MHC-RESTRICTED MINIMAL REGULATORY CIRCUIT INITIATED BY A CLASS II–AUTOREACTIVE T CELL CLONE

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The concept of an immune circuit is based on the fact that an antigenic input induces a train process where functionally different subsets of T cells are sequentially activated to produce an output that neutralizes the initial input (1). Because of the existence of such a regulatory circuit, the natural steady state of the immune response is maintained even with the continuous exposure to external and internal antigenic stimuli. Such a circuit should be preprogrammed during the early differentiational history of T cells.

A number of theoretical and experimental models have been proposed (1–6), yet several points still remain that await critical examination. One of the major issues in the circuit concept is how the mobile cells can find the appropriate partner cells for meaningful and unmistakable communications. It has been recognized that the polymorphisms of the MHC and Ig heavy chain variable region (IgV_H) restrict the interactions among functionally different subsets of T cells. It has often been wondered how some of the T–T cell interactions in the circuit are restricted by MHC genes although the majority of murine T cells do not express class II antigens.

Taking advantage of a class II–autoreactive Th clone, we have been successful in demonstrating an MHC-restricted minimal regulatory circuit. This Th clone with L3T4 and I-J^k markers was able to activate an MHC-restricted Ts cell in vivo, which, in turn, suppressed heterogeneous Th cells, provided that the latter had the same MHC-restriction specificity as that possessed by the initial Th clone. This paper describes the MHC-restricted minimal regulatory circuit visualized with this cloned Th cell line.

Materials and Methods

Animals. Female C3H/HeN, C57BL/6 (B6), (C57BL/6 × C3H)F$_1$ (B6C3F$_1$), C57BL/10 (B10), and B10BR mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan. C3H.SW, B10.A, B10.A(4R), B10.A(5R), and B10.MBR mice were raised in our animal facility.

Antigens. KLH was purchased from Calbiochem-Behring Corp., San Diego, CA. Fowl

gamma-globulin (FGG)[1] was prepared from chicken serum by ammonium sulfate precipitation. BSA was obtained from Sigma Chemical Co., St. Louis, MO. These proteins were coupled with DNP groups by the method of Eisen et al. (7). The degrees of substitution were DNP_{580}-KLH, DNP_{20}-FGG and DNP_{11}-BSA. *Bordetella pertussis* vaccine (BPV) was purchased from Chiba Serum Institute, Chiba, Japan.

Derivation of an Autoreactive Th Clone (MS202). An autoreactive Th clone of C3H origin (MS202) was established as follows: Splenic T cells of KLH-primed C3H mice were stimulated weekly with KLH and syngeneic APC in the presence of IL-2-containing medium (10% rat Con A–stimulated supernatant). T cell clones were established by limiting dilution. Some of the clones were shown to be self–class II–reactive, as the stimulation with syngeneic irradiated spleen cells without antigen was sufficient to maintain the IL-2-dependent proliferation. A clone MS202 could induce both the proliferation and Ig secretion of syngeneic B cells without antigen (see below). The clone has been maintained in our laboratory for >2 yr with occasional stimulations with autologous APC without antigen followed by propagation with IL-2-containing medium. The specificity of this clone was anti-I-A^k, as determined by the stimulation with spleen cells from various intra-H-2-recombinant strains. The surface phenotype of MS202 was $L3T4^+$, $Ly-2^-$, and I-J$^+$. Detailed properties of this clone will be described elsewhere (Fujisawa, I., K. Sano, R. Abe, Y. Asano, and T. Tada, manuscript in preparation).

