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Regulatory circuits and antibody responses*

Consideration of the interactions among cells and cell products involved in regulating antibody responses leads us to suggest that such interactions are organized into several discrete circular series (circuits) integrated with one another by virtue of shared circuit components. We see these circuits as individually concerned with particular aspects of regulation (carrier-specific, idiotype-specific, etc.), but together constituting an integrated, self-governing system capable of regulating all aspects of antibody production and assuring the orderly progress of the response (sequential idiotype representation, affinity maturation, isotype representation, overall or selective nonresponsiveness, etc.).

To illustrate how a circuit-based regulatory system could be constructed and expected to operate, we describe four integrated circuits here: a core regulatory circuit that determines whether a given idiotype will or will not be produced and three auxiliary regulatory circuits that respond to antigen and serum antibody (idiotype) levels by switching the core regulatory circuit into a suppression or "help" mode. These circuits incorporate the idiotype-anti-idiotype recognition system basic to the Jerne network theory but also provide for the operation of other cognitive systems that enable specific interactions between individual circuit elements (B cells, antibody, the various suppressor and helper T cells, macrophages and soluble regulatory products). As an integrated unit, the circuits constitute a detailed "working" model that depends on relatively few assumptions and is consistent with the known interactions between elements and the known properties of responses.

1 Introductory remarks

The development of a theoretical framework for integrating the various processes known to regulate antibody production has lagged substantially behind the description of these processes. Some time ago, Niels Jerne suggested that responses are regulated by a network of interactions based on idiotype-(id)-anti-id (complementary V_H) recognitions [1]. This theory provided some extremely useful insights in that it stimulated the successful search for expression of immunoglobulin (Ig) V_H regions (id on T cells) and encouraged a general exploration of the recognition mechanisms involved in regulation. But, being a child of its time, it conceived of the immune system in relatively simple terms *vis-a-vis* the variety of participating regulatory T cells and the complexity of their interactions. Therefore, although it laid the groundwork for definition of the language used among the specific constellation of T and B cells responding and regulating response to a given antigen, it failed to provide a predictive matrix either for organizing the T cell and other interactions responsible for individual aspects of regulation, (*e.g.* carrier-specific, id-specific) or for integrating these interactions into a coherent regulatory system capable of controlling response properties such as magnitude, duration, affinity maturation, selective isotype representation, overall responsiveness or nonresponsiveness (tolerance), etc.

During the years since formulation of the network theory, relatively little discussion has been aimed at filling the gaps it left. Instead, (quite appropriately, since first things should come first), attention has been focussed on describing the cells and cell interactions involved in the separate aspects of regulation.This approach has yielded fairly detailed descriptions of the individual regulatory mechanisms and sufficient generalizable information to enable initiation of discussion of the overall organization of the system. In addition, it has now generated a need for such discussion to facilitate the identification, for example, of those components within each unit that are responsible for coordination of the regulatory processes.

The integrated regulatory circuits we propose here represent a rudimentary attempt at developing this systematic view of how the immune system is organized**. Their construction derives from application of the network theory's cognitive principles to the current understanding of the interactions among B cells, helper T cells (Th), suppressor T cells (Ts), macrophages (MΦ), antigen and antibody; however, because the circuits we propose operate analogously to closed electronic circuits that can switch or be switched by one another to determine the system's response to external (antigenic) stimuli, they constitue a sharp departure from the open-ended networks suggested by Jerne and colleagues.

Much of what we propose here is testable, and much may prove to be wrong. Nevertheless, we believe that the illustrations we present, showing how a circuit-based regulatory system can account for various response properties, demonstrate

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Abbreviations: **allo:** IgH constant region allotypic determinants **allo+** and **allo−**: Complementary allotypic structures (shown as a^+ and a^- in circuit diagrams) **id:** Idiotype **id+** and **id−**: Complementary V_H determinants **Ig:** Immunoglobulin **IgH:** Ig heavy chains **MΦ:** Macrophages **Th:** Helper T cells **ThF:** Soluble factors produced by Th **Ts:** Suppressor T cells **V_H:** Variable region determinants (idiotypes) **CTh:** Carrier-specific Th **ATs:** Allotype Ts

** Our use of the term circuits is consistent with its usage by Cantor, Gershon and colleagues [2].

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an essentially valid new approach to consideration of the mechanisms of immunoregulation.

2 Rationale for circuit construction

Looked at broadly, the development of T and B lymphocytes from precursors to effector cells follow similar differentiation patterns. Initially, "virgin" precursor lymphocytes already committed with respect to effector function and antigen specificity arise in the absence of antigen. Antigenic exposure triggers the virgin precursors to differentiate to memory and effector cells. These antigen-dependent differentiation steps reguire help from (and are therefore regulated by) specific populations of Th. Th supply, in turn, is regulated by Ts populations capable of specifically depleting individual Th. Thus, the balance between Th and Ts activity should determine the magnitude of response to a given antigenic stimulus.

The picture, however, appears more complicated. Recently, Cantor, Gershon and colleagues [2] have shown that carrier-specific Ts differentiation from precursors to functional Ts also requires help from a Th population and that these Ts helpers are distinct from at least some of the Th which help precursor B cells differentiate to antibody-forming cells. Since there is no *a priori* reason why the supply of this second (Ts helper) Th population should not be specifically controlled by another Ts population which itself will require help from its own specific Th etc., *ad infinitum,* these findings appear to suggest the existance of a never-ending chain (or annular network) of Ts-Th interactions that alternate with respect to contributing towards help or suppression for a given response. In practice, however, this type of T cell organization would more likely result in a finite but indeterminate chain (or network) since the effects of distal regulatory interactions will have to be transmitted along a chain, and losses of definition can be expected at each step.

A "diminishing ripple" process such as this may indeed represent a true set of regulatory interactions among Ts and Th populations; however, it seems unlikely (to us) that the strict regulations observable in the immune system would be consigned to dependence on so imprecise a mechanism. Thus, we were moved to carefully inspect the potential interactions between the first few Ts and Th in the series. The result of these considerations form the basis of the hypothesis presented here, which sees immune responses as controlled by closed cell interaction circuits capable of being switched between responsive (help) and nonresponsive (suppression) states.

In the sections which follow, we will use the regulation of idiotype production as a context for illustration of the basic properties of circuits capable of controlling immune responses. We will describe
(a) a core regulatory circuit (CRC) consisting of two pairs of Ts and Th that regulate each other so that the circuit maintains stably in either the help or suppression mode;
(b) a MΦ-containing auxiliary regulatory circuit (ARC) sensitive to serum id levels that can switch the CRC from help to suppression;
(c) a carrier-specific Th (CTh) circuit that regulates the supply of id-specific help; and
(d) an allotype-sensitive ARC that suppresses production of id associated with an allotype-bearing IgG H chain. As our discussion progresses through these circuits, we will add details concerning recognition structures, affinities, etc., so that the overall description of the proposed circuitry will be complete only after description of the last circuit.

3 T cells active in regulation

3.1 General remarks

This brief review presents our understandings, interpretations and occasional assumptions regarding the properties and functions of Ts and Th. We have neither extensively defended nor extensively referenced this information since it is used here primarily to illustrate how regulatory circuits can be designed and expected to operate rather than as the basis for a definitive description of circuits operative in the regulatory system.

