*). Moreover, all the Gk1.5$^+$ T cells in the blast population were also KJ23a$^+$; thus, virtually all of the CD4$^+$ T cell blasts generated in response to RCS LA-12 were Vβ17a$^+$ T helper cells.

When the MLTI cultures were effected using γ-irradiated spontaneous RCS tumors as stimulator cells, we obtained comparable results with those seen using the RCS LA-12 line. As seen in Fig. 2 *A*, two-color analysis of day 4 T cells cocultured with the spontaneous RCS tumor, RCS Sp-3, shows that 47% of the total cells were both KJ23a$^+$ and Gk1.5$^+$. Interestingly, as seen above with T cells cultured with RCS LA-12, two populations of CD4$^+$ T cells were observed after coculture with RCS Sp-3; a CD4 dim population, which represented 27% of the total cells (36.4 of the total CD4$^+$ T cells), and a CD4$^+$ bright population, which was 47% of the total cells (63.5% of the total CD4$^+$ cells). All of the CD4 bright cells were also KJ23a$^+$, and represented blast cells as judged by forward angle and 90° angle light scatter (data not shown). These results demonstrate that both spontaneous and transplantable RCS tumors stimulate a similar and marked proliferation of KJ23a$^+$ T cells.

The Responsive Syngeneic RCS-specific T Cells in Tumor-bearing Mice Are Predominantly KJ23a$^+$. The RCS LA-12 line was passed intraperitoneally (5×10^3 cells) in 8-wk-old SJL/J mice, and 14 d later, tumor-bearing mesenteric lymph nodes were re-

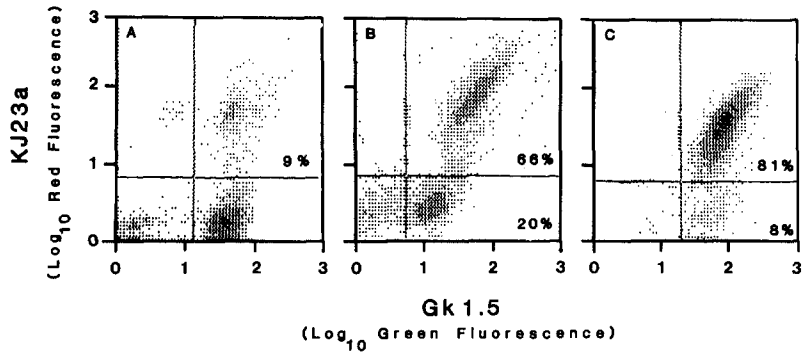

FIGURE 1. Two-color analysis of SJL/J spleen T cells cocultured with RCS tumor. T cells were incubated with KJ23a (mouse IgG2a) and Gk1.5 (rat IgG2a), washed, and then incubated with FITC-conjugated goat anti-rat IgG (F-GAR) to label Gk1.5 and phycoerythrin-conjugated goat anti-mouse IgG (PE-GAM) to label KJ23a. PE-GAM was pre-adsorbed on rat IgG sepharose to remove anti-rat IgG crossreactivity. Control staining and analysis using only the fluorochrome-conjugated secondary reagents were performed to set the red and green gains, size gates, and quadrant lines (data not shown). (*A*) The initial splenic T cell population was 56% $CD4^+$ (quadrants *2* and *4*) and 9% double positive for KJ23a and CD4 (quadrant *2*). (*B*) Total splenic T cells after a 4-d MLTI coculture with RCS LA-12. 66% of the total SJL/J T cells labeled with KJ23a and brightly with Gk1.5 (quadrant *2*), 20% of the T cells labeled dimly with Gk1.5 and were negative for KJ23a (quadrant *4*). (*C*) Blast SJL/J splenic T cells after a 4-d MLTI stimulation by the transplantable RCS LA-12 line. Blast cells were elucidated by limiting analysis to cells that exhibited both increased forward and 90° angle light scatter over a population of inactivated SJL/J spleen T cells (data not shown). Double labeling with KJ23a and Gk1.5 stained 81% of blast cells (quadrant *2*). Only 8% of the Gk1.5 blast T cells were not KJ23a positive (quadrant *4*).

moved and analyzed by two-color fluorocytometry. As seen in Fig. 2 *B*, $CD4^+$ T cells represent 51% of the total cells in the nylon wool-nonadherent fraction (T cells and RCS tumor). Further, 70% of the $CD4^+$ T cells were also positively stained by KJ23a. This is comparable with the number of $V\beta17a^+$ T cells found in the in vitro MLTI cultures (Fig. 1, *B* and *C*). Furthermore, the $KJ23a^+$ T cells were also brightly stained by Gk1.5. The remaining dull $CD4^+$ T cells (30% of the total $CD4^+$ cells) were $KJ23a^-$ (Fig. 2 *B*). We therefore conclude that $V\beta17a^+$ T cells play an important role in responding to RCS stimulation in vivo.

