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) is a spontaneous lymphatic tumor of the
SJL/J (H-2b) 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). Addi-
tionally, RCS tumors express IAα MHC-encoded polypeptides (3). RCS tumors re-
quire 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 IEα- or IEβ-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 pro-
ducts. Interestingly, only mice that lack thymic IE exhibit KJ23a+ T cells in the pe-
riphery, 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+
F1 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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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, streptomycin (all from Gibco Laboratories), and 5 x 10^-5 M 2-ME (Sigma Chemical Co., St. Louis, MO).

mAbs. The KJ25-588.1 (KJ23a), MlgG2a, 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), MlgG2a, 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, MlgM, 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 tumors mesenteric lymph nodes (8-14 d after inoculation for RCS LA-12), and separated from B cells and macrophages on 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 x 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 CO2 incubator. 50 μl of supernatant from each well was transferred to microtiter plates containing 5 x 10^3 CTLL-2 and incubated at 37°C for 16-20 h. CTLL-2 cells were then pulsed with 1 μCi of [3H]Tdr for an additional 8 h, after which cells were MASH harvested onto glass fiber filters (W. A. Bioproducts, Walkersville, MD) and [3H]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. [3H]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 x 10^5 SJL/J T cells were incubated with 5 x 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% CO2 incubator. A 1 μCi pulse of [3H]Tdr was added to each well during the last 24 h, after which the plates were MASH harvested and [3H]Tdr incorporation was assessed. For MLR cultures, 5 x 10^5 SJL/J T cells were incubated with 5 x 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 a 1:100 dilution of FITC-conjugated goat anti-mouse IgG, FGAM, (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% NaN₃ 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-II-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-containing 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 x 10^5) | Stimulator (10^5) | mAb (10 μg/ml) | [3H]Tdr incorporation* cpm ± SD |
|-----|---------------------|-------------------|----------------|-------------------------------|
| 1   | 45-2-4 RCS (LA-12)  | -                 | KJ23a          | 36,666 ± 639 (96.4)           |
|     |                     |                   | F23.1          | 34,175 ± 4,225                |
|     | Con A (10 μg/ml)    | -                 | KJ23a          | 45,280 ± 1,464                |
| 2D5-3-3 RCS                          | -                 | KJ23a          | 35,825 ± 6,351                |
|     |                     |                   | F23.1          | 1,701 ± 393 (95.2)            |
|     | Con A (10 μg/ml)    | -                 | KJ23a          | 32,007 ± 7,229                |
|     |                     |                   | F23.1          | 29,219 ± 5,136                |
|     |                     |                   | KJ23a          | 503 ± 85                      |
| 1A4-6 RCS                            | -                 | KJ23a          | 26,571 ± 4,885                |
|     |                     |                   | F23.1          | 1,957 ± 778 (92.6)            |
|     | Con A (10 μg/ml)    | -                 | F23.1          | 20,519 ± 684                  |
|     |                     |                   | F23.1          | 53,538 ± 3,536                |
|     |                     |                   | KJ23a          | 220 ± 165                     |
| IF8-11-2 RCS                          | -                 | KJ23a          | 28,032 ± 1,019                |
|     |                     |                   | F23.1          | 3,056 ± 918 (96.0)            |
|     | Con A (10 μg/ml)    | -                 | F23.1          | 67,239 ± 906                  |
|     |                     |                   | IF8-11-2 RCS   | 80,250 ± 643                  |
|     |                     |                   | KJ23a          | 2,876 ± 425                   |
|     |                     |                   | KJ23a          | 5,583 ± 1,384                 |
| 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                 |

* [3H]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.

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

5 Assay was performed as above, except 10^6 γ-irradiated DBA/2 (1E6) spleen cells were used as stimulator cells. DBA/2 cells alone did not release measurable amounts of IL-2.

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 | None | KJ23a mAb | F23.1 mAb |
|------|-----------------|------|-----------|-----------|
| 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 |
| 3    | RCS LA-12       | 49,099 ± 4,261 | 39,345 ± 503 | 52,023 ± 1,687 |
| 4    | RCS Sp-3        | 32,836 ± 1,689 | 24,267 ± 5,033 | 43,360 ± 721 |
| 5    | SJL/J spleen    | 566 ± 215  | -          | 86 ± 2     |

* Cultures are incubated at 37° for 72 h, whereupon the cultures are pulsed with 1 μCi of [3H]TdR for 24 h and then harvested by MASH. Proliferation is expressed in cpm ± SD of triplicate cultures.