3.2 Th

Two types of Th active in helping B cell differentiation to antibody have been identified. One of these recognizes (carrier) determinants present on the antigen [3, 4]. The other recognizes structural determinants (id and allotypes) on Ig present on the differentiated B cell [5–10]. Help from both types of Th is required for antibody production. Antigen is required as well.

The recognition structures used by Th appear to be drawn from the same library of variable region structures as the V_H regions found in Ig H chains [11]. Id-specific Th carry anti-id $(id^-)V_H$ structures* that enable them to recognize and help the differentiation of id^+ precursor B cells to id-producing antibody-forming cells. CTh, on the other hand, appear to carry V_H receptors similar to those in antibodies that recognize determinants on the carrier molecule.

3.3 Ts

A variety of T cell populations which suppress different types of immune responses have been described. In this discussion, however, we will confine ourselves to a narrow definition of Ts as Lyt-2,3-bearing cells that regulate responses by depleting Th populations required for expression of immune effector populations such as antibody-forming cells. Allotype, id and (most likely) carrier-specific Ts fall into this category.

Recognition receptors used by Ts, like those used by Th, appear to be drawn from the Ig V_H structure library. In id-specific regulation, this means that Ts have id-like (id^+), *i.e.* atigen-binding, receptors complementary to the id^- receptors on the Th that help id-producing B cells [5–9, 12]. Soluble suppressive factors (TsF) with antigen-binding receptors have been isolated in id regulation systems [12, 13].

The mechanisms regulating Ts differentiation and expression have not been well charted as yet; however, the recent demonstration that Th help Ts [2] suggests Ts differentiation proceeds analogously to the differentiation of other lymphocyte effector populations (antibody-forming cells, cytotoxic T). Thus, drawing together the fragmentary evidence existent in the various suppression systems and pursuing the analogy between the Ts and other immune effector cell developmental

* To facilitate discussion, we have adopted the convention of referring to complementary id as id^+ and id^-. Thus, the id^- symbol as used here defines the specificity of a V_H structure and should not be confused with the id^- symbol used ordinarily to indicate the absence of an id. Furthermore, id^- should not be read as implying a single variable region structure but rather as a collective group of id^- structures complementary to one or more id^+ V_H.

Table 1. Probable events in suppressor T cell differentiation and expression

- Virgin Ts precursors differentiate to long-lived Ts memory cells.
- Ts memory cells differentiate to Ts effector cells.
- Both of these differentiations require help from and are therefore regulated by specific Th.
- Ts memory cells, once generated, can persist indefinitely without apparent expression in the absence of the Th from which they require help.
- Failure to see Ts expression *in situ* or in adoptive transfer can be due either to the absence of Ts or the absence (depletion) of Th that help Ts expression.

pathways, the rough outlines of the mechanisms involved in regulation of Ts differentiation and expression can be discerned (see Table 1). These outlines, especially the Th regulation of Ts expression, provide the basis for construction of the core regulatory circuits proposed in this publication.

4 Organizing regulatory T cells into circuits

4.1 Basic principles

The extension of the Ts-Th-B regulatory chain to include a Th that regulates Ts is consistent with the concept of open-ended regulatory networks as proposed by Jerne; however, the introduction of this second Th also allows for the construction of limited networks that do not extend *ad infinitum.* These limited networks can be envisioned as involving a closed circle of regulatory interactions among cells and cell products, and thus are more appropriately termed "circuits". We shall illustrate several such circuits here and show how they can be integrated to constitute a rudimentary overall regulatory system capable of accounting for many of the properties of antibody responses.

The basic design of immune regulatory circuits consists of a relatively small group of cells connected in circular series of interactions such that each cell regulates the cell in front and is regulated by the cell in back. The recognitions between cells in the circuits (as between cells in a network) rely on complementary V_H interactions, although antigen and constitutive (self) determinants appear to be important cognitive elements. Cell products such as antibody and T cell factors, either secreted or transferred to other cells, also serve as links in these circuits. These recognition elements allow specific integration of circuits to control a given response.

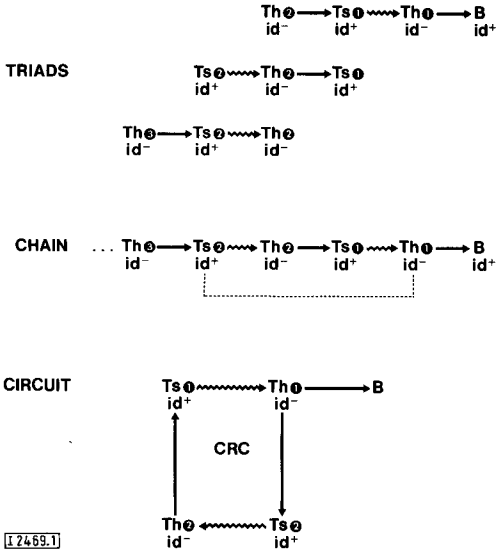
The roles of cells and cell products in the circuits we propose are kept strictly conservative in that each unit performs the same function wherever it exists in the circuit, (*e.g.* Th always help, Ts always deplete Th, etc.). These roles, furthermore, are kept consistent with the known functions of the cells and products involved. Thus, the assumptions made in designing the illustrated circuits mainly represent extrapolations from current information. (In other words, the circuits we describe do not comprise a radical departure from existing principles of regulation. Rather, they offer a new system for organizing the known cell and product interactions in the immune system into effective regulatory units.)

4.2 Distinction between helper and target Th for a given Ts

The key assumption required for circuit design states that although Ts deplete (kill, inactivate, divert) Th, recognition restrictions exist that prevent a given Ts from depleting its *own* Th. Since Ts-Th recognitions (both helper and target) must rely primarily on complementary id (id^+ to id^-) interactions to guarantee the specificity of regulations, this assumption means that a second, generic recognition system must operate between Ts and Th that enables a Ts population to distinguish its helpers from its targets.

Some support for this assumption can be drawn from recent studies of "feedback inhibition" in carrier-specific circuits [2]; however, it is also justifiable *a priori* because it defines an organizational system that prevents Ts from depleting their own helper Th. Such "short circuits," if they were allowed to occur, would inevitably result in depletion of the Th population that helps Ts and hence in the loss of Ts activity and the destruction of the regulatory capabilities of the system. Thus, we assume that in all cases, *the target Th and the helper Th for a given Ts are drawn from different Th populations.*

This assumption leads to the definition of sets of overlapping Ts-Th triads in which Ts are flanked by two different Th, one which helps the Ts and the other which is its target. Th similarly, will be flanked by two different Ts, one which is helped by the Th and the other which depletes it. Triads for the T cells expected to be involved in idiotype regulation are shown in Scheme 1.The first regulatory circuit to be described is derived from these triads.



Scheme 1. Development of the CRC.

4.3 Circuit construction

The recognitions between cells involved in id regulation have been shown to consist of complementary V_H interactions between sequential members. Therefore, in a series of id regulation triads, the two flanking members in a triad will have similar or identical V_H receptors that are complementary to the V_H receptor of the central member. Expressed in the notation system we have adopted, (in which the id structure pre-

duced by the B cell is assigned as id^+, and the anti-id V_H structure on the Th that helps the B cell is consequently assigned as id^-), the two Th in a Th-Ts-Th triad will have id^- receptors complementary to id^+ receptors on the Ts, and the two Ts in a Ts-Th-Ts triad will have id^+ receptors complementary to the id^- Th receptors (see Scheme 1).

