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Epitope-Specific Regulation: A Brief Overview*†

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Our previous studies (Herzenberg *et al.*, 1980a,b, 1982a,b, 1983a,b,c; Herzenberg and Tokuhisa, 1981, 1982) have shown that immunizing carrier-primed animals with a "new" epitope (hapten) coupled to the priming carrier severely compromises subsequent antihapten antibody responses. This immunization schedule induces fully competent memory B-cell populations that produce strong IgG antihapten responses in adoptive cotransfer assays (Table I).* Nevertheless, despite the presence of these antihapten memory cells, "carrier/hapten–carrier" immunized animals produce very little IgG antihapten antibody, even when reimmunized several weeks later with the initial hapten-carrier conjugate or with the initial hapten coupled to an unrelated carrier protein (Table II).

The diminished responsiveness in these animals is highly selective; it specifically affects IgG antibody production to the "new" hapten introduced on the carrier molecule in the immunization sequence and shows no discernable influence on antibody production to other carrier-borne epitopes. As Table II shows, *in situ* primary and secondary IgG anti-DNP antibody responses tend to remain below primary level when KLH-primed animals are immunized with DNP-KLH and then immunized again with either DNP-KLH or DNP-CGG. IgG anti-KLH responses, in contrast, rise to normal (secondary and tertiary) response

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†My co-workers in various aspects of these studies include Kyoko Hayakawa, R. R. Hardy, David Parks, Wayne Moore, and L. A. Herzenberg, Genetics Department, Stanford; Mathew Waldor and Lawrence Steinman, Pediatrics Department, Stanford; and Takeshi Tokuhisa, Masatoshi Tagawa, and Masaru Taniguchi, Chiba University Medical School, Chiba, Japan.

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TABLE I

Epitope-Specific Suppression Prevents Memory B-Cell Expression

	Immunization(s) (100 μg on alum)			IgG2a anti-DNP responses[a] [μg/ml (K)]		
	Day 0	Day 42	Day 84	Primary (*in situ*) day 14	Secondary (*in situ*) day 96	Memory B in spleen[b] (adoptive secondary) transfer on day 21
—	DNP-KLH	DNP-KLH		35 (5)	140 (100)	73 (10)
KLH	DNP-KLH	DNP-KLH		<1 (<1)	3 (<1)	75 (8)

[a]RIA 2 weeks after last indicated immunization: $K = K\,M^{-1} \times 10^6$.

[b]Splenic B cells (T-depleted spleen) plus KLH primed T plus 1 μg aqueous DNP-KLH transferred to 600 R irradiated recipients. RIA 2 weeks after transfer.

levels with each successive KLH stimulation. Similarly, IgG anti-CGG responses reach normal primary levels when KLH/DNP-KLH-immunized animals are stimulated with DNP-CGG. Thus, unprimed, KLH-primed, DNP-KLH-primed, and KLH/DNP-KLH-immunized mice produce comparable antibody responses to all epitopes on DNP-CGG except the DNP hapten itself.

These findings, repeated under a variety of conditions in different mouse strains and with different antigens (Herzenberg *et al.*, 1982a, 1983b), demonstrate that the immune system can selectively prevent (suppress) the expression of memory B cells committed to producing antibodies specific for individual epitopes on complex antigens. Furthermore, they demonstrate that carrier-specific interactions initiate the induction of this "epitope-specific" suppression but that the suppression itself is mediated by a mechanism that operates independently of the carrier molecule on which an epitope is presented, i.e., once induced (by KLH/DNP-KLH immunization), it suppresses antibody-production to DNP on DNP-CGG or DNP-KLH.