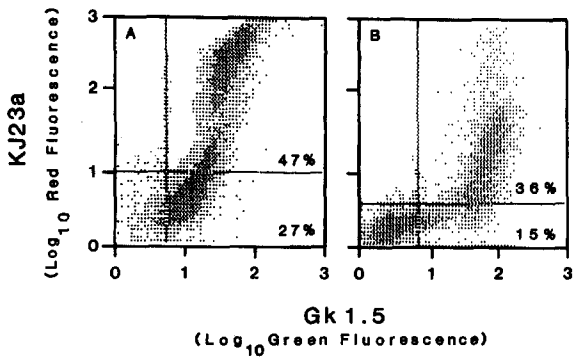

FIGURE 2. Two-color analysis of total spleen T cells in response to spontaneous RCS tumor, RCS Sp-3, and T cells from tumor-bearing MLN. (*A*) Total splenic T cells after a 4-d MLTI coculture with the spontaneous RCS tumor, RCS Sp-3. 47% of the total SJL/J T cells were double positive for KJ23a and GK1.5. The Gk1.5 dim cells were negative for KJ23a (27%). (*B*) Nonadherent cells from tumor-bearing MLN of mice given RCS LA-12 14 d prior. 51% of the nonadherent cells were $Gk1.5^+$. 70% of the $Gk1.5^+$ cells were also stained by KJ23a.

Discussion

Evidence is presented here that demonstrates the majority of the syngeneic cells responding to both primary spontaneous RCS tumors and transplantable RCS LA-12 are $CD4^+$ $V\beta17a^+$ T cells. Several lines of evidence support this conclusion. All four RCS-specific T cell hybridoma clones are $V\beta17a^+$ and show marked inhibition of IL-2 release in the presence of KJ23a mAb. Secondly, the response of SJL/J T cells to both spontaneous and transplantable RCS was markedly inhibited by the addition of monomeric KJ23a. The T cell proliferative response was inhibited on average by 54%, despite the fact that $V\beta17a^+$ T cells represent only 7–9% of the total splenic T cells in SJL/J mice. Third, we analyzed by flow cytometry the SJL/J T cells after a 4-d stimulation by either spontaneous or transplantable RCS, and found significant expansion of the $KJ23a^+$ T cells from an input level of 9% to as high as 84% of the total blast population. Concordantly, these cells were bright staining for CD4 (L3T4). The dim staining $L3T4^+$ T cells were devoid of $KJ23a^+$ cells, suggesting that all of the $KJ23a^+$ cells responded to RCS by developing into blast T cells. Lastly, we analyzed the phenotype of T cells from tumor-bearing mesenteric lymph nodes, and found that the majority of the $CD4^+$ T cells responding to RCS were $KJ23a^+$. Therefore, it appears that the in vivo response was comparable with that seen in vitro. These findings represent the first demonstration of a striking expansion of T cells bearing a single Vβ TCR family in response to a spontaneous tumor. Furthermore, these results support the notion that the RCS tumors carry IE or "IE-Like" tumor-associated antigens.

Kappler et al. (11) found that TCR that use the Vβ17a variable segment gene product react with high frequency to the IE class II MHC molecules. This bias towards IE recognition by $V\beta17a^+$ T cells was independent of the coexpressed α chain of the TCR (11). Further, mice that demonstrate intrathymic expression of IE were shown to selectively delete $V\beta17a^+$ cells during T cell maturation (12). Interestingly, previous studies using F_1 hybrid mice from matings of either SJL/J and congenic mice, or SJL/J and recombinant congenic mice found that both the in vivo growth of RCS and the in vitro RCS-specific T cell response correlated inversely with IE expression. In fact, F_1 mice that expressed IE failed to grow or respond to the tumor. This negative effect could not be overcome by increasing either tumor inoculum or by increasing the assay periods (7). Thus, in light of the evidence presented here and that IE-expressing mice debate $V\beta17a^+$ T cells, it seems attractive to speculate that the failure of IE-expressing F_1 mice to support the growth of and respond to RCS is the result of the deletion of $V\beta17a^+$ T cells in these mice.

The predominant role played by $V\beta17a^+$ T cells in the response to RCS suggests a potential therapeutic role for KJ23a mAb in the intervention and prevention of RCS tumors in SJL/J mice. Treatment of SJL/J mice with KJ23a mAb may result in the complete inhibition of RCS growth. Further, the prophylactic treatment of aged mice with KJ23a may prevent the onset of spontaneous RCS tumors. These approaches, while not radically different from our previous studies using Gk1.5 (5), have several advantages. KJ23a mAb affects only a portion of the CD4 subset and not the whole CD4 population, thereby not crippling the host's ability to mount protective humoral and cellular immunity. Furthermore, unlike Gk1.5, the KJ23a mAb is of mouse origin, which should allow for a slower clearance rate and would not elicit a potentially fatal anti-rat Ig response, as has been seen upon repeated

administration of rat IgG. Therefore, KJ23a appears to possess the intriguing potential as a tumor-specific therapeutic strategy in SJL/J mice.