5 The CRC: a circuit that tends to stabilize either in a help or suppression configuration

The CRC consists of two Th and two Ts arranged such that each Th is the target of one Ts and the helper of the other. In Scheme 1, the Th in the upper right-hand corner, called Th(1), is assigned as the id-specific helper of the B cell that produces antibody with id determinants (id^+Ig) complementary to the (id^-) V_H determinants produced by (both) Th in the circuit. (Both Ts have id^+ receptors similar to but not necessarily identical to the B cell id^+Ig). This circuit configuration assumes that a Th that helps an id^+ B cell can also help an id^+ Ts.

Inspection of the Ts-Th relationships in the CRC reveals one basic overriding property of the circuit: it tends to drive itself all the way to one side or the other and "lock" into help or suppression, depending on which Th population achieves initial dominance. For example, (see Scheme 1) if the Th(1) becomes established or activated first, it helps the differentiation and expression of Ts(2). Ts(2), once established, depletes the Th(2) in the circuit. Th(2) depletion in turn disables differentiation and expression of the Ts(2) population which, if present and active, would be capable of attacking the Th(1) and thereby suppressing id production. In the absence of an active Ts(1) population, Th(1) can increase; and this increase, because it stimulates more Ts(2) activity, will further discourage development of Ts(1) activity. Thus, if Th(1) become established first, they effectively delete the antibody-suppressive side of the circuit, and the circuit locks into a "help" configuration. On the other hand, if the Th(2) become activated first, the circuit will drive itself into the suppression configuration and lock there.

In the absence of continued inducing or activating stimuli for the dominant Th, a circuit such as this will tend to "run downhill" and become essentially dormant. During this process, stimulation of the minority Th could elevate them to dominance and thus shift the circuit into the opposite suppression/help configuration. This ability to shift means that a circuit potentially could be maintained in a poised intermediate position that would allow a stable, partially suppressed response. In practice, however, maintenance of a poised state with a circuit that tends to drive itself into one or another locked position would be more or less like trying to balance a pencil on its point on an egg, *i.e.* very difficult.

The tendency of the CRC to lock makes this circuit attractive as a basic regulatory circuit for antibody responses since it has a strong inherent stability capable of withstanding minor perturbations of the Th or Ts populations without changing its established configuration, *i.e.* help or suppression. But because the configuration of the CRC depends ultimately on the regulatory interactions that control Th(1) or Th(2) stimulation, the CRC itself must be considered mainly a cog in a more extensive system that determines the characteristics of immune response. This system, we suggest, consists of several auxiliary regulatory circuits (ARC) that control CRC Th stimulations and thereby control affinity maturation, allotype and isotype expression, maintenance of tolerance, etc. (see Table 2).

Table 2. Properties of the CRC

- Tends to lock into either suppression or help configuration.
- Can be switched from suppression to help or *vice versa* by pressures strong enough to reverse the ratio between the two Th.
- ARC composed of interactions among T cells, T cell factors, accessory cells, antibodies and antigen control the relative activities of each of the Th in the CRC.

6 ARC

6.1 Introductory remarks

An antibody response consists of the sum of the antibodies produced over a period of time by a variety of B cell clones, each producing an antibody with a defined V_H region structure (id) capable of combining with structures (haptens) on the immunizing antigen. The period of the overall response, however, appears to be considerably longer than the period during which any single clone actively produces its antibody. In general, responses begin with expansion and differentiation of B cell clones producing low-affinity antibody. These then recede as clones producing higher affinity antibodies appear, but in time the newer clones also reach ascendancy and recede as clones producing still higher affinity antibody take over the dominant position. This process (somewhat simplified here) continues until antigen or the supply of higher affinity clones becomes exhausted.

An overall regulatory system must thus have mechanisms for encouraging clones producing higher affinity antibody and suppressing those producing antibodies with lower affinities, *i.e.* for selectively increasing or decreasing help for individual id according to the hapten-combining affinity of the id. In addition, the system requires a mechanism for preventing production of undesired id (*i.e.* maintaining tolerance) and for regulating the overall isotype representation in responses.

CRC, being specific for individual id, can provide the basic regulatory system required; but since the shifting of the CRC between help and suppression depends on the relative stimulations of the dominant and minority Th, the regulatory interactions responsible for these stimulations essentially control whether a given id is produced and how long production continues. The cells and cell products involved in these interactions, we suggest, are organized into several ARC whose properties include sensitivity to V_H (id) affinity and serum isotype (allotype) levels.

6.2 Basic design

The basic design of each of the ARC is essentially the same: id^+Ig produced by a B cell combines either directly or indirectly (through antigen) with T cell factors adhering to MΦ. The presence of the id then allows formation of a recognition bridge between the MΦ (or adherent T cell factor) and an id^-

Th in the CRC regulating the B cell that produced the Ig. Formation of the recognition bridge promotes stimulation of the Th; however, in each ARC, a second recognition requirement between MΦ and/or T cell factor restricts the stimulation to only one of the two CRC Th, *i.e.* Th(1) or Th(2); thus, operation of one ARC drives the CRC toward help while another drives it toward suppression (see Table 3).

The inclusion of id (*i.e.* antibody) in the ARC reintroduces the concept of feedback regulation of responses. This concept has fallen somewhat into disuse with the ascendance of regulatory T cells but still appears to us to have considerable validity. We differ with earlier conceptions, however, in that we see the antibody as active principally in complexes containing T cell factors and exerting its regulatory influence through its id determinants as well as its antigen-combining activity. Furthermore, we see antibody as operating both in the ARC that stimulates help and in the ARC that stimulates suppression, and thus providing both negative and positive feedback signals at different stages in the response.

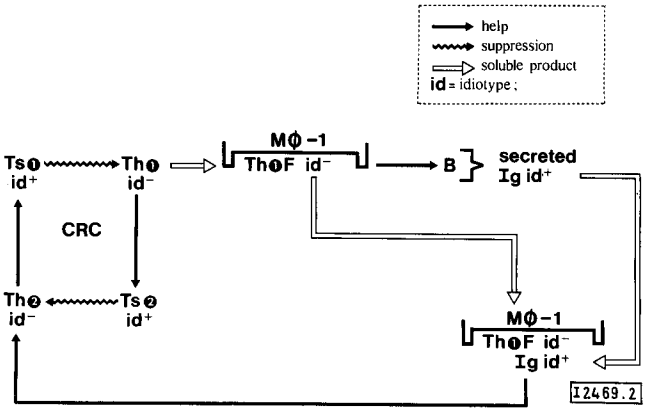
The inclusion of ThF in the ARC is less contentious, if only because relatively little is known about how these factors work. We see the factors as functional only when their (V_H) receptors are engaged. In this condition, we suggest, they serve either to activate MΦ to enable delivery of a stimulation signal or to deliver stimulation signals themselves to target cells when an appropriate recognition bridge joins the MΦ-ThF-antibody complex to the target cell. The inclusion of MΦ and their assigment as carriers of T cell factors and potential stimulators of Th in the CRC proposes a role for MΦ that is again consistent with known function [14–18]. This role could also be played by other accessory cells capable of binding T cell factors and antigen; thus, its assigment to MΦ is somewhat arbitrary, and the assumptions made in later sections concerning MΦ should be considered with this point in mind.

