

TUBULAR ANTIGEN-DERIVATIZED CELLS INDUCE A
DISEASE-PROTECTIVE, ANTIGEN-SPECIFIC, AND IDIOTYPE-
SPECIFIC SUPPRESSOR T CELL NETWORK RESTRICTED
BY I-J AND Igh-V IN MICE WITH EXPERIMENTAL
INTERSTITIAL NEPHRITIS

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The characterization and evaluation of antigen-induced suppressor T (Ts)¹ cell networks have considerably evolved in the last several years (1-4). While there are now a variety of ways to induce such Ts cells, one of the most intriguing approaches has been to intravenously inject ligand-derivatized lymphocytes into naive recipients, and to later use these recipients as suppressor cell donors (1, 3). This technique has been successfully used with haptens (5-7), polysaccharides (8), and immunoglobulins (9, 10) in mice. While suppressor networks generated by ligand-derivatized lymphocytes can have features that are inherently unique to a particular system, they also tend to share several characteristics. They typically involve several sets of sequentially activated Ts cells with complementary specificities; they often operate under H-2 and Igh-1 restrictions; and they usually secrete soluble suppressor factors that mediate complex interactive functions (1, 3, 11).

We have used an experimental model of interstitial nephritis (anti-tubular basement membrane [anti-TBM] disease) to determine if tubular antigen-derivatized lymphocytes induce or activate a disease-protective Ts cell network in an autoimmune setting. Interstitial nephritis can be induced in prototype SJL and BALB/c mice with an injection of renal TBM in adjuvant (12-14). Anti-TBM antibodies (Ab) appear by 10 d and interstitial lesions typically develop by 6-10 wk. Susceptibility to disease is defined by genes in H-2K and Igh-1 (15), and is mediated by an effector T cell repertoire containing cytotoxic and delayed-type

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¹ *Abbreviations used in this paper:* Ab, antibody; ABA, azobenzenearsonate; CFA, complete Freund's adjuvant; CTX, cyclophosphamide; DTH, delayed-type hypersensitivity; GAT, L-glutamic acid-L-alanine-L-tyrosine; NP, 3-nitro-4-hydroxyphenyl acetyl; PBS, phosphate-buffered saline; PPD, purified protein derivative; RE-Id, anti-TBM Ab eluted from nephritic kidneys; RTA, renal tubular antigen; SLA, soluble liver antigen; SRTA, soluble RTA; TBM, tubular basement membrane; Ts, T suppressor cell.

hypersensitivity (DTH)-reactive, Lyt-2⁺, RE-Id⁺ T lymphocytes (RE-Id, anti-TBM Ab eluted from nephritic kidneys) (12, 15). Such Lyt-2⁺ lymphocytes are H-2K restricted, will acutely transfer disease, can be found within the interstitial infiltrate, and produce DTH reactions to tubular antigen when transferred into naive recipients (15). These H-2K-restricted effector T cells are only found in disease-susceptible mice, and the DTH response they produce is a reliable measure of effector T cell function leading to renal injury.

We now report that tubular antigen-derivatized lymphocytes activate a Ts cell network that markedly attenuates tubular antigen-reactive DTH effector cells and the development of interstitial lesions. These suppressor cells function in both the afferent and efferent phases of the anti-TBM immune response. This network is composed of RE-Id⁺ Ts-1 cells (antigen-binding, afferent phase Ts cells) which, in the presence of antigen, induce RE-Id-binding Ts-2 cells (idiotype-binding, effector phase Ts cells) that are restricted by I-J and Igh-V gene products.

Materials and Methods

Animals. SJL (H-2^s; Igh-1^b), A.SW (H-2^s; Igh-1^e), B10.A (H-2^a; Igh-1^b), B10.A(5R) (H-2ⁱ⁵; Igh-1^b), and BALB/c (H-2^d; Igh-1^a) mice were purchased from The Jackson Laboratory, Bar Harbor, ME. B10.S (H-2^s; Igh-1^b), B10.S(9R) (H-2ⁱ⁴; Igh-1^b), B10.TBR2 (H-2^{at2}; Igh-1^b), and B10.HTT (H-2ⁱ³; Igh-1^b) mice were obtained from Dr. Chella David, Mayo Clinic, Rochester, MN. SJA/20 (H-2^s; Igh-1^a) and BAB/14 (H-2^d; Igh-V^a/C^b) mice were obtained from Dr. Roy Riblet, Institute for Cancer Research, Fox Chase, PA. B10.S(8R) (H-2^{as}; Igh-1^b) mice were a generous gift from Dr. Martin Dorf, Harvard University, Boston, MA; C.B-20 (H-2^d; Igh-1^b) mice were from Dr. Carol Cowing, University of Pennsylvania; and BALB.K (H-2^k; Igh-1^a) mice were from Dr. Robert Korngold, Wistar Institute, Philadelphia, PA. (SJL × B10.A)_{F1} hybrids were bred by the investigators.

Preparation of Renal Tubular Antigen (RTA). Rabbit renal tubular basement membranes and rabbit liver basement membranes were isolated by a differential sieving technique, sonicated, lyophilized, and stored at -70°C (12). Soluble renal tubular antigen (SRTA) and soluble liver antigen (SLA) were made from these lyophilized membranes by collagenase digestion, as previously described (15). The nephritogenic moiety in this digestion is 3M-1 glycoprotein (16).

Induction of Disease. Groups of mice were immunized with 2 mg of RTA in complete Freund's adjuvant (CFA) by footpad and/or subcutaneous injection (15). Control mice received SLA in CFA, or CFA alone. Some groups were also treated with 30 mg/kg of cyclophosphamide 24 h after immunization (17).

Induction and Elicitation of DTH Responses. DTH responses to SRTA were induced by subcutaneously immunizing mice with RTA in CFA (15). 6 d later groups of mice (four to six mice per group) were challenged in one footpad with 25 μg of soluble antigen in 25 μl of phosphate-buffered saline (PBS). Swelling as an index of DTH was measured 24 h later using a spring-loaded engineer's micrometer (Schlesingers For Tools Ltd., Brooklyn, NY). The magnitude of swelling was expressed as the mean increment, in inches × 10⁻³ ± SEM, between the antigen-challenged footpad and the other footpad injected with PBS. All measurements were cage-blind.

