Journal of Supramolecular Structure 14:175–182 (1980)
Control of Cellular Division and Development: Part A: 185–192

Polyclonal Activation of Ts Cells With Antiserum Directed Against an IGH-1 Linked Candidate for a T-Cell Receptor Constant Region Marker

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An anti-T cell serum raised in allotype congenic mice recognizes the product of a new locus coding for a heavy chain-linked polypeptide found on a subpopulation of T cells. Anti-Ts^d raised in BALB/cAnN mice against selected C.AL-20 T cells reacts with a cell surface antigen in virgin animals that is found on 25% of mature thymocytes and Lyt-bearing T cells, but not on prothymocytes, Lyt1 T cells or B cells. The antigen is restricted to strains bearing the Ig-1^d and Ig-1^e heavy chain allotype haplotypes, and is expressed in the F_1 animal. The antigen is unlinked in expression to the Lyt2, H-2, or kappa light chain loci. The antigen is not detected in the hematopoietic cells in the bone marrow and appears to mark only the mature peripheral pool of T cells. As previously reported, the antiserum blocks the binding of suppressor T cells to the cross-reactive idiotype for arsonate, while reagents specific for Fab, Fc and Ig were ineffective. It seems probable that the marker may represent a T cell constant region marker analogous to the Igh products on immunoglobulin. Antiserum against this marker induces in vivo triggering of Ts cells for a wide variety of T-dependent antigens. All subclasses of anti-hapten antibodies are suppressed; no affinity restrictions or clonotype specificity is observed in suppressed adult mice. Results suggest that precursor T cells regulating major serum idiotypes regulate individual idiotypes.

Key words: T cell, constant region, receptor, suppressor, lymphocyte surface antigen

T-cell receptors for defined antigens have been shown to bear molecules cross-reactive with many of the idiotypes on antibody molecules [1–4]. In addition, framework structures of antibody V_H [5, 6] and V_L [7] chains have been found in close association with T-cell determinants. These pieces of evidence suggest that T cells share overlapping V_H gene repetoires with their B cell counterparts and utilize these structures for antigen recognition. The existence of T-cell constant region determinants which may be analogous to the Igh-1 markers on immunoglobulin subclasses has been hypothesized [8, 9]. Recent studies have shown that Ts^d, a T-cell surface molecule closely associated with the antigen binding site, is a likely candidate for the first constant region marker [9–11]. This marker is expressed on 25–35% of the Lyt2 peripheral cells of mice, restricted to the most mature thymocyte population and present on a high frequency of band I

Received March 6, 1980; accepted June 6, 1980.

0091-7419/80/1402-0175$02.50

(26% BSA) Con A-induced T-cell blasts. Antisera directed against this determinant inhibits the binding of the Ars-IdX anti-idiotype [12] bearing suppressor T-cell (Ts) to its idiotype bearing target under capping conditions [9], suggesting that there is a physical association between Ts^d and the antigen specific part of the T-cell receptor. This antigen (Ts^d) has been shown to be allotype linked [9] but has no apparent linkage to Lyt2 or to H-2 in contrast to other studies which have shown anti-Lyt2 to block cytotoxic T-cell receptors [13] or H-2 linked determinants in association with allotypic T-cell receptors [14]. Recombinant inbred lines [15] with recombination events between Igh-V and Igh-C have shown this marker to be coded for by a gene(s) located between Igh-5 (δ) and a recombination event between Igh-1 and prealbumin, consistent with the possibility that this marker could be a constant region marker analogous to Igh-1 [11].

If Ts^d is a constant region marker, then all suppressor T-cells with a similar function should express Ts^d as a part of their antigen-binding structures. Since antisera directed against IgM can act as an in vitro polyclonal trigger for cells [16, 17], the possibility that anti-T-cell receptor serum could trigger T-cells was considered. If the antiserum recognizes a conserved determinant, then crosslinking of the membrane and induction of a triggering signal should be possible. If true polyclonal activation were achieved, one would expect to see no antigen restrictions in suppression, and no subclass, idiotype, or affinity alterations, in antibody-suppressed mice. Experiments described here support this hypothesis. However, it was observed that only T-dependent and not type I or II T-independent antibody responses are suppressed, suggesting that the target of that activated suppressor T-cell is preferentially a T-helper cell.