Table 3. Basic assumptions for ARC

- Separate ARC control stimulation of Th(1) and Th(2).
- Antigen V_H regions on T cell factors, and V_H regions on serum Ig create recognition bridges in the ARC.
- Accessory cells (MΦ) carry factors and Ig between lymphocytes.
- Accessory cells of T cell factors deliver stimulation signals to the appropriate Th.
- Accessory cells or T cell factors are responsible for distinguishing Th(1) from Th(2).

7 Shifting the CRC from help to suppression: an ARC for eliminating production of unwanted id

Shifting the CRC from help to suppression requires that stimulation of Th(2) exceed stimulation of Th(1). In this section, we describe an ARC that would increase Th(2) stimulation when serum id levels are high and thus serve to suppress id production when a critical serum id level is reached. An ARC which could control Th(1) stimulation will be discussed in the next section. Since we believe MΦ are involved in these ARC, we shall use the description of the suppressive ARC, which is basically simpler, to detail the role(s) that would be played by these cells.



Scheme 2. Suppressive ARC. Secreted id (Ig id^+) combined with MΦ-bound soluble product from Th(1) stimulates the suppressive side of the CRC.

MΦ have been shown to be involved in stimulation of T cells [14–16]. In addition, they appear to be involved in triggering B cell division and differentiation [17–18]. The molecules responsible for stimulation may be produced by other cells and passively carried by the MΦ or may be products of the MΦ itself. In either event, the MΦ recognition receptors which single out T or B cells for stimulation and bring the MΦ into close contact with the target are most likely to be passively carried molecules with V_H segments attached to different "constant regions" depending on the cells that produced and donated them to the MΦ. The ARC shown in Scheme 2 incorporates these properties. Our assumptions for this ARC are fairly simplistic; however, they serve our purposes here in allowing illustration of an ARC which could serve to shift the CRC from help to suppression when serum id levels are high.

MΦ, we assume, have generic receptors that allow them to carry Th(1)-produced V_H-containing molecules, *i.e.* Th(1)F, from Th(1) to their target B cells. In id-specific help, these Th(1) molecules contain id^-V_H segments complementary to the id^+V_H of the Ig produced by the target B cell. Thus, MΦ carrying Th(1)F have adherent id^- receptors capable of combining with id^+ surface Ig on B cells or id^+Ig in circulation.

In the absence of serum-borne id^+ Ig molecules, these MΦ will deliver their id^-Th(1)F to id^+B cells and trigger the B cells to differentiate to id^+-producing antibody-forming cells. Later, as the Id^+Ig concentration rises in serum, the circulating Ig molecules will compete with B cell surface-bound id^+Ig for the id^-Th(1)F on the MΦ and directly reduce help for the B cells. More important with respect to shifting the CRC from help to suppression, however, the complexing between id^+ serum Ig and MΦ-bound id^-Th(1)F creates a recognition bridge that completes an ARC which allows stimulation of id^-Th(2) to dominance in the CRC.

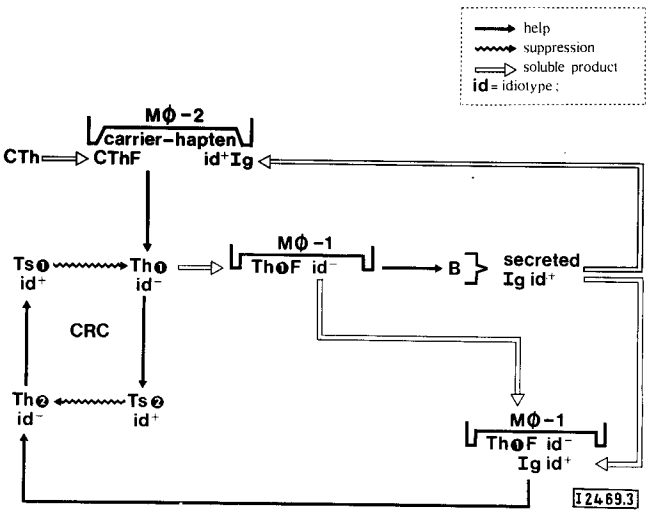
The operation of this ARC requires either that MΦ which carry Th(1)F complexed with serum id^+Ig become activated and can stimulate id^-Th(2) but not id^-Th(1), or that Th(1)F itself when complexed with id^+Ig, specifically stimulates Th(2). Under these conditions, stimulation of Th(2) will increase with serum id^+Ig concentration until a critical level is reached that will tip the balance of Th in the CRC in favor of suppression. Once this occurs, the circuit will drive itself to lock into the suppression configuration, depleting Th(1) and thus in time terminating the production of id^+Ig.

The assumption that Th(1)F-carrying MΦ do not trigger Th(1) expansion appears valid for the same reason that Th which help Ts are unlikely to be the targets of the Ts they help, *i.e.* the avoidance of "short circuits". Without this assumption, the presence of Th(1)F-

bearing MΦ and serum id$^+$Ig would result in the autocatalytic rise of Th(1) populations.

The question of whether the macrophage or the T cell factor actually delivers the stimulation signal to the target Th has been left unresolved in the ARC we propose, since both options appear equally viable and construction of the ARC is not substantially influenced by a decision between them. Similarly, the question of which element carries the recognition determinants that allow specific stimulation of Th(2) is irrelevant to the ARC construction. In the ARC we have drawn, we arbitrarily assume two subsets of MΦ, one (operating in this ARC) which is specific for Th(2) and a second (operating in the next ARC) which is specific for Th(1).

The existence of ARC sensitive to serum id$^+$Ig levels allows explanation of several properties of antibody response regulation: the sequential production of id would be expected because, as production of each id peaks, its CRC is shifted to the suppressive conditions leaving other (more recently expanded) id to maintain the response. Furthermore, if antigen selectively removes id with higher affinity for the antigen from circulation, the CRC regulating these id would be maintained in a help condition at the expense of CRC regulating lower affinity id (which will shift sooner into suppression). Thus, the integrated ARC-CRC circuit described could significantly contribute to the process of avidity maturation; however, as we shall show in the next section, the ARC responsible for Th(1) stimulation is suited to play a more important role in this process.



Scheme 3. Help-stimulating ARC. Secreted id (Ig id$^+$) combined with MΦ-bound complex of antigen and soluble product from CTh stimulates the help side of the CRC.

8 Establishing the CRC in a help configuration: an ARC which integrates the roles of carrier-specific and id-specific Th

For many years, carrier-specific Th (CTh) were the only T cells known to be required to help B cells respond to antigen. Hapten and carrier determinants on the antigen thus appeared to create a recognition bridge between B cells and CTh (or CTh products on MΦ) that enabled triggering of sufficient B cell expansion and differentiation to account for the large numbers of antibody-forming cells obtained in the response. This state of innocence, however, was shattered when allotype and id regulation studies showed that B cells could not respond in the presence of adequate numbers of CTh unless help was also available from a second Th population specific for individual B cell Ig determinants [5–11]. The demonstration of two Th populations (CTh and IgTh) with clearly distinct specificities reinforced conclusions form earlier studies suggesting that two separate Th were active in supporting initial B cell responses in adoptive secondary assays [19, 20]. Thus, it became increasingly clear that the original view of how Th help B cells had to be expanded to provide nonoverlapping roles for each of two distinct Th populations.

