ANTIGEN-SPECIFIC, I-A-RESTRICTED SUPPRESSOR HYBRIDOMAS WITH SPONTANEOUS CYTOLYTIC ACTIVITY

Functional Properties and Lack of Rearrangement of the T Cell Receptor \( \beta \) Chain Genes

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Since their discovery in 1971 (1, 2), T suppressor (Ts) cells have been the object of numerous studies. Particularly helpful in the characterization of Ts cells was the establishment of cloned T cell lines, such as T cell hybridomas, long-term T cell lines, or transformed T cells (for a survey see 3). A surprisingly large number of Ts subpopulations have been distinguished. Ts and suppressor factors (TsF) derived from them belong either to the inducing arm of the suppressor circuit or to the effector arm that ultimately mediates suppression. They appear to be either antigen specific or nonspecific, H-2 restricted, and heavy chain variable region (\( V_{H} \)) restricted or nonrestricted (reviewed in 4–6). Recent advances (7–17) in the molecular and genetic characterization of antigen-specific receptors of T cells, consisting of two chains, \( \alpha \) and \( \beta \), have not only revealed some degree of homology to immunoglobulin but also a basic similarity of receptors on T helper cells (Th) and cytotoxic T lymphocytes (Tc). In contrast, the nature of the antigen receptors on Ts remains elusive. The rearrangement of \( \beta \) chain genes described for Th and Tc have not been observed in most antigen-specific Ts cells (18), suggesting that the receptor molecule of the Ts cell is unique. However, none of the murine Ts cells that have been investigated for rearrangement were class II or class I major histocompatibility complex (MHC) restricted, as were the Th and Tc lines, respectively.

In the present report we describe the establishment and functional characterization of Ts hybridomas that are antigen specific and I-A restricted. In this respect the Ts hybridomas are similar to Th cells. In addition, they display

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*Abbreviations used in this paper: BSA, bovine serum albumin; C region, constant region; D region, diversity region; FCS, fetal calf serum; GSA, goat serum albumin; HSA, human serum albumin; J region, joining region; MHC, major histocompatibility complex; NC, natural cytotoxicity; NK cell, natural killer cell; OVA, ovalbumin; PSA, porcine serum albumin; Tc, cytotoxic T lymphocyte; Th, helper T cell; Ts, suppressor T cell; TsF, T suppressor factor; \( V_{H} \), variable region heavy chain.
spontaneous cytotoxicity against various tumor targets. No rearrangement of the T cell receptor β genes was found in this class of Ts cells.

Materials and Methods

**Mice.** CBA (H-2<sup>k</sup>) and BALB/cJ (H-2<sup>d</sup>) mice were obtained from The Jackson Laboratory, Bar Harbor, ME. B10 (H-2<sup>b</sup>), B10.A(1R) (H-2<sup>b4</sup>), B10.A(5R) (H-2<sup>b5</sup>), and B10.AQR (H-2<sup>q</sup>) were from the central animal breeding facility of the German Cancer Research Center, Heidelberg, FRG.

**Antigens.** Ovalbumin (OVA) and bovine (BSA), human (HSA), porcine (PSA), and goat (GSA) albumins were purchased from Sigma Chemical Company, St. Louis, MO. Deaggregation of albumin was achieved by centrifugation of a solution containing 10–30 mg/ml in phosphate-buffered saline (PBS) for 2 h at 100,000 g. The upper one-third of the suspension contained the deaggregated protein.

**Ts Hybridomas.** Ts cells were generated by induction of low zone tolerance as described (19, 20). Briefly, CBA mice were injected with 2.5 mg hydrocortisone (Hoechst, Frankfurt) intraperitoneally, rested for 2 d, and then injected intraperitoneally with 10 µg deaggregated BSA daily for the next 7 d. Splenic T cells from immunized mice were enriched by panning on goat anti-mouse Ig-coated petri dishes. Nonadherent cells were fused in polyethylene glycol 4000 with the III/4 T hybridoma (derived from fusion of strain CBA concanavalin A blasts with the AKR thymoma BW 5147 [21]) at a cell ratio of 5:1. Cells were seeded in hypoxanthine, aminopterin, thymidine (HAT) medium into 24-well Costar plates (Costar, Data Packaging, Cambridge, MA) without a feeder cell layer. After 3–4 wk there was growth in 90% of the wells. Hybridoma cells were tested for inhibition of BSA-specific proliferation and positive wells were cloned.