Data from a more recent series of studies (still in progress) suggest that epitope-specific suppression can also be induced for IgG responses to protein epitopes. Using a modified carrier/hapten–carrier immunization protocol in which (responder) animals were immunized sequentially with GAT and GAT-MBSA or MBSA and GAT-MBSA, Drs. Carl Waltenbaugh and Huan-yao Lei at Northwestern University (Chicago, Illinois) showed that antibody responses to the "new" epitopes

*Abbreviations: KLH, keyhole limpet hemocyanin; CGG, chicken γ globulin; DNP, dinitrophenylhapten.

TABLE II

Epitope-Specific Suppression Induced by Presenting "New" Epitopes on a Carrier Protein To Which the Animal Has Already Been Primed

Immunizations (100 μg each antigen on alum)			IgG2a antibody responses[a]		
Day 0	Day 42	Day 84	Anti-DNP (μg/ml)	Anti-KLH (units)	Anti-CGG (units)
KLH	DNP-KLH[b]	DNP-KLH	<1	330	
—	DNP-KLH[b]	DNP-KLH	140	120	
—	—	DNP-KLH[b]	25	7	
KLH	DNP-KLH[b]	DNP-CGG	5		7
—	DNP-KLH[b]	DNP-CGG	70		8
—	—	DNP-CGG[b]	20		10

[a]Measured in serum by radioimmune assay (RIA) on day 96.

[b]Normal anti DNP memory B-cell activity present 3 weeks after indicated immunization (detected by cotransferring splenic B cells and KLH primed T cells to 600 R irradiated recipients given 1 μg DNP-KLH at time of transfer; see Table I).

are selectively impaired in each case, i.e., to MBSA in GAT/GAT-MBSA-immunized animals and to GAT in MBSA/GAT-MBSA-immunized animals (Table III). Thus, as a general rule, epitope-specific suppression appears to be induced for responses to epitopes introduced on a carrier to which the animal has already been primed.

The mechanism responsible for the induction of suppression under these conditions has yet to be defined precisely; however, Drs. Takeshi Tokuhisa, Masatoshi Tagawa, and Masaru Taniguchi at Chiba University (Chiba, Japan) have shown that carrier-specific suppressor T cells and factors (Tada and Okumura, 1979; Taniguchi *et al.*, 1979) injected in conjunction with the appropriate hapten–carrier conjugate can initiate the epitope-specific suppression (Herzenberg and Tokuhisa, 1981, 1982; Herzenberg *et al.*, 1983b, and manuscripts in preparation). Therefore, it is reasonable to suspect that the maturation of these carrier-specific regulatory cells shortly after priming with a carrier molecule (Sercarz *et al.*, 1978) contributes to the *in situ* induction of suppression for antibody responses to "new" epitopes introduced on the priming carrier.

This concept would appear to have little import for ordinary antibody responses since it derives from carrier/hapten–carrier immunization studies that intentionally distort the time at which a carrier-borne epitope is presented relative to the time at which carrier priming is completed. However, the clear separation between carrier priming and

TABLE III

Epitope-Specific Suppression Induced by Presenting "New" Epitopes on a Carrier Protein To Which the Animal Has Already Been Primed[a]

	Immunizations[b]		Ag-specific PFC per spleen (× 10^3)	
			GAT	MBSA[c]
Experiment I				
BALB/c	—	GAT-MBSA	16 + 3	NT
BALB/c	MBSA	GAT-MBSA	2 + 1	NT
BALB/c	MBSA	GAT	26 + 2	NT
Experiment II				
BALB/c	—	GAT	13 + 5	NT
BALB/c	—	GAT-MBSA	12 + 1	26 + 1
BALB/c	GAT	GAT-MBSA	10 + 2	11 + 1
BALB/c	MBSA	GAT-MBSA	3 + 2	28 + 1

[a]Studies by Drs. Carl Waltenbaugh and Huan-yao Lei, Northwestern University, Chicago.
[b]Mice in Experiment I were primed with 100 μg MBSA and challenged with 20 μg GAT or GAT-MBSA with Maalox and pertussis. In Experiment II mice were primed with 10 μg GAT or MBSA and challenged with 10 μg GAT or GAT-MBSA with Maalox and Pertussis.
[c]Methylated bovine serum albumin (MBSA).

epitope presentation possible with this protocol basically permits exploration of a time-dependent process that, in principle, occurs whenever animals are primed with a complex antigen; that is, since it is likely that some epitopes on the antigen will induce antibody production more rapidly than others, it is quite possible that certain epitopes will effectively be treated as if they were "new" epitopes on the carrier protein because they fail to make their presence known (by initiating antibody production) before the carrier-specific suppressor population has matured to full function (Herzenberg *et al.*, 1982a, 1983b).