Division of labor between the Th offered a reasonable solution to the problem: CTh could be assigned to trigger B cell expansion and Ig-specific Th assigned to trigger differentiation or *vice versa.* Since each type of Th recognizes a different type of B cell surface determinant (Ig or bound antigen), the two Th can comfortably be assigned to delivering different types of signals; even though there is no firm evidence demonstrating either that B cell expression requires two separate and different signals or for that matter, that CTh, IgTh or their products interact directly with B cells. On the whole, this model seems more acceptable than alternate models in which interaction between the two Th is required to provide effective help for the B cell, since the specificity of CTh for antigen and/or IgTh for id appears to leave little ground for specific interaction between the Th.

Rejection of Th interaction models, however, is perhaps premature. Consideration of auxiliary circuits that potentially could connect the two Th and make B cell response dependent on just one signal from a circuit-regulated Th suggests that, when antigen and antibody are included in the ARC, plausible circuits can be drawn in which interaction between the Th constitutes a major, or perhaps the only, mechanism through which CTh regulate antibody responses. For example, an ARC can be drawn in which CTh serve only to stimulate specific expansion of the id$^-$Th(1) populations that help id$^+$ B cells produce antibody to haptenic determinants on the antigen (see Scheme 3).

Antigen and antibody in this ARC create the recognition bridge between CTh and Th(1) that results in stimulation of Th(1). Initiation of the ARC requires complexing between CTh-produced carrier-specific receptor molecules (CThF) and antigen. This CThF-antigen complex most likely resides on the surface of a MΦ and may serve to activate the MΦ so that it is ready to provide a stimulatory signal to Th(1). Alternatively, the complex may serve to juxtapose haptenic determinants and CThF so that the CThF can deliver a stimulatory signal when the recognition bridge is complete.

The next step in the ARC requires the binding of antibody to the haptenic determinants on the CThF-antigen complex. The antibody can be drawn from circulating Ig during an ongoing response; however, at the beginning of a response, the antibody could instead be "stolen" from B cell surface Ig. In either event, once antibody binds to the hapten, the MΦ will carry id determinants that can be recognized by the Th(1) in an id-specific CRC.

If we now assume, in parallel with our assumptions for the first (id suppressive) ARC described, that the MΦ that receive CThF can stimulate Th(1) but not Th(2) when an appropriate recognition bridge is made, then a specific Th(1)-stimulating ARC can be completed because antibody bound to a CThF-antigen complex on macrophages will allow recognition and stimulation of Th(1) specific for the id determinants on the bound antibody.

The operation of this ARC obviates the need for direct stimulation of B cells by CTh. Instead, it consigns CTh to regulating B cell responses indirectly through regulation of the IgTh, *i.e.* Th(1) supply. This subordination of Th(1) to CTh has several practical consequences. It makes the stimulation of Th(1) that are specific for individual id dependent on the binding of antibody carrying those id to the CThF-antigen complex on MΦ. Thus, stimulation will be restricted to those Th(1) populations capable of helping B cells present in the animal and available to participate in a response. It also favors stimulation of Th(1) for B cells that produce antibodies which compete more successfully for antigen. Thus, well represented V_H regions in the initial B cell populations, or V_H regions with higher antigen-binding affinities will tend to stimulate proportionately more Th(1) activity for their own id. (The significance of this preferential stimulation will be discussed in Sect. 9).

A less radical version of the Th(1) stimulating ARC, which still retains the above described advantages, would allow CTh to trigger a modest B cell response but require that Th(1) provide the help for most of the antibody production. In this case, B cell surface Ig would not be required to initiate the response, since serum antibody in combination with the CThF-antigen complex on the MΦ would be sufficient to account for stimulation of the Th(1) required to support the response. The only stipulation in allowing both CTh and Th(1) to help B cells would be to require that the size of the CTh-helped component of the response be small enough to be undetectable under those conditions where allotype or id-specific suppression or help can be demonstrated, since results from these studies indicate that responses fail unless both CTh and IgTh are present.

Whether the carrier-specific ARC as described has any grounding in reality remains to be determined. Current findings are consistent with its operation, but these findings are also consistent with the "division of labor" hypothesis. We propose it mainly to show how antigen and antibody can connect two apparently independent circuits and provide an antigen-dependent stimulus for specific expansion of desired id-specific Th. But, having now described the circuit, we shall take our discussion one step further and show how its operation could provide a positive selective force for affinity maturation.

9 Regulation of affinity maturation of antibody responses: cooperation between two ARC

In a previous section, we pointed out that affinity maturation should be favored by the operation of the (suppressive) id-specific ARC which shifts the CRC from help to suppression when the serum level of the circuit-regulated id becomes high. In this ARC (see Scheme 2), MΦ bearing Th(1)F specifically bind serum Ig with id^+ determinants complementary to the id^- receptors on the Th(1)F. This binding creates a recognition bridge between the MΦ and the id^-Th(2) and thus allows stimulation of the Th(2) to dominance in the CRC and a consequent CRC shift to suppression. Antigen does not play a direct role in this ARC; however, if id with high antigen-binding affinities are preferentially removed by sequestered or circulating antigen, the serum id spectrum will tend to become biased toward id with lower antigen-binding affinities. This bias will in turn tend to induce more rapid shifting of the CRC regulating the low-affinity id to suppression.

Selective suppression of low-affinity antibodies would account for the disappearance of these antibodies as the response matures and, by relieving the system of the need to support production of "unnecessary" antibody, would allow expansion of higher affinity clones. But this mechanism, relying as it does on preferential depletion of high-affinity antibody from circulation, seems rather unreliable for assuring the extraordinarily regular occurrence of affinity maturation in antibody responses. This process more likely requires the systematic selection of progressively higher affinity clones and thus could be expected to be regulated by circuits that increase help for these clones. The CTh ARC described in the previous section provides this capability.

Table 4. ARC control of affinity maturation

- The requirement for antibody-hapten binding in the CTh ARC that stimulates Th(1) tends to selectively increase help for B cells that produce high-affinity antibodies.
- The greater likelihood that lower affinity antibodies remain free (not bound to hapten) and therefore available to participate in the ARC that stimulates Th(2) tends to selectively shift CRC for low-affinity antibodies from the help to the suppression mode.

In the CTh ARC, CThF-antigen complexes bound to MΦ bind antibody from circulation (or possibly from B cell surfaces). The bound antibody then creates a recognition bridge between the MΦ and the id-specific Th(1) that specifically enables stimulation of Th(1). Since high-affinity antibodies should successfully compete for sites on the CThF-bound antigen, Th(1) which can help B cells that produce these antibodies will be preferentially stimulated, especially toward the end of a response when antigen becomes limiting.

The ability of the CTh ARC to "positively select" higher affinity clones suggests that this or a similar circuit plays a dominant role in affinity maturation. Coupling its activity with the Th(2)-stimulating ARC that tends to suppress lower affinity clones, however, provides a mechanism that more completely accounts for the properties of the affinity maturation process, *i.e.* the shift to higher affinities with concomitant loss of low-affinity representation in the response (see Table 4). Thus it is likely (if these circuits are real) that affinity maturation involves operations of both ARC as they regulate individual CRC within the response.

