Brief Definitive Report

DOWNREGULATION OF T CELL RESPONSES BY ANTIBODIES TO THE T CELL RECEPTOR

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Murine T cells respond to at least three different types of antigens: (*a*) H-2 alloantigens, (*b*) conventional antigens seen in association with self H-2 determinants (self + X), and (*c*) minor lymphocyte-stimulating (Mls) determinants. The fact that individual T cell clones can respond to three diverse types of antigens raises the question whether recognition of these antigens involves one, or more than one, type of T cell receptor (TcR) molecule. With the availability of monoclonal antibodies (mAb) specific for allotypic determinants of the TcR α-β heterodimer, e.g., KJ16 (1) and F23.1 (2), it has been possible to examine whether such antibodies can inhibit the response of dual-reactive T cell clones, i.e., clones reactive with more than one of the above classes of antigens. The invariable finding has been that anti-TcR antibodies, including anticlonotype antibodies, are able to inhibit responsiveness of dual-reactive clones and hybridomas to both ligands (1, 3–6). These data have been interpreted as providing strong support for the view that T cell specificity for all three classes of antigens is controlled by a single set of TcR molecules.

We show here that KJ16 and F23.1 mAb each inhibited the responses of a triple-reactive T cell clone to all three classes of antigens. Significantly, however, the anti-TcR mAb also inhibited the response of the clone to IL-2, a ligand that is not recognized by the TcR. These data suggest that the blocking effects of anti-TcR antibodies must be interpreted with considerable caution.

Materials and Methods

Mice. B10.P, B10.S, and D1.LP mice were obtained from the Scripps Clinic and Research Foundation breeding colony. All other mice were obtained from The Jackson Laboratories, Bar Harbor, ME.

Preparation of Clones. B10.D2 (H-2^d, Mls^{a,d}) mice were first immunized with FγG in vivo; lymph node T cells from these mice were then stimulated in vitro with allogeneic B10.P (H-2^p, Mls^b) spleen cells. This line was restimulated at 2-wk intervals, first with fowl γ globulin (FγG) plus irradiated syngeneic spleen cells as a source of antigen presenting cells (APC) and then with F23.1 antibody in the presence of syngeneic APC. The line was cloned in the presence of Mls-disparate DBA/2 (H-2^d, Mls^{a,d}) irradiated spleen cells.

Cell Culture. T cell lines and clones were maintained in vitro by culturing $2–3 \times 10^5$ cells with 4.0×10^7 irradiated (3,000 rad) spleen stimulator cells in 10 ml of RPMI 1640 medium containing 10% FCS (Hyclone, Logan, UT), 5% NCTC 109 (Whittaker M. A. Bioproducts, Walkersville, MD), glutamine, 2-ME, and antibiotics. When appropriate, 100 μg/ml FγG was added. Viable cells were restimulated at 14-d intervals. Cloning was

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TABLE I

Specificity of Triple-reactive T Cell Clone K3.2.1.c9

Stimulators*	H-2/Mls	Proliferative response‡ ([^{3}H]TdR uptake [cpm × 10^3])		
		Exp. I	Exp. II	Exp. III
B10.D2	d b	4.5	0.2	0.4
B10.D2 + FγG	d b	29.0§	NT	18.5
B10.BR	k b	0.5	0.1	0.3
B10.P	p b	70.5	24.0	23.0
C57BL/10	b b	NT	NT	13.2
B10.S	s b	NT	NT	0.4
B10.PL	u b	NT	1.3	NT
CBA/Ca	k b	NT	0.3	0.7
C3H/HeJ	k c	NT	0.1	0.5
BALB/C	d b	NT	0.1	NT
C57BR	k b	NT	0.2	0.4
CBA/J	k d	63.5	57.5	20.6
AKR/J	k a	57.5	40.7	11.5
SM/J	v a	136.3	17.8	NT
DBA/2	d a	73.7	16.3	7.9
PL/J	u x	NT	6.8	19.1
D1.LP	b a	28.7	NT	7.5

* Stimulators were anti-Thy-1 + C′-treated spleen cells; 1,500 rad.