**Assay for Suppressive Activity (Inhibition of Proliferation).** CBA mice immunized with 100 µg of BSA or control antigen in complete Freund’s adjuvant at the base of the tail were sacrificed after 10–14 d. Paraaortic lymph nodes from these mice were the source of BSA responder lymphocytes. Filler cells were normal CBA spleen cells irradiated with 3,300 rad. For suppression hybridoma cells irradiated with 3,300 rad were used, or culture supernatants as a source of suppressor factor (TsF). Test culture wells consisted of 40 µg antigen, 2 × 10<sup>4</sup> responder cells, 2 × 10<sup>4</sup> filler cells, 2 × 10<sup>4</sup> Ts hybridoma cells, or 5–100 µl TsF in a total volume of 200 µl in RPMI 1640/5% horse serum. In some experiments 1% CBA mouse serum was used instead of 5% horse serum. For most experiments 50 µl TsF was used. Triplicate cultures were incubated 4 d and pulsed for 18 h with 1.0 µCi [³H]thymidine. In the absence of suppression, generally 10,000 cpm per well were obtained, which is satisfactory for the small number of responder cells (2 × 10<sup>4</sup>/well) used.

**Cytotoxic Assay and Cell Lines.** A standard ⁵¹Cr release assay (22) was performed with minor modifications. Target cells as listed below and hybridoma clones were incubated for 4–8 h in RPMI 1640 containing 5% horse serum at different target-to-effector cell ratios. Results given are for 4 h and a 10:1 effector/target ratio. Tumor targets included the natural killer (NK) cell–sensitive T lymphoma YAC (H-2<sup>b</sup>), the EL-4 T cell leukemia, the IE7 fibrosarcoma from (C3H × C57BL/6)F<sub>1</sub> mice (23), the NK-sensitive DBA/2 T lymphoma L5178 (generously provided by Drs. C. Brooks and C. Henney, Fred Hutchinson Cancer Center, Seattle, WA), and the NK-resistant but NC-sensitive line W164 (provided by Dr. O. Stutman, Sloan-Kettering Institute, New York).

**Southern Blot Hybridization.** Southern blot hybridization was performed as described (24). Briefly, high molecular weight DNA was isolated from Ts hybridomas according to Wigler et al. (25) and digested with a fivefold excess of restriction endonucleases Pvu II and Hind III (5 U enzyme/µg DNA) overnight at 37°C. DNA fragments were separated by electrophoresis through 0.9% agarose gels and blotted on nitrocellulose. The probe used for hybridization was 86T5, which is mainly specific for the constant regions (C<beta>1 and C<beta>2) of the T cell receptor β chain but also hybridizes with diversity and joining regions Dβ1 and Jβ1 (14). Another probe, J15, contains the 900 basepair (bp) fragment located ~90 bp downstream of the Jβ2 cluster (26). Hybridization with ⁹⁰P-labeled cDNA...
was done at 42°C in 50% formamide, 5x SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 5x Denhardt's, 100 µg/ml denatured herring sperm DNA, and 10% dextran sulfate. After hybridization, filters were washed twice with 2x SSC, 0.1% sodium dodecyl sulfate at 65°C for 30 min. Labeled DNA fragments were detected by autoradiography.

