TITLE: Hapten-specific Regulation of Heterogeneous Antibody Responses: Intersection of Theory and Practice

L. A. HERZENBERG, T. TOKUHISA, D. R. PARKS

and L. A. HERZENBERG

Department of Genetics

Stanford University School of Medicine

Stanford, California, 94305, USA

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This opportunity to acknowledge our intellectual debt to the particular genious of Niels Jerne is especially timely because we have just completed a series of studies showing that 'hapten-specific' mechanisms akin to idiotype regulation control the spectrum of antibody combining sites and isotypes produced in 'ordinary' antibody responses (e.g., to the dinitrophenyl hapten (DNP)) (1). Our work in this area, which introduces a substantially new perspective on the mechanisms regulating heterogeneous antibody responses, has been strongly influenced by concepts of regulatory interactions involving the kinds of idiotype-anti-idiotype recognitions proposed by Jerne and colleagues (2) and by the evidence and ideas from idiotype suppression studies that grew from these proposals (3-5). Thus we see the following discussion essentially as offering fruit (hopefully sweet) from the orchard Prof. Jerne thoughtfully planted many years ago.

In a recent theoretical article (6) that preceded inception of our current studies, we had combined and reassorted information available from a variety of idiotype and allotype regulatory systems to construct a model of antibody-response regulation based on 'circuits' of interactions among regulatory and regulated cells. This model proposed a system of central (Core) 'circuits,' each composed of two helper and two suppressor T cells connected by cognitive interactions that include complementary Vh (combining site) interactions such as those involved in idiotype regulation (see Fig.1) and Ig heavy chain constant region recognitions such as those apparently involved in allotype suppression (not shown in Fig.1). Individually, each of these circuits provides the basic 'on-off' regulation for production of antibodies carrying a particular antibody-combining site coupled to a particular Ig heavy chain (isotype/allotype). Thus together, they regulate the composition of a typical heterogeneous antibody response.

The decision as to whether a given Core circuit permits or suppresses production of the antibody it regulates is partially internal to the circuit. In addition, it depends on the relative strengths of positive and negative signals transmitted to the Core circuit from a series of functionally distinct auxiliary regualatory circuits that directly sense the antigenic environment. For example, an auxiliary circuit containing carrier-specific helper T cells can be activated by antigen to issue signals that favor antibody production. This model thus places Ig-specific regulation proximal to the B cell being regulated and establishes a system whereby a variety of positive and negative auxiliary stimulatory signals are integrated (by the Core circuits) to determine whether one or more of the possible antibody populations shall be represented in a response.

The theoretical configuration we suggested for the cells in the Core circuit endow it with a novel regulatory capabilty prophetic for our studies on the 'hapten-specific' supression described below. By allowing each suppressor cell in the circuit to attack one of the helper cells and be helped (to differentiate and expand) by the other (see Fig. 1), we arrived at a set of cell interactions that approximates the behavior of an electronic binary ('flip-flop') circuit in that the circuit tends to stabilize in a help or suppression mode but nevertheless can shift to the opposite configuration in response to a dramatic shift in stimulatory conditions.

We would be pleased to claim that the experiments uncovering the 'hapten-specific' regulatory system and showing that it approximates the behavior of the the model circuits were actually instigated by ideas generated during our 'theoretical exercise'; but, if truth be told, these experiments had a much more mundane beginning. They began, quite simply, as an attempt to explain a suprising anti-hapten response failure in a group of control animals added to an experiment mainly to assure ourselves that carrier-primed animals would make the generally expected augmented anti-hapten response when stimulated subsequently with a hapten on the priming carrier. Contrary to expectations, these animals produced both a smaller and lower affinity anti-hapten response than animals exposed only to the hapten-carrier conjugate (1).

Of course, we should should not have been surprised, since both

Rajewsky et al. (7) and Mitchison et al. (9), in the first papers demonstrating carrier-specific help interactions, reported that *in situ* anti-hapten responses fail when mice are exposed first to a carrier protein and then to hapten on that carrier (i.e., to what we call the "carrier/hapten-carrier" immunization sequence); however, like most immunologists, we had removed this odd response failure from the "anomalies to be remembered" category some time ago because it appeared to be due to interference with (what we would now call) anti-hapten memory B cell development (10). Thus, being unprepared for the results we obtained, we inadvertently reopened an old question; and, being armed with a modern arsenal of techniques for measuring both memory B cell development and the amount and affinity of each isotype represented in *in situ* responses from a large number of individual animals (11), wound up some six months later with new evidence excluding any substantial interference with B cell priming and demonstrating instead that the observed non-responsiveness is due to the carrier-specific induction of a "hapten-specific" suppression mechanism whose properties match those defined for the theoretical regulatory circuits we had proposed earlier.