‡ Proliferative response of 1.5 × 10^4 K3.2.1.c9 cells measured on day 3. Mean cpm of triplicate cultures; SD were usually between 10–20% of mean.

§ Positive responses are underlined.

carried out at limiting dilution (~0.3 cells/well) 48 h after the last restimulation in 96-well round-bottom microtiter plates with irradiated spleen cells. Rat Con A culture supernatant (SN) was added as a source of IL-2. The clone K3.2.1.c9 has been subcloned repeatedly over the last two years; all subclones have always shown the same specificity as the parent in proliferation assays.

For proliferation assays, 2 × 10^4 cloned T cells were cultured in round-bottom microtiter wells with the indicated number of irradiated (1,500 rad) anti-Thy-1 + C′-treated spleen cells in 200 μl culture medium. Responses were measured by pulsing with [^{3}H]thymidine ([^{3}H]TdR) 16–18 h before harvesting. In inhibition assays, the indicated concentration of antibody was added on day 0 and remained in the culture for the duration of the experiment.

Antibodies Used. KJ16.133 hybridoma cells were kindly provided by P. Marrack and J. Kappler, National Jewish Hospital, Denver, CO (1). The F(ab′)$_2$ fragments were prepared from ascites using pepsin as described by Parham (7), and were purified using an ACA 44 column. The Fab (papain) fragments were prepared according to the procedure of Kaye (3) and then purified on a DEAE column. The samples were analyzed on a 7% SDS polyacrylamide gel and showed no contamination with uncleaved antibody. The F23.1 ascites fluid, as well as the F(ab′)$_2$ fragments of this antibody were kindly provided by Drs. U. Staerz and M. Bevan, Scripps Clinic and Research Foundation.

IL-2. Recombinant human IL-2 (rIL-2) was kindly provided by Cetus Corp., Emeryville, CA.

Results

As described in Materials and Methods, we have isolated a B10.D2 (H-2^d) clone, K3.2.1.c9, with specificity for (*a*) FγG + H-2^d, (*b*) allo-H-2^{p,b} and (*c*) Mls^{a,d} determinants (Table I). This clone is L3T4$^+$, Lyt-2$^-$, F23.1$^+$, and KJ16$^+$. Experiments comparing the degree of inhibition seen with limiting concentrations of anti-L3T4 mAb and limiting numbers of APC suggested that the relative avidity of the clone for the three ligands was not demonstrably different (data not

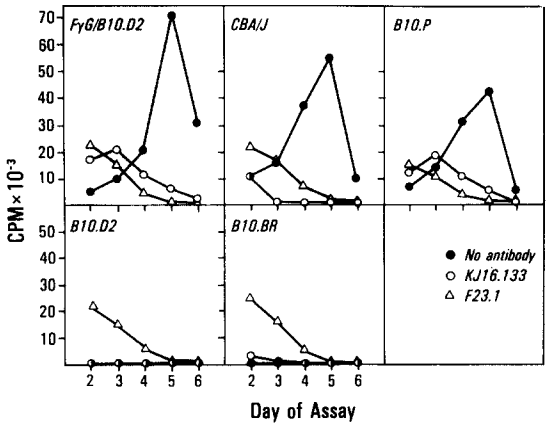

FIGURE 1. Anti-TcR inhibition of the proliferative response of the T cell clone, K3.2.1.c9, to: FγG in the presence of syngeneic B10.D2 (H-2^d Mls^b) spleen; Mls, CBA/J (H-2^k, Mls^{a,d}) spleen; allo-H-2^p, B10.P, (H-2^p, Mls^b) spleen; syngeneic B10.D2 spleen; and B10.BR (H-2^k, Mls^b) spleen. All spleen cell preparations were anti-Thy-1 + complement–treated. The responses were measured in the absence of added antibody (●) or in the presence of KJ16 (○) or F23.1 (△) mAb.