Results

Suppressive Activity of Ts Hybridoma. Splenic T cells from CBA mice suppressed with subimmunogenic doses of BSA were fused with the III/4 T hybridoma. For a screening assay we used the ability of the resulting hybridomas to inhibit antigen-specific proliferation of lymph node cells from CBA mice immunized with BSA or OVA (as control). Several antigen-specific Ts hybridomas were isolated and cloned by limiting dilution. Table I shows representative individual experiments and the average suppression observed in four independent experiments (last column). Some clones proved slightly more efficient in their suppressive activity, i.e., D9.15/H1 (81% suppression) vs. C11.5/C1 (55%). The hybridomas were as effective in suppressing a proliferative response to BSA as splenic T lymphocytes from mice tolerized to BSA (Exp. 4, Table I). Suppression was

| Exp. No. | Lymph node cells immunized with: | Ts hybridoma | Percent suppression | Percent mean suppression ± SD* |
|----------|---------------------------------|--------------|---------------------|--------------------------------|
| 1        | BSA                             | D9.15/H1     | 942 13,423 2,198 90 | 81 ± 12 |
|          | OVA                             | D9.15/H1     | 1,024 8,864 8,944 -1 | 1 ± 2  |
|          | BSA                             | D9.15/E3     | 942 13,423 2,289 89 | 81 ± 14 |
|          | OVA                             | D9.15/E3     | 1,024 8,864 8,715 2 | 1 ± 3  |
| 2        | BSA                             | C11.5/C1     | 1,039 10,379 3,961 69 | 55 ± 13 |
|          | OVA                             | C11.5/C1     | 1,475 15,881 16,455 -4 | -3 ± 5 |
|          | BSA                             | C14.2/A12    | 1,039 10,379 4,209 63 | 61 ± 2  |
|          | OVA                             | C14.2/A12    | 1,475 15,881 15,978 -4 | -4 ± 4  |
| 3        | BSA                             | D2.22/F1     | 1,770 11,052 4,992 65 | 64 ± 11 |
|          | OVA                             | D2.22/F1     | 1,541 7,981 8,136 -2 | 1 ± 2  |
|          | BSA                             | III/4        | 1,770 11,052 12,135 -8 | -2 ± 4 |
|          | OVA                             | III/4        | 1,541 7,981 7,452 3 | 2 ± 5  |
| 4        | BSA                             | Splenic Ts   | 984 7,492 2,840 71 | 73 ± 2  |
|          | OVA                             | Splenic Ts   | 892 8,872 10,738 -23 | -33 ± 7 |

Paraortic lymph node cells were obtained from CBA mice immunized 10-14 d previously with 100 µg BSA or OVA in CFA at the base of the tail. 2 × 10⁴ responder cells were cultured in round-bottomed wells without or with 40 µg corresponding antigen and 2 × 10⁴ irradiated syngeneic spleen cells in 200 µl RPMI 1640 containing 5% horse serum. For suppression, 3,300-rad-irradiated Ts hybridoma cells were included, or 2 × 10⁴ Ig-negative spleen cells from CBA mice suppressed with subimmunogenic doses of BSA (designated splenic Ts, see Exp. 4). Cultures were incubated 4 d and pulsed for 18 h with 1.0 µCi [3H]thymidine. For the individual experiments the average of triplicate cultures is given; the standard deviations (SD) were 5-15% (not shown).

* Percent mean suppression + SD for four independent experiments for each experimental group.
TABLE II

Antigen Specificity of Ts Hybridoma D9.15/H1 and TsF

| Lymph node cells immunized with: | Antigen in culture | Proliferation | Percent suppression |
|---------------------------------|--------------------|---------------|---------------------|
|                                 |                    | Without Ts | With Ts | With TsF | Without Ts | With TsF |
| cpm                            |                    |            |         |          |            |          |
| BSA                            | BSA                | 13,882     | 1,571   | 5,557    | 99         | 67       |
| OVA                            | OVA                | 9,330      | 8,592   | 12,011   | 10         | -36      |
| HSA                            | HSA                | 11,975     | 12,910  | 13,195   | -8         | -11      |
| GSA                            | GSA                | 9,298      | 10,885  | 10,379   | -22        | -37      |
| PSA                            | PSA                | 18,624     | 18,547  | 12,011   | -8         | -11      |
| OVA                            | OVA + BSA          | 9,330      | 4,118   | 3,142    | 78         | 87       |
| HSA                            | HSA + BSA          | 11,975     | 2,842   | 1,589    | 84         | 92       |

CBA mice were immunized with albumins from various species as indicated. Proliferation with homologous antigen was measured in vitro in the presence or absence of irradiated D9.15/H1 Ts hybridoma cells or D9.15/H1-derived TsF. The test was done in RPMI medium containing 1% normal CBA mouse serum. Results are the mean values for triplicate wells. Background without antigen ranged between 877 and 2,217 cpm. Standard deviations were 5-15% (not shown).