Antibodies. Anti-Thy-1 (30-H12) (8) and anti-L3T4 (GK1.5) (9) mAbs were kindly provided by L. A. Herzenberg (Stanford University, Stanford, CA) and F. W. Fitch (University of Chicago, Chicago, IL), respectively. Anti-Ly-1.1 and anti-Ly-2.1 mAbs were purchased from Cedarlane Laboratories, Ltd., Ontario, Canada. Polyclonal anti-Ia^k and anti-I-J^k antisera were prepared in our laboratory by reciprocal immunization of A.TH and A.TL, and B10.A(3R) and B10.A(5R). Anti-I-J^k mAbs (1G8, KN34, and JK10-23) were those established in our laboratory as described elsewhere (10; Asano, Y., T. Nakayama, M. Kubo, J. Yagi, and T. Tada, manuscript in preparation). Polyclonal anti–mouse Ig (MIg) antibodies were obtained by repeated immunizations of goats with MIg prepared from normal mouse serum (NMS), and were purified by affinity chromatography with MIg-conjugated Sepharose beads.

Primed Lymphoid Cells. Mice were immunized intraperitoneally with 100 µg DNP-KLH or DNP-FGG in 4 mg of aluminium hydroxide gel (alum) and 10^9 BPV. They were used as the sources of DNP-KLH- or DNP-FGG-primed spleen cells and DNP-primed B cells. DNP-primed B cells were prepared from DNP-KLH-primed spleen cells by the treatment with rabbit antiserum against the mouse brain–associated T cell antigen (anti-BAT) and C. KLH-primed T cells were obtained from mice immunized intraperitoneally with KLH and 10^9 BPV 4–8 wk previously. T cells were separated by adsorption of B cells to anti-MIg-coated plastic dishes (Fisher Scientific Co., Springfield, NJ). The nonadherent cells containing >95% of Thy-1$^+$ cells were used as either helper or regulatory T cells.

Induction of Ts Cells by MS202. C3H or B6C3F$_1$ mice were injected intraperitoneally with 10^7 MS202 twice at an interval of 2 wk. T cells from the mice were obtained 2 wk after the second injection of MS202 by incubating with anti-MIg-coated dishes.

In Vitro Antibody Responses. DNP-KLH- or DNP-FGG-primed spleen cells (5 × 10^5/well) or a mixture of DNP-primed B cells (5 × 10^5/well) and KLH-primed Th cells (2 × 10^5/well) were cultured in the presence of the titrated optimal doses of relevant antigens (0.05 µg/ml of DNP-KLH or 0.1 µg/ml of DNP-FGG) in 200-µl round-bottomed microculture wells (No. 25850; Corning Glass Works, Corning, NY) in 5% CO_2. Culture medium used was RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% FCS, 2 mM L-glutamine, and 5 × 10^{-5} M 2-ME. 4 d after the initiation of the culture, 150 µl of culture supernatant in each well was replaced with fresh medium. The culture was maintained for an additional 3 d. Anti-DNP antibody in the culture supernatant was determined by ELISA (11) as described below.

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[1] *Abbreviations used in this paper:* alum, aluminium hydroxide gel; anti-BAT, rabbit antiserum against the mouse brain–associated T cell antigen; BPV, *Bordetella pertussis* vaccine; FGG, fowl gamma-globulin; MIg, mouse Ig; NMS, normal mouse serum.
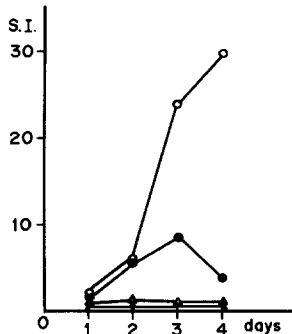
FIGURE 1. Proliferative response of syngeneic but not allogeneic T and B cells to MS202. 2 × 10⁵ B (●, ▲) or T cells (O, △) of C3H (O, ●) or B6 (△, ▲) mice were cultured with 10⁴ irradiated MS202. Cells were harvested different times after the onset of the culture. Proliferative response was assessed by [³H]TdR uptake pulsed for the last 14 h.

