REGULATION OF ANTIBODY RESPONSES

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INTRODUCTION

In proposing that recognitions between complementary immunoglobulin (Ig) variable region receptors (idiotype and anti-idiotype) play a major role in conversations between regulatory and effector lymphocytes, Niels Jerne and colleagues (1974) provided the theoretical basis for what has since been demonstrated to serve as a basic mechanism of communication among T cells and B cells involved in immune responses. The Jerne network model, however, failed to provide a framework for consideration of how functionally different lymphocytes such as suppressor and helper T cells and the effector populations they regulate are organized into functional units capable of governing the orderly progress of the immune response. This question has been addressed piecemeal during discussions of the series of cell and cell product interactions that regulate individual immune responses; however, there now appears to be a need for discussion of the underlying principles of immunoregulatory organization, if for no other reason than to facilitate identification of those components within each series that are responsible for coordination of different types of regulation in a single response (e.g., carrier, specific, idiotype-specific).

The prototypes of the integrated regulatory circuits discussed here represent a rudimentary attempt at developing this type of systematic view based on the application of the network theory's cognitive principles to the current understanding of the interactions among B cells, helper T cells, suppressor T cells, macrophages, antigen, and antibody. Each circuit individually consists of a closed series of interaction in which each element (cell or cell product) regulates the one in front and is regulated by the one in back. Shared elements between the circuits, however, enforce their integration so that they

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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. As will be shown, integrated circuits such as these, can account for the various properties of antibody responses (isotype and idiotype representation, affinity maturation, selective or general nonresponsiveness, etc.). Much of what we propose here is testable, and much may prove to be wrong. Nevertheless, we believe that the illustrations presented, showing how a circuit-based regulatory system can account for various response properties, demonstrate an essentially valid approach to consideration of the mechanisms of immunoregulation.

In the sections which follow, we will use the regulation of idiotype production as a context for illustration of basic circuit properties. 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 macrophage-containing auxiliary regulatory circuit (ARC) sensitive to serum idiotype levels that can switch the CRC from help to suppression; (c) a carrier-specific Th circuit that regulates the supply of idiotype-specific help; and (d) an allotype-sensitive ARC that suppresses production of idiotypes associated with an allotype-bearing IgG heavy 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.

T CELLS ACTIVE IN REGULATION

This brief review presents our understandings, interpretation and occasional assumptions regarding the properties and functions of suppressor and helper T cells. 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. Much of the material upon which our ideas are based is covered in detail in other chapters in this volume.

Helper T Cells (Th)

Two types of Th active in helping B cell differentiation to antibody-forming cells (afc) have been identified. One of these recognizes (carrier) determinants present on the antigen (Rajewsky *et al.*, 1969; Mitchison, 1971). The other recognizes structural determinants (idiotypes and allotypes) on immunoglobulins present on the differentiated B cell (Hetzelberger and Eichmann, 1978a,b; Eichmann *et al.*, 1978; Woodland and Cantor, 1978; Adorini *et al.*, 1979; Herzenberg *et al.*, 1976; Black *et al.*, manuscript in preparation). 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 immunoglobulin H chains (Krammer and Eichmann, 1978). Idiotype-specific Th carry anti-idiotype (id^-) V_H structures* that enable them to recognize and help the differentiation of id^+ precursor B cells to idiotype-producing afc. Carrier-specific Th, on the other hand, appear to carry V_H receptors similar to those in antibodies that recognize determinants on the carrier molecule.