These studies show that the carrier/hapten-carrier exposure sequence generates a suppression which largely prevents primary IgG anti-hapten responses to the injected hapten-carrier conjugate and then continues to suppress IgG responses to the hapten whether presented subsequently on the initial or, more significantly, an unrelated carrier. This suppression does not interfere with responses to either carrier molecule, i.e. it is specific for anti-hapten responses. Furthermore, it tends to preferentially suppress the high-affinity component of this response. Thus our studies demonstrate the existance of a suppression mechanism, operative in heterogeneous responses, that can be induced to selectively suppress production of nearly all of the antibodies reactive with an individual haptenic determinant on a complex antigen, without reference to the carrier on which that determinant is subsequently presented.

This suppression mechanism clearly prevents the expression, rather than the development, of anti-hapten memory . Adoptive transfer studies (1) show that memory populations in suppressed mice are indistinguishable from memory populations in non-suppressed mice (exposed only to the hapten-carrier conjugate). Spleen cell populations from the suppressed mice, however, must be depleted of T cells prior to transfer since total (non-T-depteted) splenic populations yield suppressed anti-hapten responses in adoptive recipients (Herzenberg and Tokuhisa, unpublished observations). Thus the hapten-carrier conjugate induces normal anti-hapten memory populations in the carrier primed animal, but it also induces a T cell-dependent suppression-effector mechanism that specifically prevents the expression of these memory cells.

The induction of the effector mechanism, in contrast with its operation, involves both carrier-specific and hapten-specific interactions with the inducing hapten-carrier conjugate. Suppression is induced only when mice are primed with a carrier and then exposed to hapten on that carrier. It is not induced when carrier-primed mice are exposed to hapten on an unrelated carrier, (even though subsequent responses to hapten on an unrelated carrier are suppressed once suppression has been induced). Thus, for example, DNP-KLH induces suppression for anti-DNP responses in KLH (keyhole limpet hemocyanin) primed mice but not in CGG- (chicken gamma globulin) primed mice while DNP-CGG induces suppression in CGG-primed mice but not in KLH-primed mice. Therefore, carrier-priming must generate a population(s) of carrier-specific cells that, once present, induce the hapten-specific mechanism to suppress anti-hapten responses when the animal is confronted with a new hapten on the priming carrier. (The well known 'carrier-specific' suppressor T cell population could be a major component of this suppression-induction mechanism, since the available data are ambiguous as to whether these cells induce hapten-specific suppression or, as commonly believed, mount a direct attack on carrier-specific help.)

Once hapten-specific suppression is induced, it can persist through multiple restimulations with hapten (on the priming or an unrelated carrier); however, there is a clear hierarchy among isotypes vis-a-vis the induction and maintenance of suppression. That is, IgM responses are not suppressed; IgG1 are suppressed initially but frequently escape from suppression after a second hapten stimulation and consistently escape after a third; and IgG2a, IgG2b and IgG3 responses usually remain suppressed in doubly stimulated animals and escape only after a third or fourth hapten stimulation. These multiple stimulations are each given at dose levels capable of stimulating a primary response (100 ug of hapten-carrier conjugate on alum), indicating that suppression can persist against a strong immunization schedule but ultimately is reversible (more easily for IgG1 than for the other IgG isotypes) as stimulatory conditions favoring response mount.

Response is also 'reversible' in that anti-hapten antibody production initiated following priming with hapten on one carrier can later be suppressed by completion of a carrier/hapten-carrier sequence with the hapten on a different carrier. The initiation of response, however, markedly impairs subsequent suppression induction. IgG1 responses remain essentially normal under these conditions and only about half the animals show dramatic suppression for the IgG2a, IgG2b and IgG3 responses. Thus, as a group, initially responding animals exposed subsequently to a carrier/hapten-carrier immunization sequence show a supression pattern roughly comparable to that in groups of initially suppressed animals after suppression has been largely reversed by several subsequent exposures to hapten.