Enzyme-linked Immunosorbent Assay (ELISA). A 96-well flexible polyvinyl chloride microtiter plate (Dynatech Laboratories, Inc., Alexandria, VA) was coated with 50 μl of 100 μg/ml DNP-BSA at 4°C overnight. The wells were blocked with 100 μl of 5% horse serum in PBS. 40 μl of culture supernatants or appropriately diluted sera were added to each well and incubated for 1 h at room temperature. Unbound materials were washed thoroughly with PBS. Plates were reacted with peroxidase-labeled rabbit anti-MIg, followed by the reaction with 0.1% orthophenylenediamine in 0.1 M citrate buffer (pH 5.0). The amount of anti-MIg bound was measured by the colorimetoric analysis using a Microelisa Automatic Reader (Corona Electric Co., Ibaraki, Japan). The result was converted into the antibody concentration (nanograms per milliliter) using the titration curve of standardized anti-DNP antibodies. All points shown in this report were expressed as the mean concentration of anti-DNP antibody (nanograms per milliliter) ± SEM of three to five identical wells.

In Vitro Proliferative Responses. Normal B cells were prepared by treating unprimed spleen cells with anti-BAT and C. T cells were enriched by passing spleen cells through a nylon wool column (Leuk-Pak; Fenwall Laboratories, Morton Grove, IL). 2 × 10⁵ B or T cells were cultured with 10⁴ irradiated (2,000 rad) MS202 in 96-well flat-bottomed microculture wells (No. 3072; Falcon Labware, Oxnard, CA). Cells were harvested different times after the onset of the culture. Proliferative response was assessed by [³H]TdR uptake pulsed for the last 14 h.

Radiation Bone Marrow Chimeras. Radiation bone marrow chimeras were prepared by transferring 15 × 10⁶ T cell–depleted bone marrow cells from the donor into lethally irradiated (950 rad) recipients. They are designated as bone marrow donor → irradiated recipient. Chimeric mice were immunized with antigen >8 wk after the irradiation and reconstitution.

Results

Self Class II Reactivity of T Cell Clone MS202. MS202 has been maintained by occasional stimulations with syngeneic APC followed by the propagation with IL-2. Antigen was not needed to maintain the clone. MS202 was found to have a strong stimulatory activity for both syngeneic B and T cells in the absence of antigen (Fig. 1). When B cells were mixed with irradiated MS202 (2,000 rad), both [³H]TdR uptake and Ig secretion were observed. The same irradiated MS202 induced a strikingly high proliferative response of syngeneic T cells, which often showed the stimulation index of 30. No stimulatory activity for allogeneic B or T cells was observed (Fig. 1), indicating that the activation of both T and B cells by MS202 is H-2-restricted. By the use of intra-H-2-recombinant strains, the restricting element to induce T and B cell proliferation was found to be associated with A^k allele (Table I).

TABLE I

I-A^k-restricted Proliferation of B and T Cells by MS202

Responder	H-2 identity	[³H]TdR uptake (Δ cpm)	
		B cells	T cells
C3H	K,A,E,D	65,723	39,217
C3H.SW	None	2,178	1,753
B10.A	K,A,E	52,598	20,088
B10.A(4R)	K,A	50,237	12,380
B10.A(5R)	E	347	291
B10	None	917	236
B10.MBR	A,E	36,745	24,169

2×10^5 B or T cells were cultured with 10^4 irradiated (2,000 rad) MS202 for 3 d. Proliferative response was assessed by [³H]TdR uptake pulsed for the last 14 h.
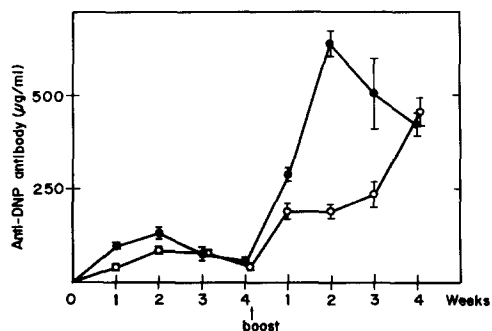

FIGURE 2. Suppression of the in vivo anti-DNP response by MS202. C3H mice were immunized intraperitoneally with 100 μg DNP-KLH and 10^9 BPV, either with (○) or without (●) the intraperitoneal injections of 10^7 of the autoreactive Th cell clone MS202 twice at an interval of 4 wk. The anti-DNP antibody in the serum was determined weekly by ELISA. Each point represents a mean anti-DNP antibody response calculated from five similarly treated mice.