Preparation of SRTA-derivatized Cells. SRTA was coupled to normal syngeneic spleen cells using a carbodiimide procedure as previously described (10, 18). Briefly, spleen cells from selected strains of mice were treated with Tris-buffered 0.83% ammonium chloride (pH 7.2) to lyse the erythrocytes. 4 × 10⁸ cells were incubated with 1 mg SRTA and 25 mg 1-ethyl 3-(3-diethylaminopropyl)carbodiimide in 1 ml 0.9% saline. The mixture was gently rotated at 4°C for 90 min and then washed three times with normal saline. Under these conditions, 15–18 μg of SRTA (trace labeled with ¹²⁵I) was bound to 5 × 10⁷ viable

cells. Cell viability at the end of this coupling procedure averaged 80% by trypan blue exclusion. 5×10^7 tubular antigen-coupled cells were intravenously injected into naive recipients and, after 7 d, the spleens of these recipients were used as a source of donor Ts.

Adoptive Transfer of Suppression. 5×10^7 viable splenic Ts cells from mice that had received SRTA-derivatized cells 7 d earlier were intravenously transferred into recipients either on the day of their immunization with RTA in CFA (afferent phase) or 5–7 d after such an immunization (effector phase) (19). Selected groups of mice receiving afferent phase Ts cells were challenged for DTH 6 d later, and mice receiving effector phase Ts cells were challenged for DTH 36 h later. Other groups of mice received similar Ts cells and were followed 8–10 wk for the histologic development of disease. In some experiments the transferred inoculum of Ts cells were pretreated with antibodies plus C' or were separated into subpopulations by indirect panning.

Preparation of T Cells and Their Subpopulations. Cell suspensions were depleted of T lymphocytes with monoclonal anti-Thy-1.2 antibody (from J1j hybridoma [20] provided by Dr. Jon Sprent, University of Pennsylvania) and a mixture of rabbit and guinea pig complement (12, 15). Anti-Lyt-1.2 monoclonal antibody (from C3PO.7 hybridoma [21], prepared by Dr. Jan Klein, Max Planck Institute, Tubingen, Federal Republic of Germany), anti-Lyt-2 monoclonal antibody (from 3.168.8 hybridoma [22], provided by Dr. Frank Fitch, University of Chicago, Chicago, IL), and anti-L3T4 monoclonal antibody (from GK-1.5 hybridoma [23], provided by Dr. Fitch) were used with a mixture of guinea pig and rabbit complement for T cell subpopulation depletion. Anti-I-J^s ([A.TL \times B10.S(9R)]F₁ anti-B10.HTT; NIH Y1-9-09-19-01) and anti-I-J^t ([B10.A(3R) \times A.BY]F₁ anti-B10.A(5R); NIH Y1-0-01-09-02) antibodies were used at optimal concentrations with a mixture of rabbit and guinea pig complement to deplete I-J-bearing lymphocytes (17). Polymorphic idiotypes (RE-Id) were prepared by acid-eluting anti-TBM Ab from SJL nephritic kidneys using glycine HCl (pH 2.6) (15, 18). RE-anti-Id antibodies were prepared by hyperimmunizing a rabbit with RE-Id. The serum containing RE-anti-Id antibodies was extensively absorbed against SJL gamma globulin and normal mouse kidney eluates coupled to activated Sepharose 4B. The specificity of binding was determined by competitive inhibition radioimmunoassay using SRTA, RE-Id, control antigen, and antibody as previously described (15). By this radioimmunoassay, the RE-anti-Id antisera was specifically inhibited by RE-Id but not by SRTA. In direct binding studies, RE-anti-Id also did not bind to normal mouse kidney eluates. The RE-anti-Id antisera was used at a final concentration of 1:200 in the presence of rabbit and guinea pig complement to deplete RE-Id⁺ cells (15).

Selected aliquots of cells were also purified using panning separation techniques (15, 18). 3×10^7 erythrocyte-free spleen cells were first incubated in 60×15 mm polystyrene culture dishes previously coated with affinity-purified rabbit anti-mouse Ig (Zymed Inc., San Francisco, CA) at 1 mg/ml. The incubations were performed at 22°C for 45 min interrupted once at 20 min by gentle swirling. The nonadherent cells were gently removed at 45 min in three washes, while adherent cell removal required cold media, 30 min incubation at 4°C, and vigorous pipetting. Nonadherent cells routinely contained 92–96% T cells by indirect fluorescence criteria. In some experiments, such T cells were further incubated with optimal concentrations of RE-Id at 4°C for 30 min. After three washes the cells were panned on dishes previously coated with affinity-purified rabbit anti-mouse IgG. Adherent and nonadherent cells were then used in selected experiments. In other studies panning-purified T cells were also separated on SRTA-coated dishes (1 mg/ml).

Assessment of Renal Disease. Kidney tissue was prepared for direct immunofluorescence and light microscopy by the standard methods of this laboratory. The degree of interstitial involvement was qualitatively graded from 0 to 4 on coded sections and expressed as a mean \pm SEM for each group (12, 15).

Statistical Analysis. Differences between experimental groups were determined by Student's *t* test.

Results

Assessment and Characterization of Ts cells that Inhibit Nephritogenic T Lymphocytes. We have previously observed (15) that the DTH response to SRTA in disease-susceptible SJL mice is a reliable measure of nephritogenic effector T cell function. The nephritogenic 3M-1 glycoprotein in RTA and SRTA (16) is the principal determinant eliciting the DTH reaction, since mice immunized with RTA/CFA strongly respond to challenge with 3M-1, but only very slightly with SRTA-depleted 3M-1, compared with control (manuscript in preparation). In the present series of experiments we wished to determine if donor lymphocytes (Ts) from SJL mice primed with syngeneic SRTA-derived cells would subsequently suppress this DTH reaction to tubular antigen. In Table I such donor cells substantially inhibited the DTH response to SRTA when transferred either at the time of recipient immunization with RTA in CFA (day 0; afferent phase) or 5 d later (effector phase suppression). Control cells provided no inhibitory effect. The specificity of these suppressor cells was further examined (Table II). Ts donor cells from mice primed with SRTA-derived lymphocytes, after adoptive transfer on days 0 and 5, inhibited the DTH response to SRTA in recipients immunized with RTA in CFA, with no concomitant effect against purified protein derivative (PPD). Such an inhibitory effect was not observed in recipients immunized with SLA in CFA, suggesting that the Ts effect was specific for the SRTA-reactive response. The phenotype of these Ts cells was determined both on day 0 and day 5 (Table III). Ts donor cells from mice primed with SRTA-derivatized cells were pretreated with various antibodies to phenotypic T cell determinants just before adoptive transfer. The results at both time points indicate that suppression was mediated by Thy-1.2⁺, I-J⁺ cells. The afferent phase suppressor cell, however, was Lyt-1⁺, L3T4⁺, while the effector phase suppressor was Lyt-2⁺. A variety of functional T cells in different experimental systems also express idiotypic determinants that are detectable by serologic analysis (24). We have previously observed that nephritogenic effector T cells

