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Lack of immune response gene control for induction of epitope-specific suppression by TGAL antigen

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Immune response genes in the *I* region of the mouse major histocompatibility complex (MHC) have been divided operationally according to whether they control responses to antigens which do or do not induce active suppression in non-responder animals (IS and IR genes respectively)[1,2]. Studies presented here, however, show that the synthetic terpolymer poly-L-(tyrosine, glutamic acid)-poly-DL-alanine-poly-L-lysine

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Table 1 C3H (Ir-1a) mice are 'non-responders' to TGAL

Strain	TGAL immunizations*		Anti-TGAL response (normalized)* IgM	IgG1	IgG2a	IgG3
C3H	Primary	TGAL	18	2	<3	5
	Primary	TNP-TGAL	7	1	<3	2
	Secondary	TNP-TGAL, TNP-TGAL	7	1	<3	2
C3H.SW	Primary	TGAL	100	100	100	100
	Primary	TNP-TGAL	93	113	65	102
	Secondary	TNP-TGAL, TNP-TGAL	51	319	74	115
	Secondary	TGAL/TNP-TGAL	40	409	85	116

Responses measured by radioimmunoassay (RIA)[20,21] 2 weeks after the last indicated immunization are reported relative to the anti-TGAL primary response of each isotype obtained in C3H.SW mice.

* See Table 4 legend for antigen dosage and immunization schedule.

(TGAL)[3,4], commonly considered the prototype of antigens which do not induce suppression, actually serves as a potent inducer of epitope-specific suppression for antibody responses in non-responder mice.

The induction of epitope-specific suppression is a general (albeit recently recognized) immunoregulatory process readily demonstrable by sequential immunizations with carrier proteins and hapten–carrier conjugates[5–7]. Primary immunization with a carrier protein induces the delayed appearance of carrier-specific suppressor T cells (CTS)[8,9] which persist and induce specific suppression for IgG antibody responses to 'new' epitopes presented subsequently on the priming carrier. The 'epitope-specific' mechanism responsible for this suppression also persists (once induced) and continues to suppress antibody responses to the 'new' epitope, whether presented subsequently on the priming carrier or on a second, unrelated carrier molecule. (We have previously referred to this system as hapten specific, using the term 'hapten' in its more general sense (synonymous with epitope) to indicate a relatively small structure which induces antibody production when presented on a larger (carrier) molecule. The term 'hapten', however, is also commonly used to distinguish artificially added structures, such as the dinitrophenyl group (DNP), from the native epitopes on a carrier molecule (antigen). Therefore, to avoid ambiguity, we have now substituted the term 'epitope-specific' for the previous nomenclature.)

For example, priming with keyhole limpet haemocyanin (KLH) and subsequent immunization with the DNP-KLH (dinitrophenyl hapten conjugated to KLH) induces specific suppression for IgG anti-DNP antibody production[5–9]. This KLH/DNP-KLH-induced suppression then serves with equal effectiveness to prevent antibody responses to DNP presented either on KLH or on chicken γ-globulin (CGG). It does not, however, interfere with antibody production to either carrier molecule.

Reversing the carriers in the immunization sequence yields similar results, that is, CGG/DNP-CGG-induced suppression also prevents subsequent anti-DNP responses to either DNP-KLH or DNP-CGG. Mismatching carriers (such as KLH/DNP-CGG immunization) results, however, in anti-DNP antibody production rather than suppression (ref. 6 and L.A.H. and T.T., in preparation). Thus epitope-specific suppression is induced by carrier-specific interactions but is mediated by an effector mechanism that operates without reference to the carrier on which a hapten (epitope) is presented.

Further selectivity displayed by this effector mechanism generates a characteristic anti-DNP response pattern in suppressed animals: high-affinity anti-DNP antibody production is preferentially suppressed; IgM anti-DNP responses are unaffected; IgG1 responses are usually suppressed initially and generally 'escape' suppression after a second or third stimulation with DNP; and the remaining IgG isotypes (IgG2a, IgG2b, IgG3) are universally suppressed at first and generally require three or four sequential stimulations with DNP to induce substantial escape from suppression (ref. 6 and L.A.H. and T.T., in preparation). Therefore, epitope-specific suppression is recognizable by both its specificity for the inducing hapten and its differential effects on the various immunoglobulin isotype responses produced to that hapten.