* Not done.

only observed when the Ts were added within the first 48 h after onset of the responder culture (data not shown). In all cases, Ts hybridoma cells did not inhibit a proliferative response to OVA (Table I). Culture supernatants of Ts hybridomas also proved to be suppressive in the same assay (see below). Neither III/4 cells (Table I) nor III/4 supernatant (data not shown) was suppressive. According to cytofluorometric analysis or immunoprecipitation, the phenotype of the Ts hybridomas was H-2 k+, Thy-l.2 +, Lyt-2 +, Lyt-1-, I-A-, I-E- (data not shown).

Further verification of the antigen (BSA)-specific nature of suppression by a selected hybridoma clone (D9.15/H1) and TsF was undertaken by testing for potential inhibition of proliferation to other albumins (Table II). No suppressive activity of hybridoma cells or TsF was seen with responder cells immunized and restimulated with albumins other than BSA. However, when OVA and BSA were admixed to OVA-primed cells, the OVA response was also suppressed by both Ts and TsF (Table II). Likewise, an HSA response could be suppressed in the presence of BSA. Possible explanations are discussed below. It should be mentioned that the effective titer of the TsF was low (maximal 1:10 dilution) and that we did isolate (data not shown) Ts hybridoma clones that are suppressive but do not release TsF into the culture medium.

I-A Restriction of Ts Hybridoma and TsF. The evaluation of H-2 restriction was performed using the D9.15/H1 clone and TsF with a panel of mouse strains immunized with BSA (Fig. 1). D9.15/H1 hybridoma cells and TsF suppressed proliferation in CBA, B10.A, B10.A(4R), and B10.AQR mice, but not in B10, BALB/c, and B10A(3R). The results demonstrate the requirement for I-A-compatible responder cells in effective suppression of proliferation by both Ts cells and TsF.

Spontaneous Nonspecific Cytotoxic Activity of Ts hybridomas. We observed that clones D9.15/H1 and D9.15/E3 were not only suppressive but also nonspecifically
H-2 Restriction of Ts Hybridoma Cells and Factor

**Figure 1.** I-A restriction of Ts hybridoma and TsF activity. Lymph node cells from mice immunized with BSA were cultured alone, with BSA, with BSA plus irradiated D9.15/H1 cells, or with BSA plus D9.15/H1-derived TsF, as indicated.

**Table III**

Spontaneous Cytotoxic Activity of Ts Hybridomas

| Effector cell     | Ag8.653 | 1E7 | YAC-1 | III/4 | L5178 | W 164 |
|-------------------|---------|-----|-------|-------|-------|-------|
| NK cells          | —       | 0   | 88    | 0     | 35    | 0     |
| D9.15/H1          | 32      | 69  | 52    | 9     | 0     | 4     |
| D9.15/E3          | —       | 70  | 61    | 4     | 0     | —     |
| D9                | 98      | 64  | —     | —     | —     | —     |
| C14               | 21      | 92  | —     | 11    | —     | —     |
| C17               | 29      | 47  | —     | 5     | —     | 3     |
| D2                | 27      | 47  | —     | 13    | —     | 10    |
| D9.15/H1D−        | 87      | 98  | —     | 0     | —     | —     |
| C17.8             | 59      | 91  | —     | 0     | —     | —     |
| C17.9             | 4       | 0   | —     | 8     | —     | —     |
| TsF*              | 0       | 0   | 0     | 0     | —     | 72    |

*Not done.