TABLE I
Functional Presence of Afferent and Effector Phase Suppressor Cells

Donor cells*	Recipient immunization	DTH Response to SRTA [‡]	
		Afferent (Day 0)	Effector (Day 7)
—	RTA/CFA	17.1 ± 0.6 [§]	15.5 ± 2.4 [§]
Ts	RTA/CFA	4.8 ± 1.0	4.0 ± 0.4
Control	RTA/CFA	17.5 ± 0.8 [§]	16.3 ± 1.2 [§]
—	CFA	4.7 ± 0.4	3.2 ± 0.7
—	—	4.3 ± 0.5	3.6 ± 0.5

* 5×10^7 donor Ts cells from SJL mice primed with SRTA-derivatized lymphocytes were transferred into recipients either at the time of immunization (afferent phase) or 5 d after immunization (effector phase).

[‡] Incremental DTH responses were measured in the recipients in inches $\times 10^{-3} \pm$ SEM. Recipients were challenged for DTH 6 d after donor cell transfer (afferent phase) or 36 h after donor cell transfer (effector phase). DTH responses were recorded 24 h after challenge.

[§] $P < 0.001$ compared with unimmunized controls.

TABLE II
Specificity of Induction and Effector Phase Suppressor Cells

Donor cells*	Recipient immunization	DTH Responses after cell transfer [‡]					
		Induction (day 0)			Effector (day 7)		
		SRTA	SLA	PPD	SRTA	SLA	PPD
—	RTA/CFA	18.0 ± 0.8 [§]	4.8 ± 0.5	18.8 ± 0.8 [§]	17.8 ± 0.6 [§]	5.3 ± 0.6	19.3 ± 0.3 [§]
Ts	RTA/CFA	5.0 ± 1.4	4.0 ± 0.6	18.4 ± 2.3 [§]	4.8 ± 1.4	3.7 ± 1.3	18.0 ± 1.7 [§]
Ts	SLA/CFA	5.1 ± 0.5	16.5 ± 1.2 [§]	16.7 ± 1.5 [§]	—	—	—
Control	RTA/CFA	21.0 ± 2.5 [§]	3.3 ± 0.9	22.0 ± 2.0 [§]	17.0 ± 1.3 [§]	5.3 ± 0.6	23.6 ± 4.2 [§]
—	CFA	3.0 ± 1.0	3.8 ± 0.3	22.0 ± 1.9 [§]	5.0 ± 0.6	5.3 ± 0.3	19.0 ± 0.9 [§]
—	—	4.1 ± 0.6	4.4 ± 0.4	4.0 ± 0.4	5.5 ± 0.3	4.3 ± 0.2	4.8 ± 0.5

* 5×10^7 donor Ts cells from SJL mice primed with SRTA-derivatized lymphocytes were transferred into recipients either at the time of immunization (induction phase) or 5 d after immunization (effector phase).

[‡] Incremental DTH responses were measured in the recipient in inches $\times 10^{-3} \pm$ SEM. Recipients were challenged with soluble antigens to produce DTH 6 d after donor cell transfer (induction phase) or 36 h after transfer (effector phase). DTH responses were recorded 24 h after challenge.

[§] $P < 0.001$ compared with unimmunized controls.

TABLE III
Phenotypic Characterization of Ts Cells

Suppressor cell transfers*		Recipient immunization	DTH Responses to SRTA [‡]	
Donor cells	Treatment		Afferent (Day 0)	Effector (Day 7)
—	—	RTA/CFA	15.0 ± 0.8 [§]	14.0 ± 1.2 [‡]
Ts	—	RTA/CFA	3.0 ± 2.2	5.0 ± 1.0
Ts	C'	RTA/CFA	4.1 ± 0.7	4.8 ± 1.3
Ts	Anti-Thy 1.2 + C'	RTA/CFA	15.3 ± 1.0 [§]	15.5 ± 1.3 [§]
Ts	Anti-Lyt-1.2 + C'	RTA/CFA	15.0 ± 1.1 [§]	4.5 ± 0.6
Ts	Anti-L3T4 + C'	RTA/CFA	17.3 ± 1.1 [§]	6.2 ± 0.9
Ts	Anti-Lyt-2 + C'	RTA/CFA	7.0 ± 0.4	16.0 ± 0.4 [§]
Ts	Anti-J ^h + C'	RTA/CFA	14.8 ± 0.7 [‡]	14.5 ± 0.5 [‡]
Ts	Anti-J ^k + C'	RTA/CFA	4.0 ± 0.5	4.0 ± 0.4
Control	—	RTA/CFA	17.5 ± 1.0 [§]	15.0 ± 0.8 [§]
—	—	CFA	3.5 ± 0.4	4.8 ± 1.0

* 5×10^7 donor Ts cells from SJL mice primed with SRTA-derivatized lymphocytes were transferred into recipients either at the time of immunization (afferent phase) or 5 d after immunization (effector phase). Some donor cells were pretreated with antibody plus C' before transfer.

[‡] Incremental DTH responses were measured in the recipients in inches $\times 10^{-3} \pm$ SEM. Recipients were challenged for DTH 6 d after donor cell transfer (afferent phase) or 36 h after transfer (effector phase). DTH responses were recorded 24 h after challenge.

[§] $P < 0.001$ compared with CFA controls.