This can be understood as follows: in general, a certain proportion of epitopes introduced initially on a priming carrier initiate stable antibody production. Thus, the subsequent emergence of carrier-specific suppressor cells that can induce epitope-specific suppression for carrier-borne epitopes usually has no effect on these established antibody responses. DNP, for example, appears to be a particularly potent epitope in this sense in that it almost always induces stable antibody production when introduced initially on a carrier molecule.

Other epitopes, however, tend to be less efficient in inducing antibody production. As immunogeneticists well know, a certain proportion of the epitopes on a given antigen never succeed in inducing anti-

body production, even after repeated immunizations with the antigen at optimal doses. The responses to these epitopes, we suggest, are initiated more slowly and tend frequently to fall prey to the emerging carrier-specific suppressor cell population (Herzenberg *et al.*, 1982a, 1983b). Thus, this population can be viewed as a kind of scavenger that goes about inducing suppression for antibody responses to all epitopes that did not succeed in initiating antibody production within the first few days of priming.

The activity of such scavengers would also explain the epitope-specific suppression that occurs following primary immunization in animals that either lack B cells or lack the ability to initiate rapidly an antibody response from the B-cell populations they have. Our earlier studies demonstrated this type of suppression induction in young allotype-suppressed mice temporarily unable to produce (Igh-1b) antibody responses (Herzenberg *et al.*, 1983a). Later we demonstrated similar suppression induction in nonresponder mice immunized with TNP coupled to an antigen (TGAL) under IR gene control. And, most recently (in studies still in progress in collaboration with Mathew Waldor and Dr. Lawrence Steinman, Pediatrics Department, Stanford Medical School), we showed that epitope-specific suppression is induced in anti-I-A-treated mice primed and boosted with DNP-KLH while anti-I-A is present and B cells are largely depleted (see Table IV and Fig. 1 and 2). In each of these cases, the suppressed antibody responses appear to be defined by the antibody responses that fail initially (in comparison with controls).

TABLE IV

Epitope-Specific Suppression for Anti-DNP Responses Induced by Immunizing Anti-I-A-Treated Mice with DNP-KLH[a]

Treatment[b] (4 × 0.5 ml)	Immunization(s)		IgG1 antibody responses	
	2 × 100 μg	100 μg	Anti-DNP (μg/ml)	Anti-CGG (units)
Anti-I-A	DNP-KLH	DNP-CGG	130	30
No	DNP-KLH	DNP-CGG	1700	36
No	No	DNP-CGG		54

[a]Studies in collaboration with Mr. Mathew Waldor and Dr. Lawrence Steinman, Pediatrics Department, Stanford University Medical School.

[b]Treatments and immunizations: Anti I-A injected on days 0, 2, 7, and 9; 0.5 ml (4 mg) moNoclonal anti I-A ascites fluid (10-3.6) each time; DNP-KLH injected on days 1 and 8; DNP-CGG injected on day 62; 100 μg indicated antigen on alum ip each time; 5 animals/group; serum samples taken on day 76.