METHODS

Production of Anti-Ts^d

Details of the production and evaluation of this sera have been published elsewhere [9]. Briefly, C.AL-20 mice were treated with anti-Ars IdX serum and KLH-Ars to render them hyperimmune and subsequently suppressed for the major serum idiotype [18]. This protocol results in the generation of large numbers of Ts cells which bear receptors for the idiotype [19]. Mice were sacrificed at the peak of expression of their Ts AID cells, spleen cells treated with 5 μg/ml Con A for 48 hr and blast cells recovered on discontinuous BSA density gradients. After being washed in PBS, cells were injected into Balb/cAnN animals (5X) and serum was pooled from demonstrated positive serum samples.

Suppression with Anti Ts^d

It was previously reported that in vivo injectin of Ts^d (2 λ/mouse) day 4 before antigen lead to induction of T-suppressor cells [10]. That protocol was followed here.

Assay of PFC Response

The method of Cunningham et al [20] was used to evaluate IgM specific PFC against TNP or FITC. TNP was conjugated to sheep red blood cells by the method of Rittenberg and Pratt [21] and FITC was conjugated by the method of Möller [22].

Measurement of Anti-Hapten and Anti-Class Specific Antibodies

A solid-phase radioimmunoassay was used to evaluate the anti-hapten responses of serum antibodies [23]. Briefly, BSA-hapten conjugates were incubated in neutral buffer

with polyvinyl chloride trays (Dynatech Laboratories, Alexandria, Va.) for 2 hr at 25°C. Trays were then nonspecifically blocked with 2% horse serum in PBS. Affinity purified anti-hapten antibodies were used to generate a standard curve which was used to quantitate the anti-hapten antibody in limiting dilutions of unknown sera. After a 2-hr room temperature incubation of anti-hapten antibody with the hapten coated plate, anti-hapten antibody was washed away with PBS and ^{125}I-goat anti-isotype serum or ^{125}I-RaMIg serum was added, 150 ng of affinity purified globulin/well, for 12 hr. Plates were washed, individual wells separated using a hot wire cutter, and each well counted in a gamma counter. The assay was sensitive to 5 ng/100 λ of media. Affinity measurements of anti-hapten antibodies were taken by inhibiting the anti-hapten phase of the assay with free hapten in neutral buffer.

RESULTS

Antiserum directed against Ts^d had previously been reported to be effective in inducing Ts cells for the IgM SRBC response when 2 λ was given i.v. 4 days prior to antigen injection [10]. This protocol was used to induce suppression of the IgM anti-TNP response (Table I) or anti-FITC PFC responses. These two haptens were chosen because the carriers, KLH, Ficoll, or LPS, could be varied to induce a response which has been well characterized to be either T-dependent (KLH-TNP [24], KLH-FITC), T-independent type II (Ficoll-TNP [24], Ficoll-FITC), or T-independent type 1 (LPS-TNP [25]). Only the T-dependent anti-hapten response was inhibited; antibody pretreatment reduced the mean PFC response of three mice treated wtih KLH-TNP from 40,000 PFC/10^8 cells on day 3 of the primary response to 17,000 PFC/10^8 cells, a 55% mean inhibition. The anti-KLH FITC response was suppressed 76%, from 5400 PFC to 1300 PFC/10^8 cells. T-independent antigens were not altered with the antiserum as evaluated by PFC formation in the early phases of the response.

TABLE I. Anti-Ts^d Suppression of the T-Dependent and T-Independent IgM PFC Response of A/J Mice*

T-Dependence	Antibody[a]	Antigen[b]	No. of Mice	Mean PFC/10^8 spleen cells[c]	% Suppression
T	0	KLH·TNP	3	40,347 ± 3,000	
T	2λ	KLH·TNP	3	17,450 ± 4,000	58
T	0	KLH·FITC	4	5,436 ± 200	76
T	2λ	KLH·FITC	4	1,332 ± 300	
TI_1	0	TNP·Ficoll	3	19,540 ± 4,000	
TI_1	2λ	TNP·Ficoll	3	30,378 ± 6,000	Enhancement
TI_1	0	FITC·Ficoll	4	12,471 ± 2,000	0
TI_1	2λ	FITC·Ficoll	4	13,933 ± 3,000	0
TI_2	0	TNP·LPS	3	174,333 ± 20,000	0
TI_2	2λ	TNP·LPS	3	123,903 ± 60,000	0

*Adult (5–6 week) mice were used.