Table 2 Anti-TNP responses to TNP-TGAL are suppressed in C3H.SW ('responder') mice preimmunized with TGAL and fail entirely in C3H ('non-responder') mice

Strain	Immunization(s)*	Anti-TNP response IgM (U)	IgG1 (μg ml⁻¹)	Ig2a (μg ml⁻¹)	IgG3 (U)
C3H	TNP-TGAL	144	<3	<1	10
	TNP-TGAL/ TNP-TGAL	191	<15	<3	11
	TGAL/TNP-TGAL	106	<3	<1	8
C3H.SW	TNP-TGAL	125	35	13	63
	TNP-TGAL/ TNP-TGAL	86	95	16	76
	TGAL/TNP-TGAL	96	<3	<9	12

Anti-TNP responses were measured by RIA 2 weeks after last indicated immunization. IgG1 and IgG2a responses are presented as μg antibody per ml serum; IgG3 and IgM responses as percentage of response obtained in an adoptive secondary 'standard' antiserum.

* See Table 4 legend for antigen dosage and immunization schedule.

Table 3 TGAL/TNP-TGAL immunization induces persistent epitope-specific suppression for IgG anti-TNP antibody production in C3H ('non-responder') mice

Strain	Pre-immunization*	Serum IgG2a anti-TNP (μg ml⁻¹) Subsequent immunizations* (first) TNP-KLH	(second) TNP-KLH	(third) TNP-CGG
C3H	None	35 (2)	68 (60)	85 (40)
	TNP-TGAL	28 (7)	82 (60)	70 (30)
	TGAL/TNP-TGAL	<3	9 (<1)	16 (6)
C3H.SW	None	24 (7)	110 (50)	150 (50)
	TNP-TGAL	250 (140)	370 (220)	160 (50)
	TGAL/TNP-TGAL	14 (<1)	180 (70)	160 (50)

Anti-TNP levels in serum were measured by RIA[20,21] 2 weeks after each indicated immunization. Relative affinity (shown in parentheses) is calculated from ratios of antibody bound to differently substituted TNP conjugates (TNP7-BSA/TNP24-BSA) based on a standard curve developed for absolute measurements of anti-DNP binding affinities (K_a)[21].

* Antibody responses to TGAL and TNP-TGAL in these animals are shown in Tables 1 and 2 respectively. See Table 4 legend for antigen dosage and immunization schedule.

We have now used similar immunization sequences and response criteria in MHC congenic mice to investigate the influence of IR genes on epitope-specific suppression induction by TGAL. The C3H (Ir-1a) mice used are typically 'non-responsive' to the TGAL antigen[3,4] in that they produce no detectable IgG anti-TGAL responses and only a small IgM anti-TGAL response, even when stimulated twice with 100 μg TGAL on alum. The congenic C3H.SW (Ir-1b) mice, in contrast, produce both IgM and IgG anti-TGAL primary antibody responses and show a strong IgG secondary response to this antigen (see Table 1). Similarly, C3H mice do not produce detectable amounts of IgG antibody to the trinitrophenyl (TNP) hapten presented on TGAL whereas C3H.SW mice stimulated with TNP–TGAL produce typical primary and secondary anti-TNP responses (see Table 2).

Surprisingly, this classical difference in responsiveness to TGAL disappears when response is measured in terms of epitope-specific suppression induction by TGAL/TNP–TGAL immunization. Both the 'non-responder' and 'responder' mice respond strongly to this immunization sequence by developing a persistent suppression for IgG antibody production to TNP (see Tables 3 and 4). In fact, the 'non-responder' strain (C3H) responds even more vigorously than the 'responder' in that its

Table 4 Epitope-specific suppression in C3H mice shows typical isotype selectivity

Strain	Immunizations				% Of control anti-TNP response*			
					IgM	IgG1	IgG2a	IgG3
C3H	TGAL	TNP-TGA	TNP-KLH		110	19	<11	10
	TGAL	TNP-TGAL	TNP-KLH	TNP-KLH	86	120	11	25
	TGAL	TNP-TGAL	TNP-KLH	TNP-CGG	53	200	23	10
C3H.SW	TGAL	TNP-TGAL	TNP-KLH		120	16	6	50
	TGAL	TNP-TGAL	TNP-KLH	TNP-KLH	94	100	47	20
	TGAL	TNP-TGAL	TNP-KLH	TNP-CGG	44	70	100	13

Immunizations of 100 μg each antigen were given intraperitoneally on alum at 0, 5, 7, 10 and 14 weeks respectively.
* Serum anti-TNP levels were measured by RIA[20,21] 2 weeks after last indicated immunization. Data are presented as the percentage of the anti-TNP response obtained in corresponding control groups immunized initially with TNP-TGAL (once) rather than with the TGAL/TNP-TGAL sequence. Absolute control responses for IgG2a are shown in Table 3.