This protocol was used in the early 1970's to show that hapten-carrier help interactions can occur *in situ* (10), since these interactions had previously been demonstrated only in adoptive systems. The observed restoration of responsiveness appeared to indicate that inadequate B cell priming was responsible for the inability of carrier primed mice to hapten (on the relevant carrier); however, in retrospect, the predominantly IgG1 response reported suggests that the "restoration" observed was actually due, as we have shown here, to interference with the induction of hapten-specific suppression.

These findings, aside from explaining an apparent discrepancy between our work and earlier reports, reveal one of the basic similarities between the observed properties of the hapten-specific mechanism and the theoretical properties of the Core regulatory circuit in the model discussed above. The theoretical cell interactions predict that responses will tend to stabilize in the initially induced configuration (suppression or help) so that more stimulation will be required to shift the circuit to the opposite configuration than would have been required to establish that configuration initially. Our experimental evidence demonstrates that initially suppressed and initially responding animals can be brought to roughly the same level of suppression, but that one or two additional stimulations with hapten are required to reverse an initial suppression to the level that is induced in initially responding animals subsequently exposed to the carrier/hapten-carrier sequence. Thus, as the model suggests, initiation of response conditions the animal to resist suppression induction and vice versa.

The isotype (and occasional allotype) specificity of the hapten-specific suppression mechanism reflects another of the theoretical properties of the Core circuit. The allotype specificity introduced into this circuit to account for allotype-suppression offers a rational ground for explaining the observed regulation of antibody production according to Ig heavy chain structure. In addition, it predicts a connection between allotype and hapten-specific suppression which explains how (as we have recently shown) immunization of allotype suppressed mice during their initial period of suppression (between 6 and 12 weeks of age) can induce a hapten-specific suppression for allotype-marked responses to all determinants on an antigen so that these responses later fail when animals are re-exposed to the immunizing determinants (on the same or an unrelated carrier) during "remission," (between 12 and 20 weeks of age) when these responses would otherwise proceed normally. (Minor modification of the circuit as drawn is required, however, to accommodate the isotype/allotype specificity of the hapten-specific mechanism.)

Finally, the demonstration that carrier-specific interactions determine whether suppression or response is established and/or maintained also recalls the model's auxiliary circuits that sense the antigenic environment and signal the Core circuit in favor of response or suppression. Although the published version of the model did not include carrier-specific auxilliary circuit which would induce the Core circuit to suppression, this type of circuit can readily be added. Thus with surprisingly little revision, the hypothetical cell interactions we proposed earlier can account for the unusual properties of the hapten-specific regulatory system.

Whether this model truly approximates reality cannot be determined from existing data. Nevertheless, the integration of concepts from studies of idiotype regulation and regulatory T cells on which it is based have now proven to define a theoretical system whose properties match the actual properties of the regulatory system controlling heterogeneous antibody responses. The development of this model, we submit, owes much to the groundwork laid by Prof. Jerne, whose imaginative view of the possible regulatory interactions in the immune system brought us and so many others to fruitful investigations of response regulation.

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LEGENDS FOR FIGURES

Figure 1.* Theoretical Core Regulatory Circuit

The B cell carrying id^+ V_H Ig surface receptors is helped by a T cell, Th ①, with complementary (id^-) receptors. This Th, which is analogous to an idiotype-specific helper T cell, is depleted by a suppressor T cell, Ts ①, analogous to an idiotype suppression T cell. It carries id^+ receptors similar to the B cell and therefore could bind the same haptenic determinant. Th ② and Ts ② are distinguished from Th ① and Ts ① by non-V_H related surface determinants. The configuration of this circuit is such that it will tend to stabilize either with Th ① and Ts ② dominant or Th ② and Ts ① dominant. Thus it will tend to maintain itself either in a help or suppression configuration depending on the initial conditions of antigenic stimulation.