**Note:** Effector cells were mixed with 51Cr-labeled target cells at an effector/target ratio of 10:1 in RPMI 1640 medium containing 5% horse serum. 51Cr release was determined after 4 h. As a source of NK cells, spleen from 4-wk-old C57BL/6 mice injected 2 d previously with poly I:C were used. Hybridomas D9, C14, C17, and D2 are the original parental lines and were in culture a total of 7 wk when assayed for spontaneous cytolytic activity. C17.8 and C17.9 are derived from C17 by cloning. D9.15/H1D− is a subclone of D9.15/H1 selected for its lack of suppressive activity.

™ Culture supernatant from D9.15/H1 was tested at a 50% concentration during the 4 h assay.
cytolytic against various tumor targets (see Table III), whereas III/4 parental
cells were not cytotoxic. For comparison, poly I:C-induced spleen cells from
C57Bl/6 mice with NK activity were included as effector cells. Table III shows
that both D9.15/H1 and D9.15/E3 exhibited strong spontaneous cytolytic activity
when tested in a 4 h chromium release assay against the NK target YAC-1, but
also against the NK-resistant fibrosarcoma, IE7. In comparison, lysis of the
plasmacytoma Ag8.653 was moderate and absent or minimal for III/4, W164,
and L5178, the latter of which is another NK-sensitive T lymphoma. Thus, the
target spectrum is different from that of a polyclonal NK cell population.

Next we investigated the possibility that the spontaneous activity was an in
vitro artifact induced by long-term culture (27, 28) of D9.15/H1 and D9.15/E3
cells, which were maintained in vitro for more than one year. The original
parental lines, D9, C14, C11, and D2, which had been frozen and stored in
liquid nitrogen 6 wk after initiation of the cell fusion experiment were placed in
culture. After 7 d they were assayed for spontaneous cytotoxicity and antigen-
specific suppression of proliferating T cells. All four original hybridomas were
cytolytic (Table III). The data suggest that spontaneous cytotoxicity is an intrinsic
property and not an in vitro artifact.

To determine whether the cytotoxic activity and antigen-specific suppression
of proliferating T cells are independent functions, subclones of Ts hybridomas
were screened. Table III shows a representative clone, C17.9 (derived from line
C17), which was suppressive but not cytolytic, whereas other clones were cytolytic
but not suppressive (e.g., D9.15/H1,D− and C17.8). The TsF was not cytotoxic
on Ag8.653, IE7, and YAC-1 (Table III). These data demonstrate that suppressive
and cytolytic activity can be dissociated.

T Cell Receptor Cβ Gene Is Not Rearranged in Ts Hybridomas. The observation
that the Ts hybridomas are antigen specific and I-A restricted indicates similarity
in their fine specificity to Th cells. This raised the possibility that the receptors
on both cell types are also structurally similar and derived from the same set of
C region genes. This was investigated by analysis of rearrangement of the gene
segments encoding the C and adjoining regions of the T cell receptor β chain.
At the time of DNA extraction, care was taken to assure that the Ts hybridomas
were still active. No decrease in suppressive activity was observed in the mass
culture used for DNA preparation (not shown). The probe used in Southern blot
analysis was the cDNA clone 86T5, which hybridizes to Cβ1, Cβ2, and some
Dβ1 and Jβ2 region nucleotide sequences. With this probe, rearrangement has
been observed using Pvu II–digested DNA from Th cells and Tc (14, 18).