Suppressor T Cells

A variety of T cell populations that 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 individual Th populations. Allotype, idiotype, 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 immunoglobulin V_H structure library. In idiotype-specific regulation, this means that Ts have idiotype-like (id^+), i.e., antigen-binding, receptors complementary to the id^- receptors on the Th that help idiotype-producing B cells (Hetzelberger and Eichmann, 1978a,b; Eichmann *et al.*, 1978; Woodland and Cantor, 1978; Adorini *et al.*, 1979;

**To facilitate discussion, we have adopted the convention of referring to complementary idiotypes 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 idiotype. 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.*

Krammer and Eichmann, 1978). Soluble suppressive molecules (TsF) with antigen-binding receptors have been isolated in idiotype regulation systems (Ju *et al.*, 1978; Kapp *et al.*, 1975). These molecules also carry other structural determinants (e.g., I-J) that are apparently part of the "constant region" of T cell effector molecules.

The mechanisms regulating Ts differentiation and expression have not been well charted as yet. The recent demonstration that Th help Ts differentiation, however, suggests that Ts differentiation proceeds analogously to the differentiation of other lymphocyte effector populations (afc, cytotoxic T). The circuits we propose here are based on this analogy, outlined as follows:

1. Virgin Ts precursors differentiate to Ts memory cells that differentiate to Ts effector cells.
2. Both differentiation steps require help from specific Th dedicated to this purpose.
3. Ts memory, once generated, persists indefinitely but remains cryptic in the absence of Th required for memory to effector cell differentiation.

Thus, the absence of Ts activity may be due either to the absence of Ts or the absence (depletion) of Th that help Ts memory development and expression.

ORGANIZING REGULATORY T CELLS INTO CIRCUITS

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 element performs the same function wherever it exists in the circuit (e.g., Th always help, Ts always deplete Th). These roles, furthermore, are kept consistent with the known functions of the cells and products involved so that 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.)

Our primary assumption states that, for effective operation, a circuit must contain three or more independent elements since two element circuits generate autocatalytic or self-negating interactions ("short circuits") that tend to spiral out of control to exhaustion. For example, in circuits such as those that regulate idiotype production, where Ts and Th recognitions and interactions are direct, we assume that Th which help Ts are not the targets of the Ts they help and Ts which deplete Th are not helped by the Th they deplete. Allowing Ts to deplete their own helper population would essentially consign the circuit to rapid suicide since increasing the help for Ts would only expand the Ts activity available to destroy the helping Th population. Even if the Th supply could keep ahead of the ability of the Ts population to destroy Th, the net result of Ts activity would be a constant pressure against suppression of the response regulated by the circuit. The instabilities inherent in such a system contrast sharply with the known stability of suppression once in force. Thus we believe that target Th and helper Th for a given Ts in direct interaction circuits are always 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 Fig. 1. The first regulatory circuit to be described is derived from these triads.

The recognitions between cells involved in idiotype regulation have been shown to consist of complementary V_H interactions between sequential members. Therefore, in a series of idiotype regulation triads, the two flanking members in a triad will have similar or identical V_H receptors that are complementary to the V_H receptors of the central member. Expressed in the notation

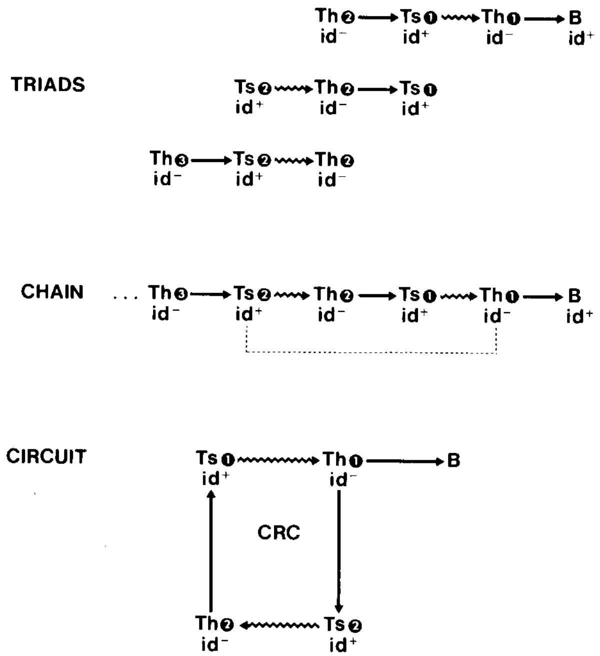

Fig. 1. Development of the core regulatory circuit (CRC).

system we have adopted, (in which the idiotype structure produced by the B cell is assigned as id$^+$ and the anti-idiotype 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 Fig. 1).