Suppression of Antibody Response by the Injection of an Autoreactive Th Clone MS202. Although the clone MS202 had been established as a self class II–reactive Th, the injection of this clone into syngeneic mice together with antigen invariably suppressed the antibody response against this antigen. Fig. 2 shows the kinetics of antibody formation of C3H mice given intraperitoneal injections of 10^7 MS202 simultaneously with primary and secondary immunization with DNP-KLH in comparison with that of normal mice immunized with the same antigen. Both primary and early secondary anti-DNP antibody responses were significantly suppressed by the administration of MS202 ($p < 0.001$ at 1–2 wk after the primary immunization and at 1–3 wk after the boost). The suppression was temporary and could not be observed later than 4 wk after the injection. The suppression was not antigen specific, as the responses to DNP-FGG and DNP-KLH were equally suppressed.

Induction of Ts Cells by Injection of MS202. To determine whether the observed transient suppression of the antibody response by the administration of MS202 is due to the induction of Ts cells, the following experiments were performed. C3H mice were injected with 10^7 MS202 twice at an interval of 2 wk. Their spleen cells were harvested 2 wk after the second injection of MS202, and T cells were aseptically obtained by panning on anti-MIg-coated plastic dishes. Graded doses of T cells were added to the culture of DNP-KLH-primed syngeneic C3H spleen cells, and the anti-DNP antibody response was assessed. The results shown in Fig. 3 indicated that the addition of T cells obtained from
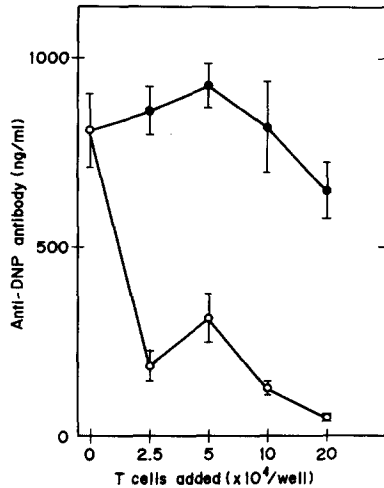
FIGURE 3. Suppression of in vitro secondary antibody response by T cells from MS202-treated mice. C3H mice were injected with 10^7 MS202 at an interval of 2 wk. 2 wk after the last injection, splenic T cells were taken and tested for their regulatory activities on in vitro secondary antibody response. Graded numbers of T cells of either MS202-treated (O) or normal (●) C3H mice were added to a culture of 5 × 10^5 DNP-KLH-primed C3H spleen cells.
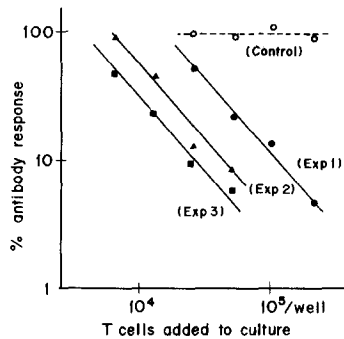

FIGURE 4. Inhibition curve with limiting numbers of MS202-induced Ts cells. Graded numbers of T cells of either MS202-treated (●, ▲, ■) or normal (O) C3H mice were added to the in vitro anti-DNP antibody response of 5 × 10^5 primed syngeneic spleen cells. Each point represents the percent anti-DNP antibody response of the culture with Ts in relation to the maximal response without Ts.

MS202-treated C3H mice strongly inhibited the in vitro anti-DNP antibody response. The addition of T cells of normal C3H mice had no effect. Repeated experiments showed that, in general, as few as 10^4 of T cells were able to induce >50% suppression of the antibody response mounted by 5 × 10^5 primed spleen cells (Fig. 4). The slope of the inhibition curve in Fig. 4 always showed the factor of 1.0, suggesting that Ts induced by MS202 was an effector that directly suppressed Th cells. The suppression was antigen nonspecific, as the MS202-induced Ts cells could suppress the response to DNP-FGG and sheep red blood cells equally well (data not shown). The results indicated that MS202, which was a Th clone per se, activated antigen-nonspecific Ts cells upon injection into syngeneic animals.