10 Maintenance of intermediate response levels: an allotype suppression ARC

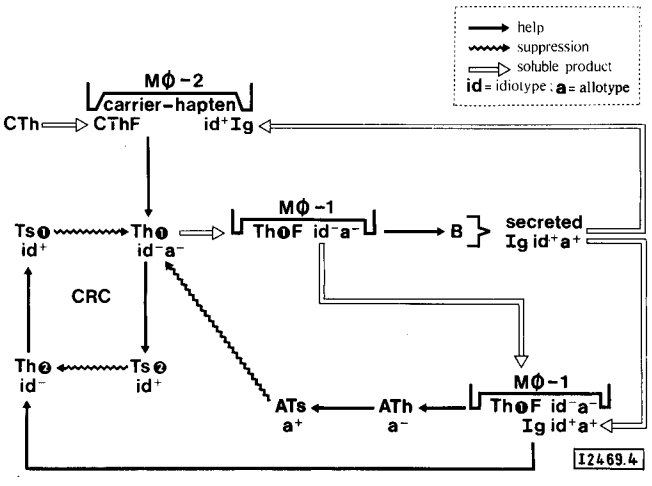
Circuits such as the id-specific CRC, as we have indicated, are useful in regulating responses that tend to be either "on" or "off" but are inappropriate for regulation of responses that persist at intermediate levels for long periods of time. Since allotype-suppressed mice often show intermediate (subnormal) serum allotype levels that fluctuate somewhat but basically remain stable over many months, our studies required design of a circuit amenable to this type of regulation. The ARC we constructed, which is integrated with the previous circuits, admittedly requires more precarious assumptions than these circuits; however, we have decided to include it in this discussion because it illustrates a different principle of circuit operation and provides a context for exploring the Ts

and Th recognition determinants necessary to reconcile the operation of id-specific and allotype-specific help in the same animal.

Considered independently, the mechanisms of allotype and id regulation appear quite similar. In both cases specific Ts attack and deplete Ig-specific Th that, in conjunction with CTh, trigger B cell differentiation to antibody-forming cells. Attempts to describe how both id and allotype regulatory systems could operate in the same animal, however, founded on apparently paradoxical predictions, *i.e.* if allotype-specific and id-specific Th coexist and provide similar signals required for B cell differentiation, then id-specific suppression could not occur because the allotype Th should be able to compensate for the specifically depleted id Th. This paradox can be resolved by assuming either that both types of the Th do not coexist or that signals from both types of Th are required for B cell expression; but since neither of these assumptions is particularly satisfactory, we propose a third: that allotype-specific and id-specific help are provided by the same Th, *i.e.* Th(1), and that Th(1) is independently depletable by allotype or id Ts (see Scheme 4).

Placing allotype and id specificity within the same Th means that the molecule(s) with which the Th recognize Ig on B cells must carry receptors for both id and allotypic determinants. Two separate Th(1) molecules could carry these receptors; however, it seems more likely that the Th(1)F carries both an id^- and $allo^-$ receptor. The $allo^-$ receptor in this case would then be expected to be located in the "constant" region of the Th(1)F, attached to the id^-V_H receptor that determines the id specificity of the help. Gene(s) on the IgH chromosome region, which donates the V_H genes expressed in Th(1)F, could code for this $allo^-$ receptor. Alternatively, the $allo^-$ structure could be determined at loci in the MHC I region since loci in this region appear to control "constant" region structures on immunoregulatory molecules and cells [12, 13 17, 21–23].

The assumption of a "constant" recognition structure for Ig H chain constant-region determinants (allotype in this case, but possibly isotype in others) on the id-specific Th(1)F is not unreasonable considering the potential restrictions on help engendered by a requirement for id-anti-id matching between Th and B. The presence of an auxiliary recognition structure would increase the number of Th available to help a given id^+B cell since the $allo^-$ to $allo^+$ binding would lower the requisite affinity of the id^- receptor for the id^+Ig. This would broaden the range of id^-V_H capable of providing effective help, making it possible for less well-matched id-anti-id pairings to result in B cell triggering. In other words, if a certain aggregate-binding strength between Th(1)F and B cell Ig is required for help, the presence of an $allo^-$ receptor on the molecule should be a decided asset.



Scheme 4. Allotype-suppressive ARC. Secreted allotype-bearing Ig ($Ig\ id^+\ a^+$) combined with MΦ-bound soluble product from Th(1) stimulates increase in allotype suppressor T cells that deplete Th(1).

Providing more leeway for successful help interactions between somewhat poorly matched id^-Th receptors and id^+Ig could explain why id-specific help can be rapidly delivered in adoptive-transfer experiments where B cells from hapten-primed donors are combined with T cells from donors primed only to the carrier. Operation of the CTh ARC described previously could be expected to select and expand id^-Th matching the id^+B cell populations available; however, sufficient id-specific help appears to be initially available from the T cell population (which has never been exposed to the hapten) to raise questions as to the source of this help. A two-site receptor (id^-allo^-) which enhanced matching at the beginning of the response could account for this disparity.

$Allo^-$ to $allo^+$ binding does not necessarily have to be viewed as an absolute requirement for help. Well matched (high-affinity) id^- to id^+ binding alone presumably could be effective. On the other hand, id^- to id^+ binding has to be essential if the id specificity of the system is to be maintained. Thus, either the $allo^-$ to $allo^+$ binding is too weak in itself to result in help or the effective delivery of the help signal from a Th(1)F molecule requires engagment of its V_H receptor. The latter conclusion is consistent with the proposed operation of the allotype regulatory ARC shown in Scheme 4 (to which we now return).

The assumption of an $allo^-$ constant region structure on the Th(1)F provides an identifying determinant that allows Th(1) recognition by the allotype Ts(ATs). In the ARC drawn in Scheme 4, the $allo^-$ determinant is abbreviated as a^-. This recognition will require complementarity between the ATs V_H and the $allo^-$ Th determinant; thus, the $ATsV_H$ will mimic the structure of the $allo^+$ determinant on the Ig molecules. The ATs, in this case, will be attacking Th(1) via a Th(1)F "constant" region determinant; however, this should not interfere with the delivery of the suppressive signal since the ATs V_H will be engaged in normal fashion, albeit in combination with a slightly displaced determinant.

Differentiation and expression of the ATs should require help from a Th (ATh) analogous to the Th(2) in the CRC. This ATh will have an $allo^-$ V_H receptor. Thus, to this point, the allotype-regulatory circuit approximates the CRC. Now, however, the circuits diverge. In the CRC, the activity of the ATh would be regulated by an ATs(2) with an $allo^+$ V_H receptor; and, to close the circuit, the ATs(2) would be helped by the Th(1) which would then have to have a complementary $allo^-$ V_H. This circuit closing condition, however, cannot be met in the allotype regulatory circuit, since the circuit design is based on the premise that Th(2) has an id^-V_H associated with an $allo^-$ constant region determinant.

For the Th(1) to help the $allo^+$ Ts(2), we should have to assume that the help signal to the Ts can be delivered without engagement of the $Th(1)V_H$. Afferent receptor engagement, however, ought to be required for signal delivery; otherwise, as indicated earlier, id specificity would be lost because of "constant" region interactions. Thus, we feel it is unlikely that Th(1) can help ATs(2) so that the circuit can close into a CRC.