Assumption of similar V_H receptors on the flanking members of a triad lead to a corollary assumption stating that a second recognition system exists among Ts and Th that identifies targets from helpers for a given Ts. Considering the wide variety of surface determinants existent on T cell subpopulations, this corollary poses no special problem. Presence or absence of QA1 determinants, for example, could serve this purpose. These markers appear to distinguish a Th population that helps a Ts from one that may be its target (Janeway, 1979); however, we suggest this (remote) possibility only as an example of a determinant that appears to be expressed on some but not all helper lymphocytes rather than as a serious extrapolation from data which in fact potentially excludes QA1 as a contender.

Returning to the development of the circuit shown in Fig. 1: Th-Ts-Th triads, when overlapped, constitute an infinite chain in which the target Th of one Ts becomes the helper Th of another, starting with the Th that helps B cells, i.e., Th(1), and working backward ad infinitum. A quick slash with Occam's razor, however, severs the first two overlapped triads from the rest of the chain, i.e., since the target Th of one Ts can be the helper Th of another Ts, two pairs of Ts and Th organized as a circuit rather than chain are sufficient to provide the help and target requirements for all four T cell types. This circuit, which we have named the core regulatory circuit (CRC), is shown at the bottom of the figure.

THE CORE REGULATORY CIRCUIT (CRC)

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 Fig. 1 the Th in the upper right-hand corner, called Th(1), is assigned as the idiotype-specific helper of the B cell that produces antibody with idiotypic 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 each other or to the B cell id^+ Ig.)

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 Fig. 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 idiotype 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.

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. However, 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 responses. This system, we suggest, consists of several auxiliary regulatory circuits (ARC's) which control CRC Th stimulations and thereby control affinity maturation, allotype and isotype expression, maintenance of tolerance, etc.

AUXILIARY REGULATORY CIRCUITS

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 (idiotype) 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 antibodies and suppressing those producing antibodies with lower affinities, i.e., for selectively increasing or decreasing help for individual idiotypes according to the hapten-combining affinity of the idiotype. In addition, the system requires a mechanism for preventing production of undesired idiotypes (i.e., maintaining tolerance) and for regulating the overall isotype representation in responses.

CRC, being specific for individual idiotypes, 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 idiotype is produced and how long production continues. The cells and cell products involved in these interactions, we suggest, are organized into several ARC's whose properties include sensitivity to V_H (idiotype) affinity and serum isotype (allotype) levels.

The basic design of each of the ARC's is essentially the same: id^+Ig (idiotype) produced by a B cell combines either directly or indirectly (through antigen) with T cell factors adhering to macrophages. The presence of the idiotype then allows formation of a recognition bridge between the macrophage (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 record recognition requirement between macrophage 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.

The inclusion of idiotype (i.e., antibody) in the ARC reintroduces the concept of antibody feedback regulation of responses. This concept has fallen somewhat into disuse with the ascendance of regulatory T cells but still appear 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 idiotypic determinant 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 helper T cell factors 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 macrophages to enable delivery of a stimulation signal or to deliver stimulation signals themselves to target cells when an appropriate recognition bridge joins the macrophage-Th factor-antibody complex to the target cell. The inclusion of macrophages and their assignment as carriers of T cell factors and potential stimulators of Th in the CRC proposes a role for macrophages that is again consistent with known function (Feldmann and Nossal, 1972; Schwartz *et al.*, 1978; Shevach, 1976; Paul and Benacerraf, 1977; Pierce *et al.*, 1976). This role could also be played by other accessory cells capable of binding T cell factors and antigen; thus its assignment to macrophages is somewhat arbitrary and the assumptions made in later sections concerning macrophages should be considered with this point in mind.