Surface Phenotype of MS202-induced Ts Cells. To determine the surface phenotype of Ts induced by MS202, the splenic T cells from MS202-injected mice were treated with alloantibodies and C. The suppressor activity of treated cells was tested by adding the residual cells to the culture of DNP-KLH-primed spleen cells. The treatment of Ts cells with anti-Ly-1 and anti-L3T4 antibodies completely abrogated the suppressive activity, whereas anti-Ly-2, conventional anti-Ia^k, and anti-I-J^k were unable to remove the suppressive activity (Table II, Exp. 1). We have further tested our available anti-I-J mAbs for their effects on MS202-

TABLE II

Phenotypic Expression of Ts Cells Induced in vivo by the Injection of MS202

Exp.	Ts cells treated with:	Anti-DNP responses
		ng/ml
1	Nothing added	386 ± 81
	C	9 ± 1
	Anti-Ly-1 + C	362 ± 28
	Anti-Ly-2 + C	58 ± 4
	Anti-L3T4 + C	318 ± 26
	Anti-Thy-1 + C	299 ± 12
	Anti-Ia^k* + C	43 ± 10
	Anti-I-J^{k}‡ + C	20 ± 3
2	Nothing added	130 ± 8
	C	19 ± 1
	Anti-I-J^k: 1G8 + C	17 ± 2
	KN34 + C	38 ± 5
	JK10-23 + C	36 ± 2
	Anti-L3T4 + C	106 ± 16

C3H Ts cells (2 × 10^5 in Exp. 1, 10^5 in Exp. 2) were cytotoxically treated with the indicated antibodies. The residual Ts cells were cocultured with 5 × 10^5 DNP-KLH-primed C3H spleen cells.
* A.TH anti-A.TL antiserum.
‡ B10.A(3R) anti-B10.A(5R) antiserum.

induced Ts cells (Table II, Exp. 2). None of mAbs were found to be effective in eliminating suppressor activity, even with high concentrations.

No Requirement of Ly-2$^+$ T Cells for the Suppression by MS202-induced Ly-1$^+$ Ts Cells. Previous reports (4–6) indicated that to induce the effector type Ts cells by the antigen-specific Ts factor the presence of Ly-1$^+$,2$^+$ cells was required. An experiment was set up to learn whether the presence of Ly-1$^+$,2$^+$ T cells was needed to induce the final suppression. MS202-induced T cells were treated with anti-Ly-2 and C, and then were added to the culture of DNP-primed B cells and KLH-primed Th cells. Th cells were also treated with anti-Ly-2 and C to obtain the complete absence of Ly-2$^+$ T cells in the experimental system. As shown in Table III, Th cells depleted of Ly-2$^+$ T cells were able to help B cells to produce good antibody response, which could be readily suppressed by MS202-induced Ts. The treatment of Ts cells with anti-Ly-2 and C did not alter the suppressive activity of Ts cells, indicating that Ly-1$^+$,2$^+$ cells are not required for the observed suppression by Ly-1$^+$ Ts cells.

MHC Restriction of MS202-induced F$_1$ Ts Cells. We examined whether or not the Ts cells induced by MS202 is MHC restricted. B6C3F$_1$ (H-2^b × H-2^k) mice were injected with 10^7 MS202 to produce Ts. Their splenic T cells were tested for their suppressive activity in the antibody response mounted by F$_1$ Th and either parental B cells. In general, graded doses of MS202-induced Ts from F$_1$ mice were added to the culture of DNP-primed parental B cells (C3H or B6) and KLH-primed F$_1$ Th cells. As shown in Fig. 5, F$_1$ Th cells were equally effective in helping the antibody production of either parental B cell. The addition of MS202-treated F$_1$ T cells was, however, capable of suppressing the