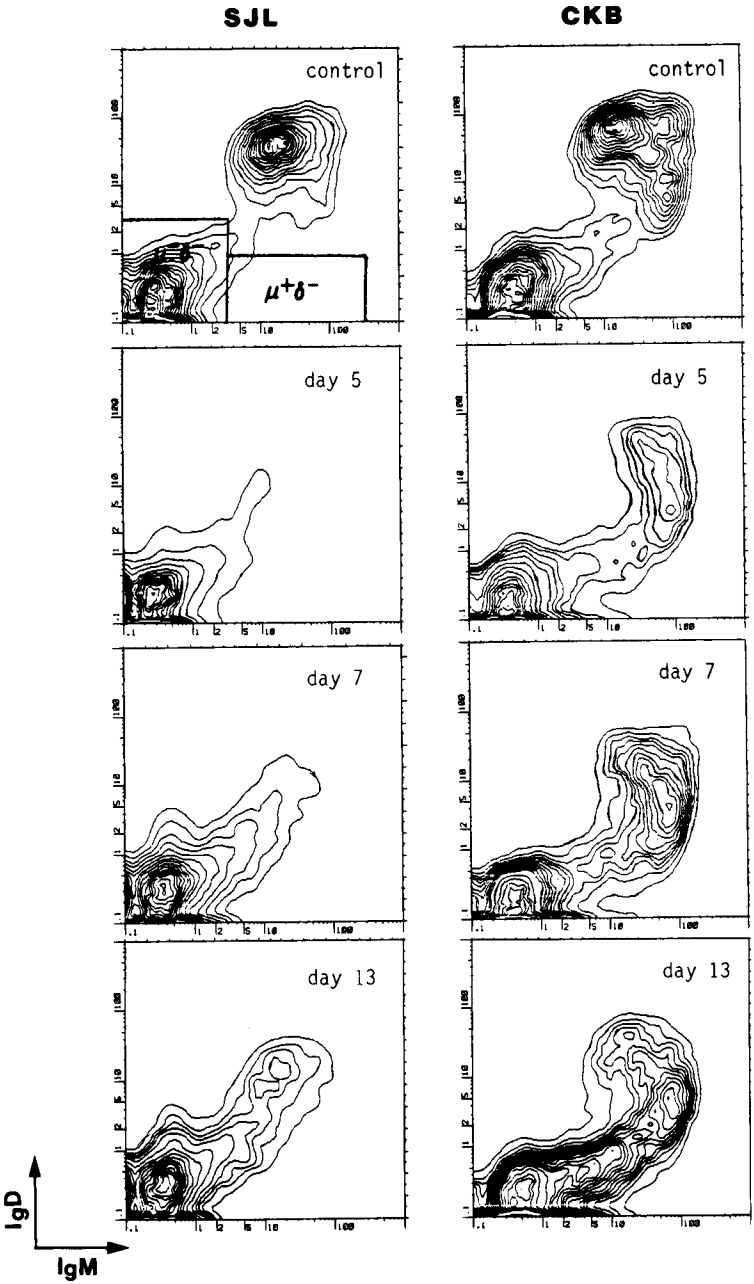

Fig. 1. Depletion and recovery of splenic B cells in adult SJL/J and CKB mice following a single injection of anti-I-A antibody. Spleen cells were stained with fluorescein anti-IgM together with biotin anti-Igh-5^b (IgD-b allotype) plus Texas Red avidin and analyzed on a dual-laser FACS equipped with the respective antigens as revealed by fluorescence intensity. The integration boundaries used to determine the frequency of IgM^+,IgD^- and IgM^-,IgD^- cells (autofluorescence background) are marked onto the plot.