Table 5. Assumptions for an allotype-suppressor ARC that generates oscillating serum allotype levels

- Th(1) delivers both allotype-specific and id-specific help.
- Th(1)F has two recognition sites for B cell Ig: an id^-V_H receptor complementary to the id^+V_H on the Ig molecule and an $allo^-$ Th(1)F constant region site[a] complementary to an ($allo^+$) allotypic determinant in the IgH chain constant region.
- Delivery of help (and suppression) signals by T cell factors requires engagement of the V_H receptor on the factor.
- Secondary interactions between factors and B cell Ig constant regions improve help delivery (*e.g.* by decreasing binding affinity required between id^+ and id^- receptors); but these secondary interactions are insufficient, in and of themselves, to enable delivery of the help signal.
- Allotype Ts (ATs) have $allo^-$ V_H receptors that recognize the $allo^-$ "constant region" determinant on Th(1).
- ATh (that help ATs) have $allo^-$ V_H receptors.
- Th(1), ATs and ATh constitute the first three cells of a chain that terminates and cannot close into a CRC because Th(1) have id^- V_H receptors and cannot help $allo^+$ ATs further down the chain.
- $Allo^+$ Ig bound to Th(1)F on MΦ can stimulate $allo^-$ ATh. This constitutes an ARC that regulates Th(1) activity in inverse proportion to serum allotype levels. Thus, operation of this ARC results in oscillating serum Ig levels.

a) $allo^+$ is abbreviated as a^- in the circuit diagram in Scheme 4.

Closing the allotype regulatory circuit into a CRC also defeats our purpose in designing (and illustrating) a circuit appropriate for allotype regulation. An allotype CRC would tend to push allotype production to all or none extremes. This would be inconsistent with the long-term maintenance of intermediate allotype levels frequently observed in allotype-suppressed mice. Thus, we shall assume that Th(1) can be suppressed by the ATs but that the circuit remains open because Th(1) does not help an ATs(2).

Removing the potential for help for ATs(2) effectively deletes this cell from the circuit, leaving a short chain consisting of Th(1), ATs and ATh(2) and freeing the ATh(2) from the severe negative regulation (deletion) that active Th(1) impose through Ts(2) in the CRC. Th(1), however, should still be able to stimulate ATh(2) through a MΦ-containing ARC similar to that described for Th(2) stimulation in the id circuits. This allows closure of a second type of ARC which, as we shall show, has the appropriate characteristics for allotype regulation (see Scheme 4).

In this circuit, ATh(2) activity will be regulated by the level of serum $allo^+$ Ig molecules capable of creating a recognition bridge between Th(1)F-bearing MΦ and ATh(2). The ARC will thus be allotype-rather than id-specific since all MΦ bearing $allo^+$ Ig bound to $allo^-$ Th(1)F will be capable of stimulating the ATh(2). In other words, any ongoing $allo^+$ Ig response in which id levels climb high enough to bind to Th(1)F will stimulate ATh and thus increase suppression for all $allo^+$ Ig responses.

The increase in suppression, however, will be relatively short-lived since it reduces Th(1) activity and therefore reduces the source of stimulation for the ATh (*i.e.* serum allotype and Th(1)F on MΦ). As ATh activity decays, ATs activity will be reduced and Th(1) activity will increase. This increase, however, will also be short-lived since it will again increase MΦ-bound Th(1)F activity and serum allotype levels, which in turn will increase ATh activity to restart the cycle. Thus, allotype levels will oscillate, and the average level over a period of time will be kept relatively constant as the circuit responds to increases and decreases in serum allotype.

The serum level around which oscillations occur will depend in part on the affinity of the Th(2) $allo^-$ V_H receptor. A high-affinity receptor will bind the the MΦ-Ig complex more easily and will thus be stimulated and provide help for ATs at low serum levels, while a low-affinity receptor will require higher serum Ig levels to trigger the suppressive cycle. The initial ratio between ATs, ATh and Th(1) will also influence the fixation of the average level. Strong antigenic stimuli, on the other hand, will increase the amplitude and perhaps prolong the period of oscillations but should not reset the initially established serum level.

This ARC satisfies the requirements for a mechanism regulating serum allotype production. It provides a servo-type regulatory system that calls for suppression or help for Ig production depending on whether serum Ig rises above or below a fixed level, and it provdies a specificity system that allows for simultaneous regulation of all idiotypes associated with individual H chain isotypes or allotypes. In a normal animal, several of these circuits, specific for the various isotypes and allotypes, could be expected to maintain characteristic levels of these Ig in serum. In allotype-suppressed mice, where neonatal exposure to anti-Ig antibodies appears to modify the normal "setting" of adult allotype levels, this type of circuit would account for the fluctuations and long-term maintenance of subnormal allotype levels by allotype Ts.

11 Carrier-specific regulatory cells: a triad in search of a circuit

Ironically, although the demonstration of two types of T cells operative in regulation of carrier-specific help led to the development of the ideas presented here, we find it difficult to propose a circuit design for regulation of this function. The consitution of the initial triad for such a circuit appears straightforward, *i.e.* a CTh, a CTs and a CTh(2) that regulates CTs function; but the recognition system that relates these T cells to each other and to the B cells whose response they regulate marks a substantial deviation from the circuits we have described.

Both CTh and CTs have V_H receptors that recognize carrier determinants on the antigen. Since these receptors should not be complementary to each other, recognition of CTh by CTs would appear to require an antigen "bridge". This raises the question of whether the recognition of the CTs by its CTh(2) helper involves a similar antigen bridge or an id^+ to id^- recognition such as those that exist in the CRC. Thus, the requirement for antigen interpolation at one or possibly more points in a potential carrier-specific regulatory circuit introduces a new element that allows design of several alternative circuits, each with its own merits and demerits. Discussion of these, we feel, would heap speculation without furthering our essential purpose in this publication, *i.e.* illustration of the basic principles of circuit design. Thus, we prefer to leave considerations of carrier-specific circuit construction for another time and focus here only on the role of CTh themselves in regulating antibody production.

12 Closing comments: perspectives on a new perspective

The inviolability of natural law rests on the principle of movement along the line of least resistance. These laws are not forces external to things but represent the harmony of movement imminent in them. That is why celestial bodies do not deviate from their orbits and all events in nature occur with fixed regularity; from the I Ching (Book of Changes), an ancient Chinese commentary [24].

In a sense, by positing the regulatory circuits described here we have come full circle in the historical development of ideas concerning the regulation of antibody responses. Originally, serum antibody was believed to be in control of responses. Then, when helper and (later) suppressor T cells were discovered, control passed to the regulatory lymphocytes, and the interactions among these cells became the major focus for regulation studies. The delineation of the mechanisms of carrier-specific, id-specific and allotype-specific regulation that followed, paved the way for our efforts here towards the creation of an overall theoretical framework for integration of these individual mechanisms into a comprehensive regulatory system. In the end, the rudimentary circuits we drew led us back to reintroduce antibody as a prime element in the regulation of its own production.

We began the process by making several assumptions based on extrapolations of findings in one or another of the individual regulatory systems.