Fig. 2A shows a Southern blot with Pvu II-digested DNA from three inde-
pendent, functional, I-A-restricted Ts hybridomas, D9.15/H1, C14.2/A12, and
D2.22/F1, liver DNA, the fusion partners BW5147 and III/4, and Ts hybridoma
D9.15H1,D−, the last of which has lost its suppressive activity but maintained
cytolytic activity (see Table III). DNA from CBA liver or the B cell line CH.1
yields two strong and one week germline band corresponding to Cβ1 (6.3 kb),
Cβ2 (6.1 kb), and D-Jβ1 (5.9 kb) segments. In agreement with previous obser-
vations, BW5147 contains only two rearranged bands, of 6.5 and 5.2 kb, but has
lost all three germline bands. III/4 is the fusion partner for the Ts hybridomas,
its derived from fusion of a CBA lymphocyte and BW5147. It has lost the 5.2
Figure 2. Southern blot analysis of T cell receptor β chain rearrangement. High molecular weight DNA from Ts hybridomas D9.15/H1, D9.15/H1D−, C14.2/A12, and D2.22/F1 was digested with Pvu II (A) or Hind III (B), and hybridized with the 32P-labeled 85T5 probe primarily specific for the β chain C region of the T cell antigen receptor. III/4 is the fusion parent derived from fusion of BW 5147 and a CBA T cell; CH1 is a B cell tumor. A consists of two different blotting experiments. (C) Hind III–digested DNA was hybridized with a labeled J15 probe specific for the Jβ2 region.
kb rearranged band from the BW5147 parent but yields a new rearranged band of ~9.2 kb and germline bands of 6.3 and 6.1 kb, probably derived from the lymphocyte parent. The most important observation is that none of the Ts hybridomas displayed bands that were not present in either liver or III/4 DNA, implying lack of rearrangement of Cβ1 and Cβ2 genes. The 5.9 kb band is seen in D2.22/F1 but not III/4, corresponding to the D-Jβ1 germline band in liver DNA, and therefore does not reflect any rearrangement. The 6.5 kb rearranged band derived from BW5147 is missing in C14.2/A12. The absence of this band could be due to a further rearrangement, deletion, or chromosomal loss.

Using the 86T5 probe and a Pvu II digest, rearrangements of the first and second J segment of the Jβ2 cluster cannot be detected. Therefore, we also used the J15 probe, which is a 900 bp Cla I/Eco RI fragment located ~90 bp downstream of the Jβ2 locus (26). Using this probe and a Hind III digest, potential rearrangements within the Jβ2 cluster should be detected (26). Fig. 2C shows that, using III/4 cells, a 5.4 kb germline band and two rearranged bands of 5.2 and 3.9 kb are obtained. None of the suppressor hybridomas displayed any bands that were not present in III/4, indicating that the Jβ2 cluster is not rearranged. We should add that the restriction pattern observed with BW5147 is different from that described by Snodgrass et al. (26), possibly because we used a different subclone of BW5147.

The data strongly suggest that the antigen receptor on the I-A-restricted Ts hybridomas does not use the same β chain as Th and Tc. The same conclusion was derived from Southern blots with Hind III–digested DNA (Fig. 2B). Again, no change from the III/4 pattern was observed, with the exception of D2.22/F1, which yielded a germline band of ~9.4 kb, probably derived from the Ts lymphocyte parent.

Discussion

The Ts hybridomas described here, of which D9.15/H1 is a representative example, are antigen specific (for BSA) and I-Ak restricted. D9.15/H1 also produced an antigen-specific and I-Ak-restricted TsF. The mechanism of suppression is still unclear. It is of interest that the TsF can also suppress third-party proliferative responses in the presence of BSA (Table II). A possible explanation is that the TsF binds to antigen-presenting cells that have been pulsed with both BSA and the third-party antigen, e.g., OVA. When these cells present OVA to OVA-specific T cells, the T cells come into contact with the TsF and are suppressed. Alternatively, BSA-pulsed macrophages or other cell types could be triggered by TsF to release nonspecific suppressor factors. These findings make it unlikely that the TsF is directed against receptor structures on the proliferating T cells, as suggested by Asano and Hodes (29) for a different type of Ts cells.

The other striking characteristic of the BSA-specific Ts hybridomas is their spontaneous cytotoxicity against various tumor cell targets. In this respect, the Ts hybridomas appear to be similar to NK cells. The respective target spectra are not, however, completely identical. The establishment of subclones that are suppressive but no longer cytotoxic, and the existence of noncytolytic TsF, indicates that suppression and cytotoxic activity are independently controlled functions.
The combined properties ascribed to these Ts hybridomas set them apart from the majority of Ts cells described in the literature, most of which are unrestricted, V₃₄ restricted, or I-J restricted (4–6, 30–40). Antigen-specific and Ia-restricted Ts hybridomas producing antigen-specific and Ia-restricted factors have recently been described by Ikezawa et al. (41, 42). These authors did not test for spontaneous cytolytic activity. A long-term Ts clone, HF1, with characteristics similar to the Ts hybridomas described here, has been isolated by Heuer et al. (20). HF1 is antigen specific, I-A restricted, and also cytolytic (20, 43). No data have been reported on antigen specificity and MHC restriction of a TsF or on the requirement of cytotoxic activity for suppression. A remarkable difference between HF1 cells and our Ts hybridomas is that the former synthesize large amounts of I-A and I-E antigens that are identical to B cell-derived Ia molecules (44).