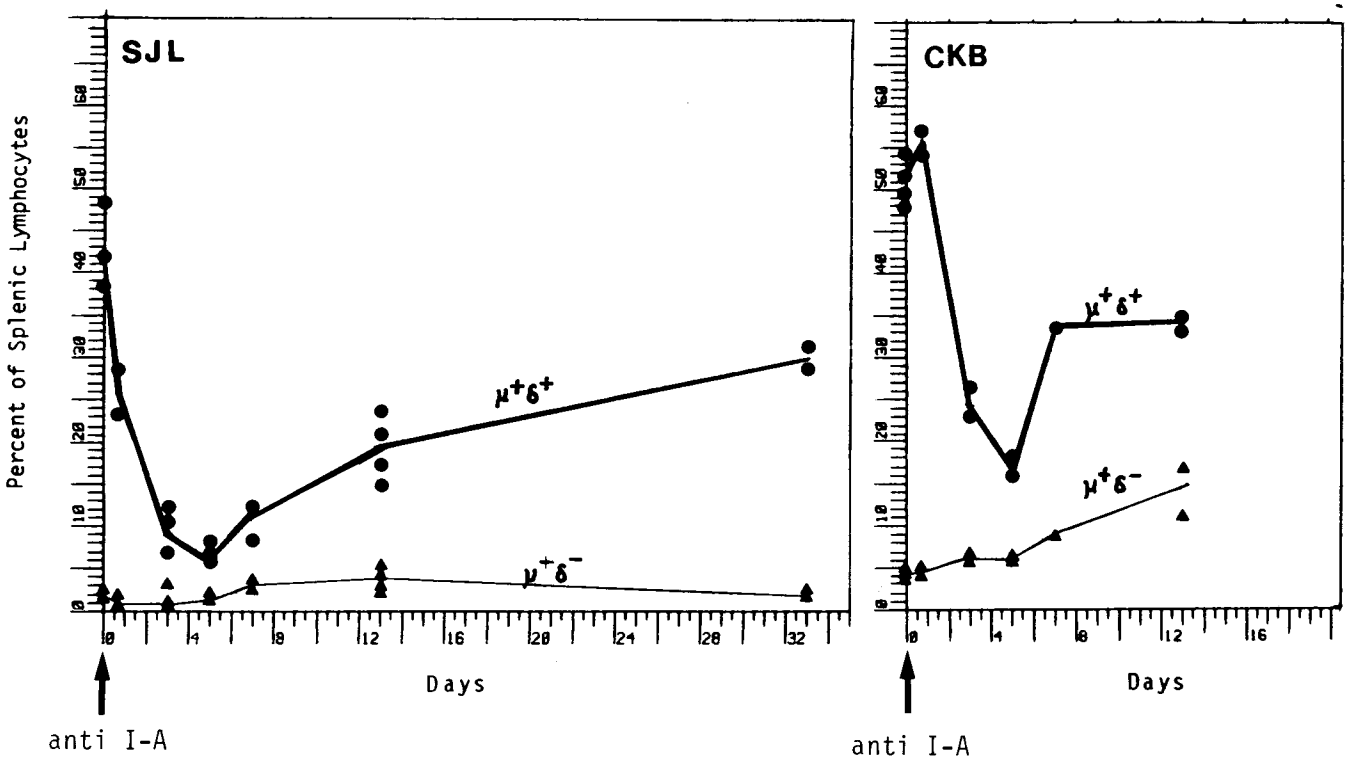

Fig. 2. Frequency of splenic B cells following a single injection of anti-I-A antibody that bear either IgM alone (▲) or both IgM and IgD (●) as determined by two-color FACS analysis. The frequency of IgM^+,IgD^+ cells was obtained by subtracting the frequency of IgM^+,IgD^- cells from the frequency of cells above the autofluorescence background (as defined by the integration boundary for double-negative cells shown in Fig. 1).

Thus, in essence, we now view antibody responses as being controlled by a central, epitope-specific regulatory system that determines whether individual memory B cells contribute to a given antibody response. Our evidence suggests that this system is bistable in that it can be induced either to support (protect) or suppress antibody production and will then tend to maintain itself as initially induced (Herzenberg *et al.*, 1982a, 1983b). Therefore, it appears to serve neutrally as a mechanism that tends to perpetuate the characteristics of the initial response to a given epitope. The integrated regulatory circuit model that we proposed some time ago (Herzenberg *et al.*, 1980a) offers a set of cell interactions that could account for the bistable properties of the epitope-specific system and many of its other properties.