1 Monomeric affinity-purified mAbs were added at the initiation of each culture at 10 μg/ml.

5 × 10^5 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.

The responsive syngeneic RCS-speck T cells in tumor-bearing mice are predominantly KJ23a+. The RCS LA-12 line was passed intraperitoneally (5 × 10^5 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° 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 Vβ17⁺ 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 Vβ17⁺ T cells play an important role in responding to RCS stimulation in vivo.
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+ Vβ17a+ T cells. Several lines of evidence support this conclusion. All four RCS-specific T cell hybridoma clones are Vβ17a+ 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 Vβ17a+ 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 Vβ17a+ 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 Vβ17a+ cells during T cell maturation (12). Interestingly, previous studies using F1 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, F1 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 Vβ17a+ T cells, it seems attractive to speculate that the failure of IE-expressing F1 mice to support the growth of and respond to RCS is the result of the deletion of Vβ17a+ T cells in these mice.

The predominant role played by Vβ17a+ 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 IF8 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(\(e\))), 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 IF8 and 2D5. However, the fact that IF8 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 V\(\beta\)17a+ T cells from SJL/J mice to IE appears to be greatest for allogeneic IE, there is a significant reactivity of V\(\beta\)17a+ T cells for self IE (19). SJL/J mice lack expression of the \(\alpha\beta\) heterodimer of IE due to a 600-bp deletion in the promoter and leader sequence of the Ea gene (20, 21). \(\alpha\beta\), 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 Ea and A\(\beta\) genomic DNA have demonstrated that cell surface expression of Ea and A\(\beta\) is possible, in what are termed mixed isotype heterodimers (22, 23). Therefore, it remains a formal possibility that \(\beta\) is expressed with \(\alpha\) in a mixed heterodimer on the surface of RCS tumors. SJL/J T cells, which are not tolerant to surface expression of \(\beta\), would therefore respond to it. Evidence presented here would suggest that this response would be predominantly by KJ23a+ T cells. Alternatively, cytoplasmic \(\beta\) protein, unable to achieve surface expression, is degraded by the RCS tumor cells and presented by \(\alpha\) in peptide form. While no direct evidence exists that the presentation of \(\beta\) peptide by \(\alpha\) 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 \(\beta\) in the context of \(\alpha\).

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\(^b\), 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 V\(\beta\)17a TCR clonotype. The V\(\beta\)17a+ 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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References

1. Murphy, E. D. 1963. SJL/J, a new inbred strain of mouse with a high, early incidence of reticulum cell sarcoma. Proc. Am. Assoc. Cancer Res. 4:46.
2. Nakauchi, H., H. Osada, H. Yagita, and K. Okumura. 1987. Molecular evidence that SJL reticulum cell sarcomas are derived from pre-B cell. Clonal rearrangement of heavy chain but not of light chain immunoglobulin genes. J. Immunol. 139:2803.
3. Brown, P. H., and G. J. Thorbecke. 1985. Characterization of the molecules on SJL/J lymphomas which stimulate syngeneic T cells. J. Immunol. 135:3572.
4. DeKruyff, R. H., P. H. Brown, G. J. Thorbecke, and N. M. Ponzio. 1985. Characterization of SJL T cell clones Responding to Syngeneic Lymphoma (RCS): RCS-specific clones are Stimulated by Activated B Cells. J. Immunol. 135:3581.
5. Ohnishi, K., and B. Bonavida. 1987. Regulation of Ia+ Reticulum cell sarcoma (RCS) growth in syngeneic SJL/J mice. I. Inhibition of tumor growth by passive administration of L3T4 monoclonal antibody before or after tumor inoculation. J. Immunol. 138:4524.
6. Sakano, T. and B. Bonavida. 1986. Inhibition of IA+ positive reticulum cell sarcoma (RCS) tumor cell growth in syngeneic SJL/J mice by passive administration of monoclonal anti-IA antibody. Cancer Res. 46:3245.
7. Katz, I. R., S. P. Lerman, N. M. Ponzio, D. C. Shreffler, and G. J. Thorbecke. 1980. Growth of SJL/J-derived transplantable reticulum cell sarcoma as related to its ability to induce T cell proliferation in the host I. Dominant negative genetic influences of the other parent haplotype in F, hybrids of SJL/J mice. J. Exp. Med. 151:347.
8. Wilbur, S. M., and B. Bonavida. 1981. Expression of hybrid Ia molecules on the cell surface of reticulum cell sarcomas that are undetectable on host SJL/J lymphocytes. J. Exp. Med. 153:501.
9. Wilbur, S. M., O. Marelli, and B. Bonavida. 1983. Serological demonstration of an al-
10. Ohnishi, K., and B. Bonavida. 1986. Mapping of the SJL/J reticulum cell sarcoma tumor associated Ia antigens by T cell hybridomas: characterization of tumor-specific and shared epitopes detected on IE+ allogeneic cells. *J. Immunol.* 137:733.