The structural relationship between the TsF and the antigen receptor on the Ts hybridomas is unclear. Since the TsF has all the functional properties displayed by the Ts cells, excluding cytolytic activity, it is possible that it is receptor material released from the cell surface. Recently (18), it has been reported that most murine Ts cells do not rearrange the genes encoding the β chain of the T cell receptor. However, all Ts cells investigated previously were either unrestricted or I-J restricted and not MHC class II or I restricted, as are Th or Tc cells, respectively, in which rearrangement was observed (14, 18). The Ts investigated for rearrangement also included the so-called haplotype Ts (45), which appear to recognize I-A directly and are therefore not I-A restricted in the way that the recognition of a soluble antigen is restricted by I-A. The Ts hybridomas described in this study were I-A restricted, as are Th cells, suggesting the possibility that both cell types recognize antigen and I-A via similar receptors. However, the observed lack of rearrangement of the β chain C region genes in these I-A-restricted Ts hybridomas (Fig. 2) implies that this class of Ts cells does not use the β chain. Since the Ts hybridomas display NK-like activity, the findings also suggest that the receptor(s) necessary for target recognition does not contain the β chain.

In contrast to results in the mouse, rearrangement of the T cell receptor β genes was recently demonstrated in human Ts cells that are able to suppress mitogen-induced immunoglobulin secretion (46, 47). However, to our knowledge, none of these Ts cells were antigen specific. Therefore it remains unclear whether the observed rearrangement is functionally meaningful and required for suppressive activity.

The lack of rearrangement described for the I-A-restricted Ts hybridomas does not exclude the possibility that the Ts receptors use the same set of V genes, and perhaps the same α chain, as Th cells or Tc. The question why Ts use different receptors remains unanswered. Perhaps the function of antigen receptors on Ts cells does not consist only of recognition of antigen. It is possible that the suppressive activity is mediated by the receptor molecule itself or by a subunit, cell bound or a soluble factor. This dual function is consistent with the existence of both specific and nonspecific suppressor factors. In contrast, antigen-specific receptors on Th or Tc are not identical to their respective effector molecules. Their only function is to recognize antigen, leading to production of
unrelated effector molecules such as interleukin 2, or to activation of the lytic mechanism. Such assumptions would explain the sharing by Th and Tc of the β chain isotypes while antigen receptors on Ts cells are different.

Summary

T suppressor (Ts) hybridomas were produced by fusion of the III/4 T cell hybridoma with splenic T cells from CBA mice tolerized with subimmunogenic doses of bovine serum albumin (BSA). Both the Ts hybridoma cells and a suppressor factor (TsF) inhibited in an antigen-specific and I-Ak-restricted fashion the in vitro proliferative response of BSA-immunized lymph node cells.

In addition to the suppressive activity, the hybridoma lines displayed spontaneous cytotoxicity against various tumor targets. The isolation of Ts subclones that are suppressive but not cytolytic, as well as the existence of the noncytolytic TsF, indicates that suppression of antigen-specific T cell proliferation is not dependent on cytolytic activity.

The Ts hybridomas were I-A restricted, as are many T helper cells. Therefore, a potential similarity with respect to antigen receptor genes was expected. Southern blot analysis with a probe specific for genes encoding the β chain of the T cell receptor on T helper and T killer cells revealed no rearrangement of the β genes in the Ts cells. The data imply that neither the antigen receptor on the I-A-restricted Ts cells nor the receptor involved in the cytolytic interaction with tumor targets use the same β chain constant region as T helper and T killer cells.

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