The cells that actually mediate epitope-specific regulation, however, have yet to be defined. An economical hypothesis would locate this mechanism within the cell populations known to be responsible for idiotype-specific regulation, since the cells in such populations clearly have the requisite specificity for specifically preventing the expression of individual memory B cells. This defies dogma in a certain sense since there is no evidence that idiotype-specific suppression can be induced by antigen-specific interactions (as opposed to antiidiotype-based induction mechanisms). This obstacle could be overcome either by viewing idiotype-suppression induction as antigen mediated in some cases or by viewing the antigen-dependent induction of epitope-specific suppression as an indirect mechanism that ultimately depends on idiotype–antiidiotype interactions. Both possibilities are consistent with current evidence.

In any event, the studies of epitope-specific suppression that we have already completed introduce a new perspective on the mechanisms regulating IgG antibody responses and provide a substantially altered framework for interpreting evidence from previous and current immunoregulatory studies. At a theoretical level, we expect that many surprises remain. At a practical level, however, we suggest that many of the surprises could be avoided by taking the findings we have presented into account, particularly in clinical settings where improved immunization procedures and methods for suppressing deleterious antibody responses are currently being sought.

In a somewhat lighter vane, we can summarize the evidence we have discussed by the following homilies:

1. Bistable regulation: A suppressed response tends to stay suppressed whereas an ongoing response tends to keep on going.

2. Specificity: One mouse's epitope can be another mouse's carrier.
3. Timing: When it comes to B cells, he who hesitates is lost.
4. Theories: The light at the end of the tunnel may be the headlight of an oncoming train. Time will tell.

References

Hardy, R. R., Hayakawa, K., Parks, D. R., and Herzenberg, L. A. (1983). *Nature (London)* (in press).

Hayakawa, K., Hardy, R. R., Parks, D. R., and Herzenberg, Leonore A. (1983). *J. Exp. Med.* **157,** 202.

Herzenberg, L. A., and Tokuhisa, T. (1981). *In* "Immunoglobulin Idiotypes and Their Expression, ICN-UCLA Symposia on Molecular and Cellular Biology, Volume XX" (C. Janeway, E. E. Sercarz, H. Wigzell, and C. F. Fox, eds.). Academic Press, New York.

Herzenberg, L. A., and Tokuhisa, T. (1982). *J. Exp. Med.* **155,** 1730.

Herzenberg, L. A., Black, S. J., and Herzenberg, L. A. (1980a). *Eur. J. Immunol.* **10,** 1.

Herzenberg, L. A., Tokuhisa, T., and Herzenberg, L. A. (1980b). *Nature (London)* **285,** 664.

Herzenberg, L. A., Tokuhisa, T., Parks, D. R., and Herzenberg, L. A. (1982a). *J. Exp. Med.* **155,** 1741.

Herzenberg, L. A., Hayakawa, K., Hardy, R. R., Tokuhisa, T., Oi, V. T., and Herzenberg, L. A. (1982b). *Immunol. Rev.* **67,** 1.

Herzenberg, L. A., Tokuhisa, T., and Hayakawa, K. (1982c). *Nature (London)* **295,** 329.

Herzenberg, L. A., Tokuhisa, T., and Herzenberg, L. A. (1983a). *Eur. J. Immunol.* (in press).

Herzenberg, Leonore A., Tokuhisa, T., and Hayakawa, K. (1983b). *Annu. Rev. Immunol.* **1,** 609.

Sercarz, E. E., Yowell, R. L., Turkin, D., Miller, A., Araneo, B. A., and Adorini, L. (1978). *Immunol. Rev.* **39,** 109.

Tada, T., and Okumura, K. (1979). *Adv. Immunol.* **28,** 1.

Taniguchi, M., Saito, T., and Tada, T. (1979). *Nature (London)* **278,** 555.