[a]Antisera or NMS was diluted in PBS to 200 λ and injected i.v. into the tail vein.

[b]Antigen was diluted to 200 λ in PBS and injected i.v. into the tail vein; KLH·TNP (50 mg), KLH·FITC (50 mg), TNP·Ficoll (10 μg), FITC·Ficoll (10 μg), TNP·LPS (10 μg).

[c]Direct PFC's were evaluated on day 3 of the response.

The serum responses of mice to TNP or FITC was compared with the PFC results. Again only the early primary response is suppressed (Table II), and under repeated antigen pressure, these mice overcame the suppressive signal. Serum from 10 mice in each group was pooled and evaluated for the total anti-hapten antibody using a sensitive solid phase radioimmunoassay. On day 10, mice preinjected with 2 λ of anti-Ts^d serum followed by KLH-TNP made 24 ng/λ of anti-TNP in contrast to 123 ng/λ for the control mice. By day 21 of the secondary response, this difference between groups was no longer observed. In contrast, the TNP-Ficoll response is not altered significantly by pretreatment of mice with antibody. In a parallel experiment (Table II), 10 mice were immunized with KLH-Ars and pretreated with either antibody or NMS. Similar results showed the anti-Ars response was 35 ng/λ for antibody-treated mice and 117 ng/λ for NMS controls (day 17). When mice were treated with anti-Ts^d followed by FITC-KLH or Ficoll-FITC the antibody was effective in suppressing the early primary response from 134 ng/λ to 80 ng/λ for FITC-KLH, but may have somewhat enhanced the T-independent Ficoll-FITC response (day 10). These experiments are consistent with the possibility that anti-Ts^d suppresses the primary response to T-dependent antigens.

A possible isotype restriction in the antibody which is suppressed was evaluated (Table III). With anti-TNP, all isotypes are suppressed, while γ_1 and γ_3 are more completely and persistently altered than μ. In contrast the anti-FITC μ response is more suppressed than γ_1 and γ_3. Quantitatively, the response to TNP is predominatly γ_1 and γ_3. In contrast to the FITC response, which is predominatly IgM on day 10; the major responding isotype is therefore altered most in each case. There is no evidence

TABLE II. Effect of Anti-Ts^d on a T-Independent Serum Response

		Pool of 10 animals/group				
		1°			2°	
		Day			Day	
Antigen[a]	Antibody[b]	10	15	20	28	31
		mean ng/λ anti-TNP				
KLH·TNP	NMS	123	776	178	302	1409
KLH·TNP	Ab	24	113	74	158	1215
Ficoll·TNP	NMS	51	67	43	61	32
Ficoll·TNP	Ab	62	75	48	63	53
		mean ng/λ anti-ARS				
KLH·Ars	NMS	N.D.	117	N.D.	4374	3947
KLH·Ars	Ab	N.D.	35	N.D.	3645	3773
		mean ng/λ anti-FITC				
KLH·FITC	NMS	134				
KLH·FITC	Ab	89				
Ficoll·FITC	NMS	15				
Ficoll·FITC	Ab	30				

[a]Antigens were administrated i.p. on day 0 in CFA. The following quantities were given: KLH·TNP, 50 μg; TNP·Ficoll, 50 μg; KLH·Ars, 50 μg; KLH·FITC, 50 μg; Ficoll·FITC, 10 μg.

[b]Anti-Ts^d (2 λ/mouse) was administered i.v. 4 days before antigen injection.

for an induction of a shift to enhanced production of another isotype in the presence of antibody [26].

The affinity differences between mice treated with anti-Ts^d and then challenged with antigen on day 15 of a primary response were compared with control mice immunized with NMS and antigen on day 15. Figure 1 shows that there are no gross differences between the affinity of anti-TNP or anti-Ars responses in suppressed or control mice.