IgG2a and IgG3 anti-TNP responses generally remain suppressed following repeated stimulation with TNP (on CGG and KLH) whereas these isotype responses escape suppression in typical fashion in the 'responder' (C3H.SW) strain (see Table 4). Thus 'non-responder' animals clearly recognize TGAL in an immunological context and can use this antigen as a carrier for presenting haptens to initiate the induction of epitope-specific suppression.

These findings suggest that TGAL is capable of generating functional carrier (TGAL)-specific suppressor T cells which induce epitope-specific suppression for TNP on TGAL (as KLH-specific suppressor T cells initiate the induction of epitope-specific suppression in KLH-primed mice)[8,9]. Thus although the IR gene controlling responses to TGAL largely prevents TGAL-dependent stimulation of T-cell proliferation and IgG antibody production, it does not seem to interfere with the full set of (TGAL-dependent) T-cell interactions involved in the development of carrier-specific suppressor T cells[10–15].

Therefore, in addition to demonstrating that both IR and IS genes permit suppression induction, our results indicate that certain TGAL-specific T-cell pathways (not readily demonstrable by T-cell proliferation assays) remain intact in 'non-responder' mice, thereby suggesting several possible roles for the apparently functionless TGAL-specific T-cell clones recently isolated from 'non-responder' mice[16].

Finally, the unhampered induction of epitope-specific suppression in 'non-responder' mice suggests that the epitope-specific system may provide a versatile effector mechanism which permits *I*-region genes to exert a selective, long-term influence on the magnitude and heterogeneity of antibody responses. This would explain how certain of these genes can modify the composition of antibody responses to epitopes on antigens that induce some antibody production in 'non-responder' animals[17,18]. The restricted heterogeneity of these antibody responses, the tendency towards production of IgG1 rather than IgG2a antibodies and the apparent involvement of suppressor T cells in the regulatory process clearly point to epitope-specific regulation. Thus we suspect that diagnostic sequential immunization protocols similar to those used here would reveal the induction of epitope-specific suppression in these partially responsive mice.

TGAL and TNP–TGAL, unlike the antigens discussed above, do not induce substantial IgG antibody production in non-responder mice (see Tables 1, 2). Furthermore, a single initial immunization with TNP–TGAL does not appear to affect subsequent anti-TNP responses to TNP on other carriers (see Table 3). At face value, therefore, the evidence presented here suggests that epitope-specific suppression induction by antigens like TNP–TGAL requires prior TGAL priming (or perhaps two sequential immunizations with the TNP–TGAL conjugate). However, should TNP–TGAL immunization prove to induce B-cell memory for TNP in 'non-responders' (work in progress), this conclusion would have to be modified since the presence of such memory B cells in TNP–TGAL-primed animals would indicate that the primary level anti-TNP response observed to TNP–KLH in these animals actually represents the weak suppression of a potential secondary anti-TNP response.

That the epitope-specific regulatory system may serve to maintain antibody response characteristics dictated initially by *I*-region-defined mechanisms is consistent with evidence from studies showing that this system maintains suppression for responses prevented initially by the allotype suppression mechanism[19] and mediates suppression initiated by carrier-specific suppressor T-cell interactions[8,9]. These data suggest that the epitope-specific system provides a common effector mechanism through which non-responsiveness, whatever its genesis, can be maintained.

Furthermore, because the epitope-specific system suppresses responses to individual epitopes regardless of the carrier on which the epitope is subsequently presented, it offers a general mechanism through which undesirable antibody responses (such as to self) can be controlled should an epitope to which non-responsiveness has been induced be encountered later on an immunogenic carrier. Thus this previously unrecognized regulatory system introduces a novel immunological memory mechanism capable of being 'educated' selectively to suppress antibody production to individual epitopes according to conditions in the regulatory environment when the epitope is first introduced.

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