[‡] $P < 0.005$ compared with CFA controls.

are RE-Id⁺ lymphocytes (15). We therefore wished to determine if either afferent or effector phase Ts cells express such a specificity. We pretreated donor Ts cells with RE-anti-Id plus C' before adoptive transfer (Table IV). Control normal rabbit serum (NRS) plus C' did not affect suppression on day 0 or day 5. Treatment with RE-anti-Id plus C', however, eliminated suppression on day 0. These findings suggest that only afferent phase Ts cells are RE-Id⁺.

Having demonstrated the existence and effect of these Ts lymphocytes on DTH reactivity towards SRTA, we wished to determine their ability to influence the histologic expression of interstitial nephritis. We have previously (12, 14) observed that the lesions are progressively fatal and that renal function is

TABLE IV
Effect of RE-Anti-Id on Ts Cell Function

Donor cells*	Recipient immunization	Treatment	DTH Response after cell transfer [‡]	
			Induction (day 0)	Effector (day 7)
—	RTA/CFA	—	16.8 ± 1.1 [§]	17.5 ± 0.9 [§]
Ts	RTA/CFA	—	3.7 ± 0.7	5.2 ± 0.8
Ts	RTA/CFA	RE-anti-Id + C'	16.8 ± 1.4 [§]	4.8 ± 0.6
Ts	RTA/CFA	NRS + C'	2.8 ± 1.0	4.7 ± 0.6
Control	RTA/CFA	—	17.5 ± 1.0 [§]	16.7 ± 1.0 [§]
—	CFA	—	4.0 ± 0.4	4.3 ± 0.3

* 2.5×10^7 donor Ts cells (panning-purified T cell fraction) from SJL mice primed with SRTA-derivatized syngeneic lymphocytes were transferred into recipients either on the day of immunization (induction phase) or 5 d after immunization (effector phase).

[‡] Incremental DTH responses were measured in the recipient in inches $\times 10^{-3} \pm$ SEM. Recipients were challenged for DTH 6 d after transfer (induction phase) or 36 h after transfer (effector phase) and the footpad swelling was recorded 24 h later.

[§] $P < 0.001$ compared with CFA controls.

TABLE V
Inhibition of Interstitial Nephritis With Ts Cells

Donor cells*	Treatment [‡]	Recipient immunization	Histology [§]	
			Afferent (day 0)	Effector (day 7)
—	—	RTA/CFA	3.8 ± 0.2 (8)	
Ts	—	RTA/CFA	0.3 ± 0.2 (15) [¶]	1.1 ± 0.4 (10) [¶]
Ts	C'	RTA/CFA	0.1 ± 0.1 (9) [¶]	0.8 ± 0.4 (7) [¶]
Ts	Anti-Thy-1.2 + C'	RTA/CFA	3.3 ± 0.2 (4)	3.1 ± 0.2 (4)
Ts	Anti-I-J ^s + C'	RTA/CFA	3.7 ± 0.3 (3)	3.5 ± 0.2 (3)
Ts	Anti-I-J ^k + C'	RTA/CFA	0.0 ± 0.0 (4) [¶]	0.4 ± 0.2 (6) [¶]
Control	—	RTA/CFA	3.4 ± 0.3 (11)	3.2 ± 0.4 (5)
—	—	CFA	0.0 ± 0.0 (8)	

* Donor Ts cells were harvested from SJL mice 7 d after priming with syngeneic SRTA-derivatized lymphocytes. These donor cells were adoptively transferred (5×10^7 cells per recipient) into SJL mice either at the time of immunization (afferent phase) or 5 d after immunization (effector phase).

[‡] Some donor cells were pretreated with antibody plus C' before transfer.

[§] Recipients were sacrificed 10 wk after immunization and their kidneys were histologically graded (0 to 4) and reported as mean \pm SEM (n).

[¶] $P < 0.001$ compared with control.

significantly decreased by 16 wk. In Table V, it can be observed that donor Ts cells from mice primed with SRTA-derived lymphocytes almost completely blocked the development of disease when they were given at the time of recipient immunization (afferent phase). Substantial inhibition also occurred when these cells were transferred 7 d after immunization (effector phase). This suppressive effect was largely abrogated if the donor cells were pretreated with anti-Thy-1.2 or anti-I-J^s antibodies plus C'. Direct immunofluorescence for kidney-bound anti-TBM Ab appeared equally strong in groups with interstitial nephritis and those protected with adoptively transferred suppressor cells; by quantitative radioimmunoassay a 17% reduction in serum anti-TBM Ab was observed in the group receiving afferent Ts cells (data not shown).

Binding Reactivity of Afferent and Effector Phase Ts Cells. One of the interesting characteristics of Ts cells, unlike other functional T cell subpopulations, is their ability to bind ligand supports in vitro (6, 25–28). We took advantage of this to examine the binding specificity of afferent and effector phase Ts cells from mice primed with SRTA-derivatized lymphocytes. Donor Ts cells were panned on SRTA-coated culture dishes (Table VI). We observed after separation that as little as 2×10^6 SRTA-binding Ts cells inhibited a DTH response to SRTA, but only in the afferent phase. The separation was largely complete, since 2.5×10^7 SRTA-nonadherent cells inhibited the DTH response only during the effector phase. The specificity of the SRTA-binding Ts cells was also suggested by their failure to suppress the DTH reaction to PPD. These cells represented ~2–4% of the total starting donor spleen cell number.

Similar experiments were also performed with donor Ts cells panned to select for RE-Id-binding lymphocytes. We observed that 3×10^6 RE-Id-binding cells inhibited a DTH response to SRTA only in the effector phase (Table VII). 2.5×10^7 RE-Id-nonadherent T cells were not able to suppress DTH under the same conditions, but did inhibit DTH during the afferent phase. As above, the specificity of this suppression was suggested by the failure of RE-Id-binding cells to inhibit DTH to PPD.