DISCUSSION

The network theory of regulation of the immune response [27] states that an extended circuit of antibodies and anti-antibodies interact with one another to control the production of antibody of any given specificity. This original concept has been expanded upon by the recent studies of those working in cellular systems with idiotypic antibodies and anti-idiotypic reagents which recognize major cross-reactive serum idiotypes, related clones which are serologically detectable. It has been shown that heterologous anti-idiotype serum, injected into adult mice can suppress the immune response by generating T-suppressor cells specific for a given idiotype [1, 28] or T-helper cells [1]. In all cases reported, this regulation is clonotype specific. However, the major ques-

TABLE III. Suppression of Class Specific Serum Immunoglobulin Response*

	IgG_1	IgG_3	IgG_{2b}	IgG_{2a}	IgM	Total Ig
			Anti-TNP			
Day 10 – 1° response						
T-cell dependent[a]:						
Ab/NMS ratio	0.14	0.20	0.16	0.21	0.39	0.16
T-cell independent[b]:						
Ab/NMS ratio	1.45	1.89	1.79	3.07	1.15	1.2
Day 15 – 1° response						
T-cell dependent:						
Ab/NMS ratio	0.18	0.19	0.23	0.49	0.50	0.15
T-cell independent:						
Ab/NMS ratio	1.56	1.80	1.56	2.48	1.07	1.10
Day 28 – 2° response						
T-cell dependent:						
Ab/NMS ratio	0.46	0.75	0.64	0.66	1.7	0.54
T-cell independent:						
Ab/NMS ratio	0.71	1.2	0.78	1.5	1.1	0.95
			Anti-FITC			
Day 10 – 1° response						
T-cell dependent[c]:						
Ab/NMS ratio	0.80	0.90	N.D.	N.D.	0.49	0.60

*Isotype specificity was determined by pulsing antigen coated plates with serum antibody and following that 2-hr pulse with ^{125}I-goat anti-isotype specific affinity purified reagents.

[a]KLH·TNP, 50 μg, was injected i.p. in CFA on day 0.

[b]Ficoll·TNP, 50 μg, was injected i.p. in CFA on day 0.

[c]KLH·FITC, 50 μg, was injected i.p. in CFA on day 0.