TABLE III

Lack of Requirement of Ly-2⁺ T Cells for the Suppression by MS202-induced Ts

Th cells treated with:	Ts cells treated with:	Anti-DNP response
		ng/ml
Nothing added	Nothing added	3 ± 1
Anti-Ly-2 + C	Nothing added	125 ± 13
Anti-Ly-2 + C	C alone	25 ± 1
Anti-Ly-2 + C	Anti-Ly-2 + C	21 ± 1

2×10^5 Ts cells from MS202-treated C3H mice were treated with anti-Ly-2 and C. The residual cells were added to the culture of 5×10^5 DNP-primed B cells and 2×10^5 KLH-primed Ly-1⁺ Th cells of the syngeneic mice.
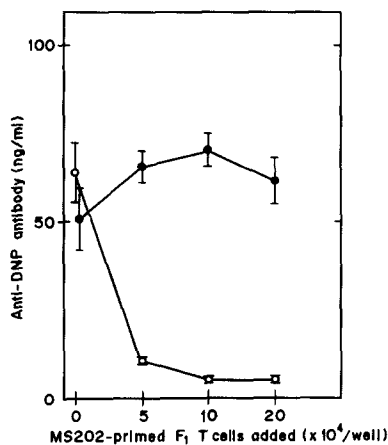

FIGURE 5. Suppression of H-2ᵏ-restricted but not H-2ᵇ-restricted antibody response by Ts cells induced in F₁ mice pretreated with MS202. 5×10^5 DNP-primed B cells from C3H (H-2ᵏ) (○) or B6 (H-2ᵇ) (●) were cocultured with 2×10^5 KLH-primed B6C3F₁ Th cells to induce the secondary antibody response. Graded numbers of MS202-induced Ts cells from B6C3F₁ pretreated with MS202 were added to the cultures at the onset of cultivation.

response of C3H B cells but not of B6 B cells helped by the same F₁ Th cells. The results indicated that Ts cells induced in F₁ by MS202 have the same H-2ᵏ restriction specificity as the inducer clone MS202.

Evidence that the Target of Ts Cells Is the MHC-restricted Th. An experiment was designed to ascertain the direct target of MS202-induced Ts using Th cells obtained from radiation bone marrow chimeras. KLH-primed Th cells were obtained from F₁ → C3H and F₁ → B6 chimeras that had gained the host type H-2 restriction specificity. They were cocultured with DNP-primed F₁ B cells to induce the secondary antibody response. As shown in Table IV, chimeric Th cells were able to cooperate with F₁ B cells that codominantly express both parental class II antigens. F₁ Ts cells, when added to these mixtures, were capable of suppressing the response mounted by F₁ B cells and H-2ᵏ-restricted Th cells from the F₁ → C3H chimera. The same Ts cells were unable to suppress the response induced by H-2ᵇ-restricted Th cells derived from the F₁ → B6 chimera. The results compelled us to conclude that MS202-induced Ts cells directly acted on Th cells having the same H-2 restriction specificity to that of MS202.

TABLE IV

*H-2^k-restricted but not H-2^b-restricted Th Cells of F_1 → Parent Bone
Marrow Chimera Origin Are Suppressed by MS202-induced
F_1 Ts Cells*

DNP-primed B cells	KLH-primed Th cells	B6C3F$_1$ Ts cells	Anti-DNP response
			ng/ml
B6C3F$_1$	F$_1$ → C3H	−	823 ± 59
B6C3F$_1$	F$_1$ → C3H	+	139 ± 41
B6C3F$_1$	F$_1$ → B6	−	583 ± 31
B6C3F$_1$	F$_1$ → B6	+	480 ± 75

5 × 10^5 DNP-primed B6C3F$_1$ B cells were cocultured with 2 × 10^5 KLH-primed Th cells of F$_1$ → C3H or F$_1$ → B6 chimera. 5 × 10^4 B6C3F$_1$ Ts cells induced in F$_1$ by MS202 were added to the responding cell cultures.