The results obtained to this point suggest that donor spleen cells, 7 d after priming with SRTA-derivatized lymphocytes, contain at least two general pop-

TABLE VI
Inhibitory Effect of SRTA-binding Ts Cells

Suppressor cells* transferred	Dose ($\times 10^7$)	Recipient immuni- zation	DTH Responses after cell transfer [‡]			
			Induction (day 0)		Effector (day 7)	
			SRTA	PPD	SRTA	PPD
—	—	RTA/CFA	17.3 \pm 1.3 [§]	16.3 \pm 2.4 [§]	18.5 \pm 0.9 [§]	19.0 \pm 0.6 [§]
Unfractionated cells	5.0	RTA/CFA	3.8 \pm 2.0	15.0 \pm 0.8 [§]	5.3 \pm 1.2	20.8 \pm 2.2 [§]
Ig plate-adherent B cells	2.5	RTA/CFA	15.8 \pm 0.3 [§]	15.8 \pm 0.3 [§]	18.5 \pm 0.9 [§]	19.3 \pm 0.3 [§]
Ig plate-nonadher- ent T cells	2.5	RTA/CFA	5.0 \pm 0.4	15.3 \pm 0.3 [§]	6.0 \pm 0.4	18.8 \pm 1.0 [§]
SRTA-adherent T cells	0.2	RTA/CFA	3.5 \pm 0.6	14.2 \pm 1.3 [‡]	21.0 \pm 1.1 [§]	21.8 \pm 0.8 [§]
SRTA-nonadherent T cells	0.4	RTA/CFA	15.5 \pm 1.4 [§]	14.5 \pm 1.2 [‡]	18.7 \pm 1.3 [§]	21.0 \pm 1.8 [§]
SRTA-nonadherent T cells	2.5	RTA/CFA	16.3 \pm 0.5 [§]	16.5 \pm 0.3 [§]	6.1 \pm 0.7	18.2 \pm 0.3 [§]
Control cells	5.0	RTA/CFA	17.7 \pm 0.9 [§]	15.3 \pm 0.6 [§]	18.2 \pm 0.7 [§]	19.0 \pm 0.2 [§]
—	—	—	3.8 \pm 0.3	4.5 \pm 2.0	4.1 \pm 0.7	3.9 \pm 0.4

* Donor cells from SJL mice primed with SRTA-derivatized lymphocytes were separated on anti-mouse Ig panning dishes and the nonadherent T cells were then placed on SRTA-coated panning dishes. Each of the resulting cell fractions were transferred into recipients either at the time of immunization (induction phase) or 5 d after immunization (effector phase).

[‡] Incremental DTH responses were measured in the recipients in inches $\times 10^{-5} \pm$ SEM. Recipients were challenged for DTH 6 d after donor cell transfer (induction phase) or 36 h after transfer (effector phase). DTH responses were recorded 24 h after challenge.

[§] $P < 0.001$ compared with unimmunized controls.

[‡] $P < 0.005$ compared with unimmunized controls.

TABLE VII
Inhibitory Effect of RE-Id-binding Ts Cells

Donor cells*	Dose ($\times 10^7$)	Recipient immuniza- tion	DTH response after cell transfer [‡]			
			Afferent (day 0)		Effector (day 7)	
			SRTA	PPD	SRTA	PPD
—	—	RTA/CFA	17.3 \pm 1.2 [§]	16.6 \pm 0.3	17.3 \pm 0.3 [§]	16.0 \pm 0.6
Unfractionated cells	5.0	RTA/CFA	4.6 \pm 0.7	17.2 \pm 0.7	4.0 \pm 1.0	16.8 \pm 0.6
Ig-plate-adherent B cells	2.5	RTA/CFA	18.3 \pm 0.9 [§]	16.8 \pm 0.3	16.6 \pm 0.3 [§]	15.6 \pm 0.3
Ig-plate-nonadherent T cells	2.5	RTA/CFA	6.5 \pm 1.2	17.8 \pm 0.5	5.0 \pm 0.6	17.6 \pm 0.3
RE-Id-adherent T cells	0.3	RTA/CFA	19.6 \pm 1.9 [§]	17.3 \pm 1.0	4.3 \pm 1.2	17.2 \pm 1.8
RE-Id-nonadherent T cells	0.3	RTA/CFA	19.0 \pm 2.0 [§]	16.8 \pm 0.4	16.6 \pm 0.9 [§]	15.0 \pm 0.6
RE-Id-nonadherent T cells	2.5	RTA/CFA	5.4 \pm 0.8	17.2 \pm 0.9	16.3 \pm 0.8 [§]	16.3 \pm 0.9
Control cells	5.0	RTA/CFA	18.6 \pm 1.0 [§]	17.3 \pm 1.4	19.3 \pm 0.6 [§]	17.3 \pm 0.4
—	—	CFA	4.8 \pm 0.4	18.3 \pm 0.3	5.0 \pm 0.6	18.0 \pm 1.1

* Donor cells from SJL mice primed with SRTA-derivatized lymphocytes were separated on anti-mouse Ig panning dishes and the nonadherent cells were then incubated with RE-Id Ab for 30 min. After washing, a second panning was performed on anti-mouse IgG dishes. Each of the resulting cell fractions was transferred into recipients either at the time of immunization (afferent phase) or 5 d after immunization (effector phase).

[‡] Incremental DTH responses were measured in the recipients in inches $\times 10^{-3} \pm$ SEM. Recipients were challenged for DTH 6 d after donor cell transfer (afferent phase) or 36 h after transfer (effector phase). DTH responses were recorded 24 h after challenge.

[§] $P < 0.001$ compared with CFA controls.

ulations of suppressor cells: (a) L3T4⁺, I-J⁺, RE-Id⁺, SRTA-binding suppressor cells that only operate at the time of immunization with RTA in CFA (afferent phase) and will be referred to as Ts-1 cells, and (b) Lyt-2⁺, I-J⁺, RE-Id-binding suppressor cells that only operate after immunization (effector phase) and will be referred to as Ts-2 cells.