TABLE II

F(ab')$_2$ and Fab Fragments of Antireceptor Antibodies Inhibit Proliferative Responses of K3.2.1.c9

Exp.	Anti-TcR antibody*	[^{3}H]TdR uptake (cpm × 10^{-3}) with stimulators on day 2 or 4‡							
		B10.D2		CBA/J		B10.P		B10.D2/FγG	
		2	4	2	4	2	4	2	4
I	0	0.2	1.9	8.9	12.1	12.0	37.6	11.0	91.1
	KJ16	—	—	3.3	0.3	9.9	8.3	17.9	13.2
	KJ16 F(ab')$_2$	—	—	1.5	0.2	15.4	10.3	16.0	14.0
	F23.1	9.9	0.4	7.5	1.2	16.4	0.6	12.1	0.5
	F23.1 F(ab')$_2$	0.4	0.1	1.6	0.3	15.9	4.9	19.7	6.1
II	0	0.5	0.2	8.5	20.4	11.7	16.5	13.0	38.2
	KJ16	—	—	0.6	0.6	11.7	1.8	19.1	1.2
	KJ16 Fab	—	—	16.1	2.5	16.1	3.6	19.2	6.9

* All antibodies were used at concentrations that did not show nonspecific inhibition of the KJ16$^-$/F23.1$^-$ clone K3.1.c7. The percentages are of the following stock antibody preparations; KJ16, 1% of culture supernatant; KJ16 F(ab')$_2$, 4.0 μg/ml; F23.1, 1:10^3 dilution of ascites; F23.1 F(ab')$_2$, 3.6 μg/ml. All responses were titrated and optimal dilution was chosen for each antibody.

‡ See footnotes to Table I.

shown). Another clone, K3.1.c7, was used as a specificity control; this clone has the same triple-specificity as K3.2.1.c9 but is F23.1$^-$, KJ16$^-$.

Inhibition with Anti-TcR mAbs. As shown in Fig. 1, the day 4 response of the K3.2.1.c9 clone to each of the three antigens, FγG, Mls (CBA/J), and allo-H-2 (B10.P), was markedly reduced by adding KJ16 or F23.1 mAb to the cultures. By contrast at earlier time points, e.g., day 2, the responses were either unaffected or slightly enhanced, especially in the case of responses to FγG and allo-H-2^p. Table II illustrates that F(ab')$_2$ fragments, as well as Fab fragments have similar effects as the whole molecule. Anti-Mls^{a,d} responses were often depressed on day 2 by anti-TcR antibodies, but definite enhancement (followed by inhibition on

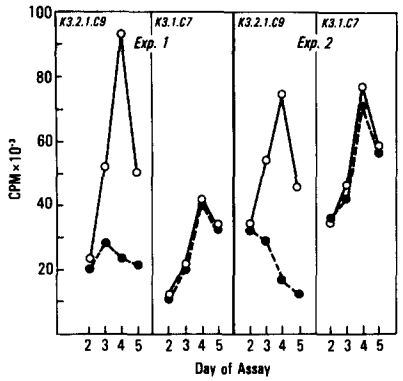

FIGURE 2. Inhibition of IL-2-induced proliferation with F23.1 mAb. K3.2.1.c9 ($F23.1^+$) and K3.1.c7 ($F23.1^-$) clones were incubated with 10 U/ml rIL-2 either with (●) or without (○) F23.1 mAb.

day 4) was seen in some experiments, e.g., with KJ16 Fab fragments (Table II, *bottom*).

Intact F23.1 mAb is mitogenic for the K3.2.1.c9 clone (Fig. 1); stimulation of the clone with intact F23.1 mAb requires feeder cells (spleen cells), but the H-2 haplotype of the feeders is irrelevant. KJ16 antibodies, on the other hand, have no demonstrable stimulatory effect on the clone.

Capacity of Anti-TcR mAb to Inhibit IL-2-induced Proliferation. The fact that the inhibitory effects of the anti-TcR mAbs on the response of the K3.2.1.c9 clone were often preceeded by enhancement suggested that the antibodies were not acting simply by blocking T cell recognition of the three antigens in question. If other mechanisms were involved, anti-TcR mAb might be expected to alter the response of the clone to a stimulus not involving TcR, e.g., a response to IL-2. In this respect, experiments with the F23.1 mAb showed marked inhibition of the response of the K3.2.1.c9 clone to rIL-2 (Fig. 2); the kinetics of inhibition were very similar to that seen above with antigen, i.e., inhibition on days 3–5 but not on day 2. Little or no inhibition was observed in control studies with an $F23.1^-$ clone, K3.1.c7. KJ16 mAb and Fab fragments also inhibited the response to IL-2, although the effect was sometimes less marked than with F23.1 mAb (data not shown).