Discussion

We have described here a minimal regulatory circuit initiated by a cloned T cell line MS202. MS202 was at first defined as a class II–autoreactive T cell clone and was characterized by its ability to induce proliferation and Ig synthesis of syngeneic B cells in vitro without known antigen specificity. However, the in vivo application of this clone was found to be suppressive for the primary and early secondary antibody responses against the simultaneously administered antigen. A series of experiments described here indicated that MS202 induced a potent Ts whose action exactly followed the same H-2 restriction specificity borne by the inducer MS202. No antigen specificity was observed in the MS202-induced Ts. The phenotype of Ts was L3T4$^+$, Ly-1$^+$,2$^-$, and I-J$^-$. It acted directly on Th without participation of Ly-2$^+$ T cells. The final figure observed here was amazingly simple, as the H-2-restricted Th clone induced Ts that, in turn, directly suppressed the heterogeneous population of Th, provided the latter had the identical H-2-restriction specificity to that of MS202. Indeed, the experiments with Th cells from F$_1$ → parent chimeras indicated that the MS202-induced Ts recognizes the very MHC restriction site of the Th receptor rather than any other genetically determined polymorphisms.

The strict MHC restriction observed in the activation of normal T cells by MS202 is most likely to be due to the direct recognition of the H-2 restriction site of MS202 by syngeneic T cells. This notion was supported by the fact that the activation of syngeneic T cells by MS202 could be induced in the presence of anti-IL-2-R mAbs and in the relative absence of macrophages (data not shown). The glutaraldehyde-fixed MS202 could also induce the H-2-restricted proliferation of T cells, indicating that the observed response is not merely due to IL-2 produced by MS202 in response to syngeneic macrophages. These results indicate that the normal T cell repertoire accommodates a high frequency of self-reactive T cells that recognize the MHC restriction site of other T cells making up an internal regulatory circuit. Such an MHC restriction imposed on interaction between T cells was recently termed "epirestriction" by N. A. Mitchison, University College, London, United Kingdom (personal communication).

Recently, several investigators became aware of direct T–T cell interactions

based on the mutual recognition of MHC restriction specificities. Direct activation of syngeneic normal T cells by autoreactive T cell clones or hybridomas through the recognition of the anti–class II site of the latter has been reported by Nagarkatti et al. (12) and Kennedy et al. (13). Although the functional roles of activated T cells have not been determined, they suggested the participation of MHC recognition sites in the expansion of clonal repertoire of T cells. Suzuki et al. (14) and Suzuki and Quintans (15) further demonstrated that self–class II–reactive sites on T cell clones can initiate a chain of regulatory interactions leading to either the stimulation or inhibition of the response. The activated T cells contain Ly-2$^+$ CTL, which inhibits the antibody response, probably by eliminating Th. In the present experiments, however, no cytotoxic activity against both MS202 and Con A–activated C3H T cells was detected in the MS202-treated splenic T cells even after restimulation in vitro (data not shown). Thus, the observed suppression is not due to the veto effect of in vivo MS202–induced T cells, unlike the situation often seen with in vitro stimulation.

Stimulation of Ts cells by T cell clones (16–18) or mixed populations of preactivated T cells (19, 20) has also been reported. Crispe and Owens (16) and Lamb and Feldmann (17) reported that the Ts induced by Th clones can suppress only the response helped by the original T cell clone. Others (18–20) reported that human T cell blasts recognizing class II alloantigens can induce Ts in vitro that suppresses the allo-MLR response. The induced Ts of the CD8 phenotype appears to recognize the T cell receptor for self in association with class I antigen. The simplicity of the situation is similar to ours except for the restriction specificity imposed on the Ts effect and for the CD phenotype of Ts. Thus, the MHC restriction sites of T cells seem to generally serve not only as the recognition element for the target cells but also as devices for the activation of the second regulatory cells. The previously observed restriction-restricted Ts cells (21, 22) may also be placed in this context.