Ts-2 Cells Do Not Induce Auxiliary Ts-3 Cells in Immunized Recipients. An auxiliary suppressor lymphocyte, or Ts-3 cell, has also been described in a variety of other suppressor cell systems (1, 3). This Ts-3 cell has typically been characterized as I-J⁺ and cyclophosphamide (CTX) sensitive. It or its precursor appears in the antigen-immunized host by 5 d, but the Ts-3 cell is only fully activated by the addition of Ts-2 lymphocytes. The Ts-3 effect is nonspecific and is thought to be a final effector cell in some suppressor networks (1, 3). We wished to determine if Ts-3 cells were also present in our system. Ts-2 cells were transferred into recipients immunized with tubular antigen, some of which had been treated with CTX 24 h after immunization (17). Effector phase suppression of DTH was measured on day 7. The results (Table VIII) indicate that CTX treatment of the recipient did not eliminate the ability of Ts-2 cells to suppress. In another experiment, DTH-reactive lymphocytes pretreated with anti-I-J⁺ plus C' were co-transferred into naive recipients with Ts-2 cells (17). Effector phase DTH under these conditions was also still inhibited compared with I-J allele controls

TABLE VIII
Failure to Observe CTX-sensitive Ts-3 Cells

Donor cells*	Dose ($\times 10^6$)	Recipient immunization	DTH Response to SRTA [§]
—	—	RTA	20.0 \pm 0.6
		RTA/CTX [‡]	22.6 \pm 1.8
RE-Id Adherent (Ts-2)	0.8	RTA	11.3 \pm 1.7 [¶]
		RTA/CTX	11.6 \pm 1.9 [¶]
RE-Id Nonadherent (non-Ts-2)	4.0	RTA	26.0 \pm 2.1
		RTA/CTX	25.6 \pm 0.9
Control [‡]	4.0	RTA	23.7 \pm 0.6
		RTA/CTX	27.6 \pm 1.4
—	—	CFA	9.3 \pm 1.8
		CFA/CTX	10.0 \pm 0.5

* Donor cells separated into subpopulations were adoptively transferred into syngeneic recipients 5 d after immunization (effector phase).

[‡] Some recipients were given CTX (30 mg/kg) 24 h after immunization.

[§] Incremental DTH responses were measured in the recipient in inches $\times 10^{-3}$ \pm SEM. Recipients were challenged for DTH 36 h after transfer and the footpad swelling was recorded 24 h later.

[¶] Controls are a pooled average from all groups.

[¶] $P < 0.005$ compared to controls.

(data not shown). From these two experiments we were not able to identify the functional presence of Ts-3 cells in our system.

Ts-1 Cells Induce Ts-2 Lymphocytes. From the previous experiments it would seem that Ts-2 cells are the final effector suppressor cell in the network described in this report. Because Ts-1 cells operate earlier in the suppressive scheme and share complementary determinants with Ts-2, we wished to determine if Ts-1 cells would induce the Ts-2 repertoire. 3×10^6 syngeneic Ts-1 cells were cotransferred into naive recipients with or without SRTA (100 μ g). Control cells were normal lymphocytes because the non-SRTA-binding lymphocytes from the Ts-1 preparation contained Ts-2 cells. Only the donor cells harvested from mice primed with Ts-1 cells plus SRTA were able to mediate effector phase (Ts-2) suppression (Table IX). This induction of Ts-2 was not only antigen dependent, but also antigen specific, as Ts-2 cells were not induced after priming with Ts-1 plus SLA. Thus it seems that Ts-1 lymphocytes can induce Ts-2 cells but that this process requires tubular antigen.

Genetic Restriction Requirements for the Ts-2 Suppressive Effect. A genetic analysis of the Igh-1 and H-2 restrictions imposed on the Ts-2 suppressor response was also performed in H-2^s and H-2^d recombinant and congenic mice. Ts-2 cells from SJA (Igh-1^a) and SJL (Igh-1^b) mice primed with syngeneic, SRTA-derivatized lymphocytes were transferred into other immune H-2 mice of different allotype to assess the Igh-1 requirement for afferent and effector phase suppression. Transfers within H-2^s mice all produced afferent phase suppression of DTH to SRTA (Table X). When compared with controls, these results suggest that Igh-1 differences between Ts-1 cells and their recipient do not influence the ability to mediate afferent phase suppression. Transfer into B10.A (H-2^a; Igh-1^b) mice, however, did not produce suppression, indicating that H-2 compatibility might be more important. Using the same recombinant mice to examine effector

TABLE IX
Induction of Ts2 Suppressor Lymphocytes by Ts1 Cells

Donor cells harvested from mice*	Recipient immunization	Recipient DTH response to SRTA [‡]
—	RTA/CFA	21.2 ± 2.0 [§]
Primed with SRTA-binding Ts-1 cells + SRTA	RTA/CFA	7.5 ± 0.9
Primed with SRTA-binding Ts-1 cells	RTA/CFA	23.0 ± 3.6 [§]
Primed with SRTA-binding Ts-1 cells + SLA	RTA/CFA	21.3 ± 1.7 [§]
Primed with control cells + SRTA	RTA/CFA	23.8 ± 2.1 [§]
Primed with control cells	RTA/CFA	21.3 ± 1.4 [§]
—	CFA	4.3 ± 2.6

* Naive SJL mice were intravenously primed with 3×10^6 syngeneic Ts-1 or control cells with or without cotransfer of 100 μ g of SRTA or SLA. 7 d later the spleen cells from these primed recipients were transferred into SJL mice previously immunized 5 d earlier.

[‡] 36 h after transfer the recipients were footpad challenged (effector phase suppression) and an incremental footpad response was measured 24 h later in inches $\times 10^{-3} \pm$ SEM.

[§] $P < 0.001$ compared with CFA controls.

phase suppression, we observed that the Ts-2 effect could not be transferred across allotype differences, suggesting that Ts-2 cells are Igh-1 restricted. We examined this latter idea more closely using BALB/c mice congenic in Igh-1. Ts cells from BALB/c and C.B-20 mice primed with syngeneic, SRTA-derivatized lymphocytes were transferred into immune BALB/c (Igh-C^a/V^a), C.B-20 (Igh-C^b/V^b), and BAB/14 (Igh-C^b/V^a) recipients to measure Ts-2 effector phase suppression. Ts-2 suppression only occurred when Ts cells and the recipient shared homologous Igh-V determinants (Table XI). As before, Ts-2 suppression was not demonstrable across a complete H-2 difference.

Since Ts-2 suppression seems to be the distal and final effector phase process in this suppressor network, we also wished to determine the H-2 restriction on this suppressive event. Ts cells from SJL and B10.S(9R) mice primed with SRTA-derivatized lymphocytes were transferred into a variety of immune recombinant, Igh-1-identical recipients to measure effector phase suppression. Ts cells from SJL mice suppressed the DTH response to SRTA in B10.HTT and B10.S(8R) recipients (Table XII), but not in B10.S(9R), suggesting that functional homology at I-J^e is required for suppression. This restriction was confirmed by the observation that Ts cells from B10.S(9R) donors suppressed in B10.S(9R) recipients but not in B10.HTT. The lack of suppression in a particular donor-recipient combination was not simply due to acute rejection, since Ts cells from (SJL \times B10.A)_{F1} hybrid donors can mediate effector phase suppression in immune SJL recipients (data not shown).