1 Ts function by specifically depleting Th.

2 Ts differentiation and expression require help from, and are therefore controlled by, specific Th.

3 The recognitions between Ts and Th are mediated by complementary V_H region receptors, i.e. id-anti-id interactions.

4 A second Ts-Th recognition system exists that establishes a directionality of interaction such that a Th which helps a Ts will not be the target of the Ts it helps, *i.e.* that a Ts cannot deplete its own helper Th.

5 The series of Th-Ts interactions that regulate a response is not infinite, but rather turns back on itself at some point to create a circuit in which each cell regulates the one in front and is regulated by the one in back.

These assumptions led to the construction of a "core" regulatory circuit (CRC) in which each Th is the helper of one Ts and the target of the other. One of these Th is assumed to be the id-specific (Ig) Th that helps B cells differentiate to antibody-forming cells; the other has no assigned function (at present) other than to help the id-specific Ts that depletes the IgTh in the circuit.

The CRC, which is analogous in operation to an electronic binary (or "flip-flop") circuit, has certain defined properties that make it attractive as a core immunoregulatory circuit but restrict its independent regulatory capability. It can provide a basic "on-off" regulation for responses that would account for the tendency of the immune system to lock into responsive or nonresponsive states; but although it lends stability to the system, it cannot in and of itself control which state becomes established and is maintained. This control, which ultimately determines the kinetics of the response, must reside in auxiliary circuits (ARC) that control the level of Th activity available to support either help or suppression.

Definition of ARC that could potentially serve this purpose brought us to an unexpected conclusion, but one which in retrospect seems extremely reasonable: the V_H regions on antibody molecules, acting as id and anti-hapten combining sites, provide the most logical recognition bridges available to allow stimulation of the Th in the CRC and integration of the circuits that control id-, allotype- and carrier-specific regulation. Thus, starting with consideration of the interactions among regulatory T cells, we find ourselves returning in a sense to validate our "immunologic" heritage by reintroducing the regulated product, *i.e.* antibody, as a key element in the control of the antibody response regulatory system.

There are, however, major distinctions between the roles originally envisioned for antibody as response regulator and the roles to which we assign antibody in the ARC described here. We treat the overall antibody response as the aggregate of the individual (anti-hapten) id responses that occur over a period of time. Therefore, we see regulation of the overall response as the composite regulation of the individual CRC that control production of the anti-hapten id. The ARC that determine whether the CRC is in its help or suppression mode utilize the id determinants of the Ig they regulate as requisite recognition bridges for ARC function. Thus, the serum level of each id specifically controls production of that id while collectively, the serum id levels regulate the overall response.

The affinity of the serum antibody for antigen, and the availability of antigen, also play a role in determining which id are stimulated and which suppressed. Direct binding between antibody and haptenic determinants on the antigen is required to consitute a recognition bridge (between IgTh and CTh) in the ARC we have drawn as responsible for stimulation of the id-specific IgTh that help B cells. Thus, when antigen becomes limiting, id on antibodies with higher affinities for the antigen will be preferentially bound, enabling these antibodies to preferentially stimulate help for production of their own (high-affinity) antibodies. Operation of this ARC therefore provides a positive selection mechanism for progressively increasing the representation of high-affinity antibodies (id) in the response, *i.e.* for affinity maturation.

The reassignment of CTh from their generally accepted role as B cell stimulators to an auxiliary regulatory role as stimulators of Th(1) is probably the most novel proposition in the ARC we have drawn. Surprisingly, this reassignment appears consistent with the bulk of the available information on the mechanism of carrier-specific help. Despite extensive attempts, direct interaction between CTh or CTh factors and B cells has proved extremely difficult to verify. Essentially, the main argument in favor of this interaction still rests on the evidence demonstrating that hapten and carrier determinants, together with antibody, connect CTh and Th(1), thus presents a viable alternative to the prevailing view of the mechanism of carrier-specific help.

Nevertheless, we are leery of stripping CTh of their ability to directly help B cells, in part because of difficulties in visualizing how the initial stimulation of Th(1) could occur in the absence of antibody, but largely because of a conservative sense that well-established ideas should not be questioned without due cause. Our proposal stems from the need to

reconcile the requirement for both CTh and Ig-specific help for effective antibody responses. As we indicated earlier, however, this requirement could also reflect a requirement by B cells for two different types of signals (*e.g.* one for expansion and the other for differentiation). Similarly, although the CTh to Th(1) ARC offers a specific set of interactions that could explain affinity maturation, such maturation could also be accounted for if CTh selectively stimulate B cells that produce high-affinity antibody because these B cells tend to capture more antigen. Thus, at present the proposed ARC stands as an example of how consideration of circuit-based immunoregulatory interactions can lead to the development of new perspectives which both challenge current interpretations and suggest directions for future work.

Throughout our discussion, in fact, we have attempted to hew to this same general principle. In demonstrating how regulatory circuits could operate, we have described interactions that we believe are consistent with current evidence and could actually function as described; however, we have also repeatedly emphasized the tentative nature of the detail with which we embroidered the basic circuit structures. Existentially, presentation of detail is inescapable since abstract concepts such as circuit design require concrete models from which essential principles can be extracted. Furthermore, detail must be included to enable design of experiments that provide grounds either for rejection of the model or for its modification to conform to reality. Thus, in our discussions here we have made a number of "educated" guesses concerning regulatory interactions among the cells and cell products involved in the control of antibody responses.

Some of the guesses we have made may prove correct, others not. Undoubtedly, the regulatory process will prove substantially more complex than we have drawn it. For example, we have not even attempted to delineate mechanisms to account for the initial (neonatal) establishment and maturation of Th, Ts and B cell populations; for the sensitivity to exogenous induction of id-, allotype- and carrier-specific suppression; for the induction and regulation of MΦ and other accessory cell populations; for the regulation of immune responses other than antibody production, etc. Thus, we have ignored major blocks of information that potentially could allow more accurate shaping of the circuits we described.

But whether the specific circuits we have drawn form part of the actual immunoregulatory structure or whether modifications will be required, we think it highly likely that the basic principles of circuit interactions they embody will prove to be generally applicable. We offer the foregoing, therefore, as a beginning, a new approach that hopefully will provide a framework for discussion and experimentation aimed at understanding the integrated systems that regulate immune responses.

The development of the ideas presented here owed a major debt to Dr. Benvenuto Pernis (Columbia University, New York) who initiated the use of the term "circuit" in helping us to clarify the similarities and differences between the cell interaction circuits we envision as controlling immune responses and the networks proposed by Dr. Niels Jerne (Basel Institute for Immunology, Basel, Switzerland) and his colleagues. We are also indebted to Dr. Moon Nahm (Washington University, St. Louis, MO) who early in our deliberations pointed out that the core regulatory circuit we propose is analogous to an electronic binary ("flip-flop") circuit, and to Dr. David Parks (Stanford) who helped us to understand the properties of this and other circuits potentially applicable in immune regulation. In addition, we are pleased to acknowledge the skillful assistance of Dr. T. Tokuhisa, Ms. Jean Anderson and Ms. Debra Parks in the preparation of this manuscript and by Mr. Milton Wise in drawing the circuit diagrams. We also particularly appreciate the detailed substantial and editorial criticism provided by Dr. H. S. Micklem (Stanford University and University of Edinburgh, Edinburgh, Scotland).

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