11. Kappler, J. W., W. Tade, J. White, E. Kushnir, M. Blackman, J. Bill, N. Roehm, and P. Marrack. 1987. A T cell receptor Vδ segment that imparts reactivity to a class II major histocompatibility complex product. *Cell.* 49:263.

12. Kappler, J. W., N. Roehm, and P. Marrick. 1987. T cell tolerance by clonal elimination in the thymus. *Cell.* 49:273.

13. Staerz, U. D., H. G. Rammensee, J. D. Benedetto, and M. J. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell receptor. *J. Immunol.* 134:3994.

14. Ey, P. L., S. J. Prowse, and C. R. Jenkin. 1978. Isolation of pure IgG₁, IgG₂a, and IgG₂b immunoglobulins from mouse serum using protein A sepharose. *Immunochimistry.* 15:429.

15. Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintans, M. R. Loken, M. Pierres, and F. W. Fitch. 1983. Characterization of the murine T cell surface molecule, designated L3T4 identified by monoclonal antibody Ok1.5: similarity of L3T4 to the human Leu-3/T4 molecule. *J. Immunol.* 131:2445.

16. Marshak-Rothstein, A., P. Fink, T. Gridley, D. H. Raulet, M. J. Bevan, and M. L. Gefter. 1979. Properties and applications of monoclonal antibodies directed against determinants of the thy-1 locus. *J. Immunol.* 122:2491.

17. North, J., and C. Henry. 1980. One-step gradient separation of rosetted cells. In *Selected Methods in Cellular Immunology.* B. B. Mishell and S. M. Shiigi, editors. Freeman Publications, San Francisco. 205–208.

18. Gillis, S., and K. A. Smith. 1977. Long term culture of tumor-specific cytotoxic T cells. *Nature (Lond.)* 268:154.

19. Marrack, P., and J. W. Kappler. 1987. The T cell receptor. *Science (Wash. DC)* 238:1073.

20. Mathis, D. J., C. O. Benoist, V. E. Williams II, M. E. Kanter, and H. O. McDevitt. 1983. Several mechanisms can account for defective Ea gene expression in different mouse haplotypes. *Proc. Natl. Acad. Sci. USA.* 80:273.

21. Jones, P. P., D. B. Murphy, and H. O. McDevitt. 1981. Variable synthesis and expression of Ea and Ae(Eb) Ia polypeptide chains in mice of different H-2 haplotypes. *Immunogenetics.* 12:321.

22. Germain, R. N., and H. Quill. 1986. Unexpected expression of a unique mixed-isotype class II MHC molecule by transfected L-cells. *Nature (Lond.)* 320:72.

23. Malissen, B., N. Shastr, M. Pierres, and L. Hood. 1986. Cotransfer of Eaα and Aβγ genes into L cells results in the surface expression of a functional mixed-isotype Ia molecule. *Proc. Natl. Acad. Sci. USA.* 83:3958.

24. Song, E. S., R. Links, C. A. Olson, M. McMillan, and R. S. Goodnow. 1988. Allospecific cytotoxic T lymphocytes recognize an H-2 peptide in the context of a murine major histocompatibility complex class I molecule. *Proc. Natl. Acad. Sci. USA.* 85:1927.

25. Marrack, P., and J. Kappler. 1988. T cells can distinguish between allogenic major histocompatibility complex products on different cell types. *Nature (Lond.)* 332:840.