Discussion

We have been interested in developing immunologic strategies to alter the natural history of immune-mediated renal disease (18, 29, 30). The present

TABLE X
Igh-1 Restriction of Ts Cells

Recipients			Cells transferred*	DTH Response to SRTA [‡]	
Strain	H-2	Igh-1		Day 0	Day 7
SJL	s	b	SJA/20 Ts	5.5 ± 0.2 [§]	18.3 ± 0.5
			SJL Ts	3.6 ± 0.7 [§]	5.0 ± 2.4 [§]
			Control	16.0 ± 0.8	19.5 ± 0.9
SJA/20	s	a	SJA/20 Ts	3.0 ± 2.3 [§]	6.5 ± 2.6 [§]
			SJL Ts	3.3 ± 0.6 [§]	19.7 ± 7.5
			Control	18.5 ± 4.3	17.0 ± 1.5
B10.S	s	b	SJA/20 Ts	5.3 ± 0.9 [§]	15.7 ± 0.7
			SJL Ts	5.0 ± 0.7 [§]	5.3 ± 1.0 [§]
			Control	16.1 ± 1.0	19.6 ± 1.3
A.SW	s	e	SJA/20 Ts	5.3 ± 0.5 [§]	17.3 ± 0.5
			SJL Ts	4.0 ± 0.4 [§]	17.0 ± 0.7
			Control	15.6 ± 0.5	16.5 ± 0.8
B10.A	a	b	SJA/20 Ts	18.8 ± 0.5	18.2 ± 1.3
			SJL Ts	21.5 ± 3.5	17.2 ± 0.2
			Control	20.9 ± 1.1	16.4 ± 0.7

* 5×10^7 donor cells from SJL or SJA mice primed with SRTA-derivatized syngeneic lymphocytes were transferred into Igh-1-similar or -disparate recipients at the time of immunization with RTA/CFA (afferent phase) or 5 d after immunization (effector phase).

[‡] Incremental DTH responses were measured in the recipient in inches $\times 10^{-3} \pm$ SEM. Recipients were challenged for DTH 6 d after transfer (afferent phase) or 36 h after transfer (effector phase) and the footpad swelling was recorded 24 h later. Controls are a pooled average from all groups.

[§] $P < 0.001$ compared with controls.

series of experiments examine the interactions and effect of a tubular antigen-induced Ts cell network that can specifically inhibit the expression of nephritogenic effector T lymphocytes producing interstitial nephritis and DTH to tubular antigen (15). Our findings indicate that at least two subpopulations of Ts cells are involved in this network, and that after 7 d both can be found in the spleens of recipients primed with tubular antigen-derivatized cells. The first subset of suppressor cells, Ts-1, is Thy-1.2⁺, L3T4⁺, and RE-Id⁺ (polymorphic idotype). These cells are tubular antigen-binding and only operate, after adoptive transfer, if they are present at the time of immunization (afferent phase suppression). In the presence of tubular antigen, Ts-1 cells also specifically induce a second subpopulation of Ts cells, Ts-2. Ts-2 cells are Thy-1.2⁺, Lyt-2⁺, and I-J⁺. They are RE-Id binding (antiidiotypic), only operate in the effector phase of the nephritogenic immune response, and are I-J and Igh-V restricted. In two additional experiments we found no evidence for the presence of a Ts-3 or auxiliary subset of suppressor cells (17).

While our suppressor network bears some resemblance to other hapten-reactive Ts cell systems induced by derivatized lymphocytes (1, 3, 7), we also observed several findings that distinguish it from these other models. Unlike the

TABLE XI
Igh-V Restriction of Ts2 Cells

Strain	Recipients			Cells transferred*	DTH Response to SRTA [‡]
	H-2	Igh-C _H	Igh-V _H		
BALB/c	d	a	a	BALB/c Ts	5.8 ± 0.8 [§]
				C.B-20 Ts	17.5 ± 2.5
				Control	15.5 ± 1.4
C.B-20	d	b	b	BALB/c Ts	17.8 ± 1.0
				C.B-20 Ts	6.8 ± 1.1 [§]
				Control	15.3 ± 0.3
BAB/14	d	b	a	BALB/c Ts	6.0 ± 1.4 [§]
				C.B-20 Ts	17.4 ± 1.6
				Control	15.8 ± 0.9
BALB.K	k	a	a	BALB/c Ts	23.0 ± 1.9
				C.B-20 Ts	19.5 ± 0.6
				Control	20.4 ± 0.8

* 5×10^7 donor cells from BALB/c or C.B-20 mice primed with SRTA-derivatized syngeneic lymphocytes were transferred into Igh-1-similar or -disparate recipients 5 d after immunization with RTA/CFA (effector phase).

[‡] Incremental DTH responses were measured in the recipient in inches $\times 10^{-3} \pm$ SEM. Recipients were challenged for DTH 36 h after transfer and the footpad swelling was recorded 24 h later. Controls are a pooled average from all groups.

[§] $P < 0.001$ compared with controls.

TABLE XII
H-2 Restriction of Effector Phase Ts-2 Cells

Strain	Recipients*							DTH Response to SRTA after suppressor cell transfer [‡]	
	H-2 Haplotype							SJL	B10.S(9R)
	K	A _β	A _α	E _β	J	E _α	D		
SJL	s	s	s	s	s	s	s	5.3 ± 0.8 [§]	—
B10.S	s	s	s	s	s	s	s	5.5 ± 1.0 [§]	18.0 ± 0.7
B10.HTT	s	s	s	s	s	k	d	5.8 ± 0.9 [§]	19.5 ± 1.4
B10.S(9R)	s	s	s	s	k	k	d	17.3 ± 1.1	4.0 ± 0.9 [§]
B10.S(8R)	k	k	k	k	s	s	s	5.8 ± 0.6 [§]	—
B10.A(5R)	b	b	b	b	k	k	d	—	4.7 ± 0.3 [§]
B10.A	k	k	k	k	k	k	d	18.3 ± 1.3	5.0 ± 0.8 [§]

* 5×10^7 donor cells from SJL or B10.S(9R) mice primed with SRTA-derivatized syngeneic lymphocytes were transferred into H-2-similar or -disparate recipients 5 d after immunization with RTA/CFA (effector phase).