What, then, is the interrelationship between the previously known regulatory circuits and those observed here? The fact that a Th clone induces Ts that inhibit heterogeneous Th is very similar to the feedback suppression by Eardley et al. (23). In fact, they demonstrated that an inducer Ts cell activates the Ts effector cells that inhibit the initial inducer signal (23). The only definite difference is that the present minimal circuit is within the Ly-1$^+$,L3T4$^+$ compartment and that the interaction is strictly H-2 regulated. The participation of soluble factors has so far not been demonstrated. We therefore believe that the minimal circuit described here is not similar to previously described ones, which may be initiated only by the external antigenic input. We have been concerned with the antigen-specific suppressor circuit (3), but the presently described MHC-driven circuit should be a core structure inherently operative even without antigenic stimulation.

A question arises whether or not the observed phenomenon is unique to this special autoreactive T cell clone, MS202. In humans, CD4 T cells have been divided into two populations that can or cannot induce CD8 Ts (24). We have performed a similar induction experiment in vivo with a typical H-2-restricted FGG-specific Th cell clone. The results were essentially similar to the present report in that the FGG-specific Th induced an antigen-nonspecific Ts. Thus, the

minimal regulatory circuit reported here can be stimulated not in particular by MS202, but is generally operative under the normal physiologic condition. In view of the known plasticity of MHC restriction specificity of Th and Ts (25), such a circuit is probably preprogrammed during the early ontogeny of T cells in the thymus to construct the core regulatory structure without further antigenic stimuli.

The clone MS202 is also of particular interest since it constitutively expresses I-J^k epitopes. Most of the MHC-restricted functions of MS202 can be blocked by anti-I-J^k antibodies (Fujisawa, I., K. Sano, T. Nakayama, Y. Asano, and T. Tada, manuscript in preparation). It has recently been demonstrated (26, 27) that I-J is not a genetic marker of Ts, but is associated with inducible T cell receptor for self (26, 27). The efficient inductive ability of MS202 for Ts can be related to the high expression of I-J epitopes.

The structural basis of the construction of this circuit is still not known. As we have proposed previously (10), MHC polymorphisms in the thymus would dictate the MHC restrictions of some of the T cells, including Th cells. However, the restriction elements of other regulatory T cells are not directly dictated by the MHC polymorphism itself but rather secondarily induced by the presence of initial H-2-restricted repertoire. We have coined terms of "antetope" for the first set of the T cell repertoire and "metatope" for the secondarily induced T cell receptors on regulatory T cells (10). The site of dictation and selection of metatopes is not known. The major point of this argument is that the regulatory circuit governed by the MHC polymorphism is programmed independently from the selection of T cell repertoire for external antigens. Although we do not want to discuss the old two-receptor hypothesis for T cell recognition, it is noteworthy that I-J epitopes associated with the self–class II recognition sites are expressed by a large number of class II–restricted T cells regardless of their antigen-specificities (Asano Y., T. Nakayama, M. Kubo, J. Yagi, and T. Tada, manuscript in preparation). Thus, the role I-J also has to be reconsidered on the basis of the synaptic structure of this minimal regulatory circuit as a restricting element.

Summary

The in vivo administration of a self–class II–reactive Th clone MS202 derived from C3H into syngeneic mice resulted in the suppression of both primary and early secondary antibody responses against T cell–dependent antigens. The suppression was due to the generation of antigen-nonspecific Ts cells in the recipient, as the splenic T cells from the mice treated with MS202 were able to strongly suppress the in vitro secondary antibody response of primed syngeneic spleen cells. The dose-response curve of suppression indicated the generation of an effector type Ts that directly suppressed Th. The surface phenotype of Ts was Ly-$1^+,2^-$, L3T4$^+$, I-J$^-$. The presence of Ly-$1^+,2^+$ T cells was not required to induce the suppression. The suppression was strictly restricted to H-2^k, as F$_1$ Ts cells were able to suppress the response of C3H but not of B6 B cells helped by the same F$_1$ Th cells. The experiments with chimeric mice indicated that the direct target of Ts is an MHC-restricted Th but not a B cell or APC. The results indicate the existence of a minimal regulatory circuit where an MHC-restricted Th induces a preprogrammed Ts that in turn directly suppresses Th with the

same MHC-restriction specificity. The induction of and suppression by Ts appeared to be due to the direct recognition of MHC restriction sites of Th cells.

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