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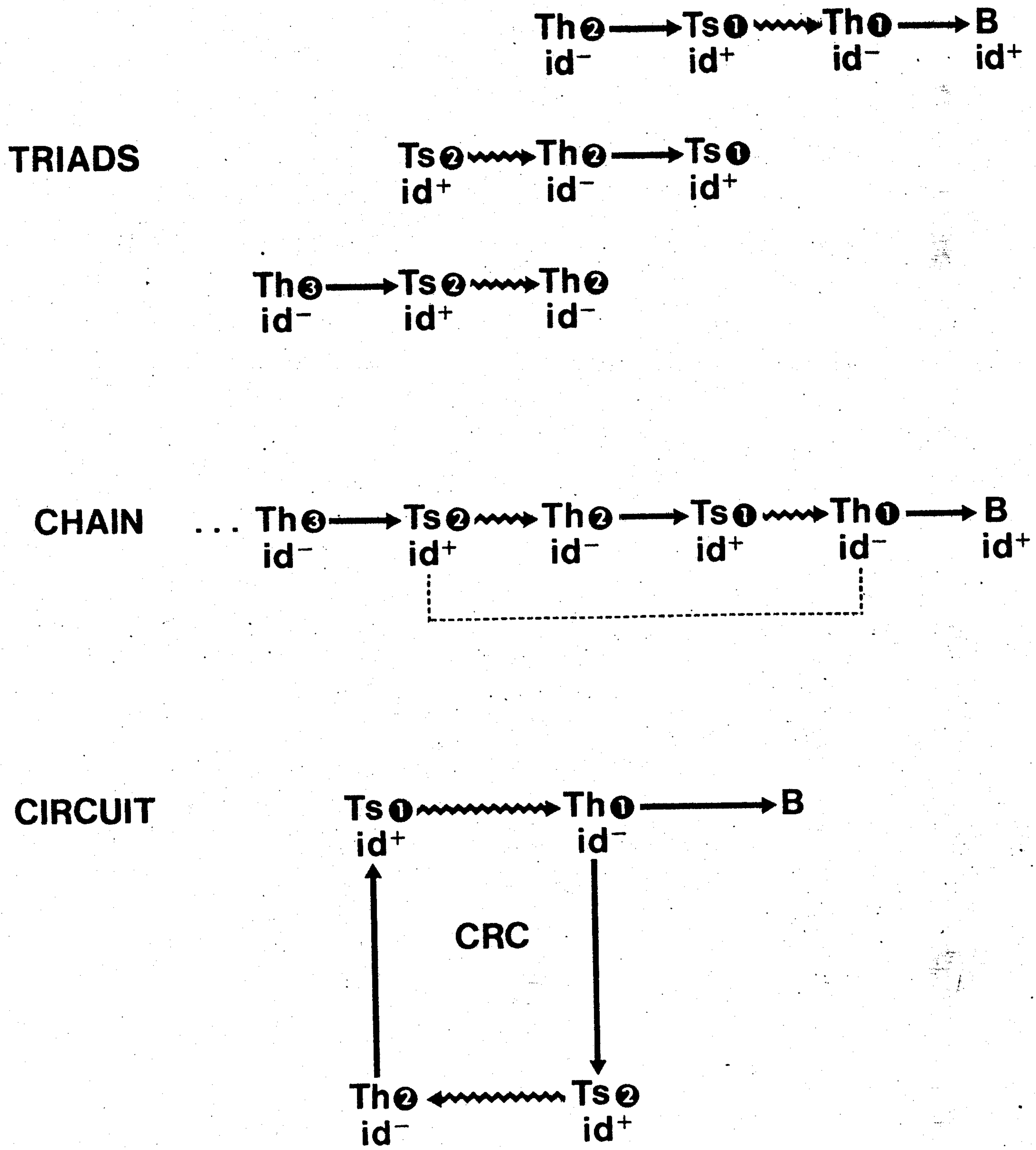
L. A. HERZENBERG, Department of Genetics
Stanford University School of Medicine
Stanford, California, 94305, USA

Fig. 1



TRIADS
Th2 id$^-$ → Ts1 id$^+$ → Th1 id$^-$ → B id$^+$
Ts2 id$^+$ → Th2 id$^-$ → Ts1 id$^+$
Th3 id$^-$ → Ts2 id$^+$ → Th2 id$^-$
CHAIN
... Th3 id$^-$ → Ts2 id$^+$ → Th2 id$^-$ → Ts1 id$^+$ → Th1 id$^-$ → B id$^+$
CIRCUIT
Ts1 id$^+$ → Th1 id$^-$ → B
CRC
Th2 id$^-$ ← Ts2 id$^+$