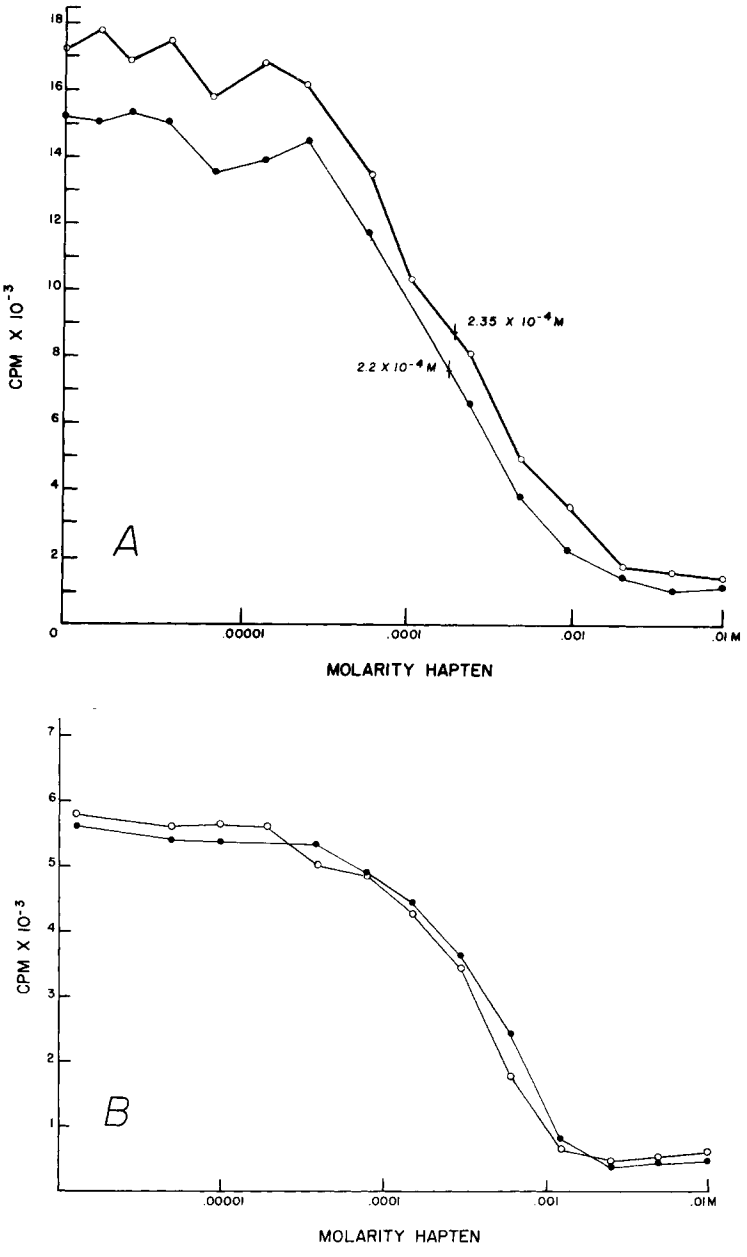

Fig. 1. Affinity measurements of anti-hapten antibodies from mice pretreated with anti-Ts^d or NMS and KLH·TNP or KLH·Ars. Ten mice immunized with TNP (frame A) and αTs^d (0) or NMS (0) showed average affinity on pooled sera at day 10 of 2.35×10^{-4}M or 2.2×10^{-4}M, respectively, when 10 ng of antibody was blocked with free hapten. The average affinities of 10 anti-Ars producing mice pretreated with Ab (0) or NMS (0) was indistinguishable at 4.5×10^{-4}M when 10 ng of day-10 pooled sera was blocked with free hapten.

tion raised by these studies is whether the mechanisms which regulate major dominant idiotypes also apply to disperse clones of cells with idiotypes recurring too infrequently to measure. One might alternatively argue that normal mechanisms which regulate the immune response may be deficient in these mice permitting the observed expression of unusually high numbers of related clones.

This study utilizes antiserum produced against a cell which regulates the anti-Ars IdX, and which appears to be specific for a T-cell receptor bearing anti-idiotype determinants. The antiserum has been shown previously to recognize a conserved determinant outside the antigen binding site which may represent a T-cell constant region marker [9]. We have shown previously that this antiserum, largely λ_1, when injected in vivo can induce T suppressor cells for the primary SRBC response [10]. This study suggests that Ts^d is in effect a polyclonal activator for a functionally restricted subpopulation of cells. The results shown here further confirm that the precursor cells which suppress all T-dependent responses we have measured, independent of clonotype, isotype, or affinity restrictions, share Ts^d as a surface determinant.

It is possible that the cells which control the immune response to T-dependent antigens are regulated more easily than the B cells responding to T-independent antigens. Therefore, the differences seen may be quantitative and not absolute. The results do suggest that the target of suppression is preferentially a T-cell. Whether the cell triggered initially with the antiserum is a suppressor cell or an inducer of that suppressor has not been shown. The NZB animal, which may be defective in the Ly1, 2, 3 antigen specific feedback suppressor, [29] appears to be fully competent in the T-cells expressing Ts^d. Therefore, the T-cell expressing Ts^d may be analogous to the Ts2 cell recently described as an acceptor for the antigen specific signal [30].

It seems probable therefore that similar precursor cells regulate the major dominant idiotypes and are also responsible for regulation of smaller clones, difficult to measure serologically. That is, each individual idiotype may have a mirror image anti-idiotype-bearing Ts cell regardless of its initial clone size. That anti-Ts^d appears to act as a polyclonal activator rather than an idiotype specific regulatory molecule is probably determined by the site of binding to the receptor. Anti-Ts^d may bind to a constant part of the T-cell receptor outside the binding site for idiotype on all Ts cells while anti-idiotype antibodies may trigger idiotype bearing Ts or Th cells which secondarily induce Ts AID bearing cells for only the clone triggered by anti-idiotype.

The availability of monoclonal antibodies directed against a determinant(s) recognized in this strain combination would greatly facilitate our own studies and lead to a reagent of general usefulness in other laboratories. A major effort is now in progress to produce and screen for such a reagent.

ACKNOWLEDGMENT

This work was supported by NIH grant AI-156262.

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