The two RCS-specific hybridomas, IF8 and 2D5, shown previously not to be stimulated by allo-IE, were nonetheless KJ23a$^+$. While we have not observed IL-2 release by 1F8 or 2D5 in the presence of a number of allogeneic stimulator cells, IF8 and 2D5 have exhibited specific binding to allo-IE spleen cells (IE^d, IE^k), which was inhibitable by anti-IE mAb of defined specificities (unpublished observation). We are currently investigating whether the specific binding, while not leading to IL-2 release, may trigger other initial activation events, such as the mobilization of cytoplasmic Ca^{2+}. Further, we have clearly not exhausted all possible allogeneic IE$^+$ strains, therefore, there may still be allogeneic strains that bind and stimulate 1F8 and 2D5. However, the fact that 1F8 and 2D5 are KJ23a$^+$ and do not release IL-2 upon stimulation by the allo-IE strains tested to date, still remains an enigma.

While the reactivity of Vβ17a$^+$ T cells from SJL/J mice to IE appears to be greatest for allogeneic IE, there is a significant reactivity of Vβ17a$^+$ T cells for self IE (19). SJL/J mice lack expression of the αβ heterodimer of IE due to a 600-bp deletion in the promoter and leader sequence of the Eα gene (20, 21). Eβ, however, is actively transcribed (20). The nature of the IE-like determinant on SJL/J RCS tumors remains unknown. However, experiments using L cells transfected with Eα and Aβ genomic DNA have demonstrated that cell surface expression of Eα and Aβ is possible, in what are termed mixed isotype heterodimers (22, 23). Therefore, it remains a formal possibility that Eβ^s is expressed with Aα^s in a mixed heterodimer on the surface of RCS tumors. SJL/J T cells, which are not tolerant to surface expression of Eβ^s, would therefore respond to it. Evidence presented here would suggest that this response would be predominantly by KJ23a$^+$ T cells. Alternatively, cytoplasmic Eβ^s protein, unable to achieve surface expression, is degraded by the RCS tumor cells and presented by IA^s in peptide form. While no direct evidence exists that the presentation of Eβ peptide by IA^s would elicit a KJ23a$^+$ T cell response, recent evidence suggests that allo-reactive CTL clones can recognize allo-MHC class I peptide in the context of self-MHC class I, and thereby reconstitute the allogeneic response (24). Therefore, it remains to be tested whether RCS tumors may process and present Eβ^s in the context of IA^s.

Recent evidence suggests that KJ23a$^+$ T cells recognize and respond to IE only when complexed with a B cell–specific peptide (25). RCS, a B cell tumor, stimulates a vigorous KJ23a$^+$ T cell response, and therefore, may express this B cell stimulatory antigen. The expression of such an antigen by RCS may explain the observed stimulation of RCS-specific T cell clones by activated syngeneic blast B cells (4). The exact nature, though, of the RCS tumor-associated antigen awaits future experimentation.

Summary

Previous studies have revealed that the reticulum cell sarcoma (RCS) of SJL/J (H-2^s, IE$^-$) mice express an "IE-like" stimulatory tumor-associated antigen, the expression of which is requisite for stimulating host T cells necessary for tumor growth. Herein, we present evidence that the predominant T cells raised in the syngeneic response to both spontaneous and transplantable RCS tumors are of the Vβ17a TCR clonotype. The Vβ17a$^+$ clonotype of T cells has been shown to interact with IE al-

logeneic specificities. We demonstrate that all four characterized RCS-specific T cell hybridomas stained positively for the anti-Vβ17a mAb, KJ23a. Additionally, KJ23a, when added to cocultures of the T cell hybridomas and RCS tumors, inhibited the release of IL-2 by the hybridomas. Further, KJ23a was shown to markedly inhibit the proliferation of SJL/J T cells when cocultured with either spontaneous or transplantable RCS tumor cells. When analyzed by flow cytometry, the T cell blast population raised in response to both spontaneous and transplantable RCS were >80% KJ23a$^+$. These T cells were brightly stained by the anti-CD4 mAb, Gk1.5, and, therefore, represent class II-responsive T cells. In corroboration of the in vitro data, T cells derived from mesenteric lymph nodes of RCS tumor-bearing mice had likewise undergone a similar expansion of Vβ17a$^+$, CD4$^+$ T cells. Together, these results indicate that KJ23a$^+$ T cells play an important and predominant role in the response of SJL/J mice to spontaneous RCS tumors and provide further suggestive evidence that the stimulatory antigen(s) on the RCS tumor is IE or an "IE-like" molecule. Significantly, the important role Vβ17a$^+$ T cells play in the response to RCS suggests a potential therapeutic role for KJ23a mAb in the intervention and prevention of RCS tumors in SJL/J mice.

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