Discussion

Although it is generally accepted that reactivity to self + X and allo-H-2 determinants reflects crossreactive recognition by a single set of TcR α-β heterodimers (1, 8), we have previously reported data suggesting that Mls determinants might be recognized by a different cell surface molecule (9). The strongest evidence against this notion has been the demonstration that Mls-reactivity can be inhibited by typical anti-TcR mAb (1, 4–6). In confirmation of this finding, we show here that all three reactivities displayed by a T cell clone, including Mls reactivity, can be blocked by anti-TcR mAb. Surprisingly, however, anti-TcR mAb also inhibited the response of the clone to IL-2, implying that at least in certain situations, anti-TcR mAb can cause nonspecific downregulation of T

cells. The capacity of anti-TcR mAb to inhibit Mls-reactivity thus provides no evidence either for or against the notion that Mls determinants are recognized by separate molecules. Downregulation of T cells has also been observed with antibodies to T accessory molecules (10–12); there is also a report that the response of a $T8^+$ suppressor T cell clone to IL 2 can be inhibited by anticlonotypic mAb (13).

In attempting to explain the paradoxical enhancing/inhibiting effects of anti-TcR, it should be noted that intact F23.1 mAb is clearly mitogenic for $F23.1^+$ T cells (Fig. 1); the response peaked early, i.e., on day 2 or 3, and then declined rapidly. Interestingly, very similar kinetics were observed when the clone was cultured with F23.1 mAb in the presence of antigen (Fig. 1, panels *1–3*), suggesting that the transient response of the clone to F23.1 mAb preempted the response to antigen. Demonstrable mitogenicity was not usually seen with $F(ab')_2$ fragments or with KJ16 antibodies (Table II). It is possible, however, that these latter preparations were covertly mitogenic; the cells received a signal from the antibodies that was insufficient by itself to promote DNA synthesis but was sufficient to prime the cells for contact with antigen. In favor of this idea, we have recently found that prior overnight incubation of the clone with KJ16 mAb (without antigen) followed by washing leads to an accelerated response of the clone to each of the three antigens (data not shown). Why anti-TcR causes late downregulation of the clone, e.g., to IL-2, is unclear. One possibility is that downregulation reflects excessive signalling through the TcR; in this respect it is accepted that high concentrations of antigen are often inhibitory for T cells (14). Another possibility is that antibody binding to the TcR prevents the T cells from receiving a protective signal from the APC, i.e., a signal that counteracts downregulation.

Although the mechanism by which anti-TcR mAb downregulate T cells is still unclear, the main conclusion to be drawn from the present studies is that blocking effects noted with anti-TcR mAb do not necessarily reflect simple occlusion of the binding sites on the TcR molecule.

Summary

We have derived a T cell clone that recognizes and responds to three different types of antigen: self + X (fowl gamma globulin + $H\text{-}2^d$), allo-$H\text{-}2^{p,b}$, and minor lymphocyte-stimulating ($Mls^{a,d}$) determinants. Anti-TcR mAb and their $F(ab')_2$ and Fab fragments were tested for their capacity to block the response of this clone. When responses were assayed on day 4 or later, addition of KJ16 or F23.1 mAb caused a marked inhibition of the response to each of the three antigens recognized by the clone. Responses measured at earlier time points however were unaffected or enhanced. This finding suggested that the inhibitory effects of anti-TcR mAb that followed the phase of enhancement might have reflected downregulation of the cells rather than simple blockade of TcR. In support of this possibility it was found that addition of anti-TcR mAb caused marked inhibition of the response of the clone to IL-2, i.e., a response that is not known to involve the TcR.

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