[‡] Incremental DTH responses were measured in the recipient in inches $\times 10^{-3} \pm$ SEM. Recipients were challenged for DTH 36 h after transfer and the footpad swelling was recorded 24 h later. Average DTH response from all groups receiving control cells was 18.3 ± 0.5 .

[§] $P < 0.001$ compared with controls.

azobenzeneuronate (ABA) and 3-nitro-4-hydroxyphenyl acetyl (NP) systems (1, 3), the nephritogenic effector T cells producing DTH and interstitial nephritis are RE-Id⁺ (15) and, therefore, share complementary specificities with Ts-2 effector phase suppressor cells. Under these conditions there might be no

theoretical necessity for a Ts-3 or auxiliary cell to co-mediate effector phase suppressive events. In support of this view we also did not find a CTX-sensitive, I-J⁺ Ts-3 cell (17). This finding must be interpreted cautiously, however, as it is a negative result, and all Ts-3 cells may not have the same sensitivity to CTX or anti-I-J plus C'. The Ts-2 suppression in our model, unlike the ABA and NP systems (1, 3), also did not produce a nonspecific or bystander suppression (Table VI). Such specificity of the antiidiotypic Ts-2 cell suggests that additional interactions with TACC cells are also unlikely (1). The precise interactions with the target cell of Ts-2 suppression, however, have not been formally defined (work in progress), so additional contributions from other lymphocytes cannot be totally excluded at this time (see below). It is also interesting to note that the amount of anti-TBM Ab produced was only slightly affected by Ts-1 suppression, and not by Ts-2 suppression (data not shown). We are currently trying to determine whether the production of anti-TBM Ab in disease-protected mice is less over a longer period of time, is of different affinity, or reflects a phenotypic shift in representation of idiotypes that were not defined by RE-anti-Id specificities.

We also observed that the Ts-2 effect on DTH to tubular antigen was genetically restricted by I-J and Igh-V determinants. A role for I-J gene products is consistent with the ABA and NP systems (1, 3), but I-A and K/D restrictions have also been reported in other models (31–32). Although a more complete definition of I-J gene products continues to evolve (1, 34, 35), their serologic presence and functional effect were clearly noted in the current study (Tables III, V, and XII). The target of the I-J restricted Ts-2 cell has yet to be established. We suspect, however, that the DTH-reactive, RE-Id⁺ cell is not I-J⁺ (work in progress), so the Ts-2 suppressive effect, in spite of our failure to implicate a Ts-3 cell (see above), may involve more than one cell-mediated interaction or cellular target (36). The Igh-V restriction of the antiidiotypic Ts-2 cell was also of interest (1, 3). We have previously observed (15) that the expression of RE-Id by nephritogenic T cells is co-defined by an interactive effect between H-2K and Igh-1 gene products in disease-susceptible mice. Antiidiotypic (RE-Id-binding) Ts-2 cells from SJL mice, in turn, can be isolated *in vitro* using either RE-Id from immune SJL (Igh-1^b) or SJA/20 (Igh-1^a) donors, so that direct binding to RE-Id by T-2 cells does not seem to depend on heavy chain or minor light chain differences. The functional effect of Ts-2 cells from SJL mice selected by RE-Id from SJA/20 is only observed, however, when such Ts-2 cells are transferred into immune SJL, as compared with SJA recipients (data not shown). This confirms that a unique non-MHC restriction determinant on chromosome 12, perhaps Igh-V (Table XI), cofunctions with I-J to facilitate the Ts-2 effect. We are presently attempting to serologically characterize this Igh-V gene product.

The induction of Ts-2 cells by purified Ts-1 lymphocytes in our model specifically requires tubular antigen. This antigen effect can be observed when used in soluble form (Table IX) or when covalently linked to derivatized lymphocytes (Table I). In contrast to the ABA and NP systems (1, 3), this requirement for antigen is supported by similar observations with L-glutamic acid-L-alanine-L-tyrosine (GAT) and trinitrophenyl (TNP) models of suppression (4, 7). The Ts-2 cells in the GAT and TNP systems, however, are antigen specific, while Ts-2 cells in the ABA and NP systems are idiosyncratic. It has

been recently proposed (7) that immune networks which provide a major idiotype produce antiidiotypic Ts-2 cells, whereas those which do not express a major or crossreactive idiotype, like GAT and TNP, express Ts-2 cells with antigen specificity (7). While we do not know yet whether the interstitial nephritis model produces a major idiotype, it is clear that the idiotypic determinants defined by RE-anti-Id antibodies are well represented on both Lyt-2⁺ nephritogenic T lymphocytes (15) and on Ts-1 cells (Table IV). Our data also indicate that only in the presence of tubular antigen can such a regulatory and protective antiidiotypic Ts-2 effect be predictably activated. Thus, the requirement for antigen in Ts-2 induction may operate under, yet to be defined rules, apart from the presence or absence of a major idiotype on Ts-1 cells.

Finally, in preliminary studies, we have observed that the Ts cell effect in this model of tubulointerstitial disease can be mediated by soluble suppressor factors (work in progress); additional biochemical analyses should clarify and enhance the present observations.

Summary

The nephritogenic effector T cell response producing interstitial nephritis in mice can be largely inhibited by the adoptive transfer of suppressor T cells before or after the induction of disease. These suppressor T cells are harvested from donor mice primed with tubular antigen-derivatized syngeneic lymphocytes, and two subsets of suppressor cells can be characterized within this donor cell population. The first suppressor cell in this network is an L3T4⁺, I-J⁺, RE-Id⁺ cell (Ts-1). Ts-1 cells are antigen-binding suppressor cells that inhibit afferent phase immune responses and, in the presence of tubular antigen, specifically induce Lyt-2⁺, I-J⁺ cells (Ts-2) that are antiidiotypic (RE-Id-binding) suppressors. The Ts-2 cell is functionally restricted in its suppressive effect by I-J and Igh-V gene products, and acts on the effector limb of the cell-mediated anti-tubular basement membrane immune response. These studies provide an experimental basis for further efforts to use immunoregulatory modulation in the control of autoimmune renal disease.

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