SHIFTING THE CRC FROM HELP TO SUPPRESSION: AN ARC FOR ELIMINATING PRODUCTION OF UNWANTED IDIOTYPES

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 idiotype levels are high and thus serve to suppress idiotype production when a critical serum idiotype level is reached. An ARC which could control Th(1) stimulation will be discussed in the next section. 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 (see Fig. 2).

We assume that macrophages carry T cell helper factors, i.e., Th(1)F, from Th(1) to target B cells. In idiotype-specific help, the Th(1)F molecules contain id^-V_H segments complementary to the id^+V_H of the immunoglobulins produced by the B cell. The id^- receptors should be capable of combining both with id^+ surface Ig on B cells and with id^+Ig in circulation.

In the absence of serum-borne id^+Ig molecules, the macrophages will deliver id^-Th(1)F to id^+B cells and trigger the B cells to differentiate to id^+Ig-producing afc. 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 macrophages 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 macrophage-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 macrophages 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). The assumption that Th(1)F-carrying macrophages 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."

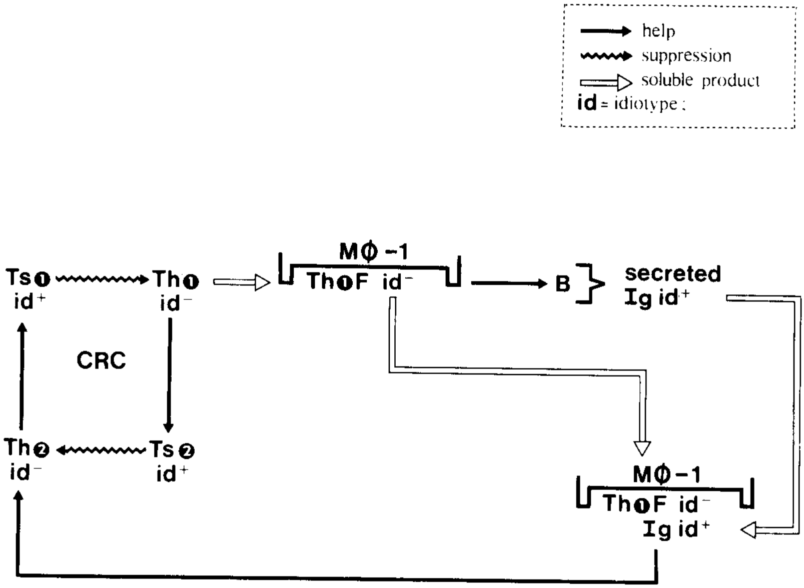

Fig. 2. Suppressive auxiliary regulatory circuit (ARC). Secreted idiotype (Ig id$^+$) combined with macrophage-bound soluble product from Th(1) stimulates the suppressive side of the CRC.

Stimulation of Th(2) by macrophage-Th(1)F-id$^+$Ig complexes 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 existence of ARC's sensitive to serum id$^+$Ig levels allows explanation of several properties of antibody response regulation: the sequential production of idiotypes would be expected because, as production of each idiotype peaks, its CRC is shifted to the suppressive condition leaving other (more recently expanded) idiotypes to maintain the response. Furthermore, if antigen selectively removes idiotypes with higher affinity for the antigen from circulation, the CRC regulating these idiotypes would be maintained in a help condition at the expense of CRC regulating lower affinity idiotypes (which will shift sooner into suppression). Thus the integrated ARC-CRC circuit described could significantly contribute to the process of avidity maturation, although as we shall show, a second ARC, responsible for Th(1) stimulation, would be expected to play a more important role in this process.

ESTABLISHING THE CRC IN A HELP CONFIGURATION: AN ARC THAT INTEGRATES THE ROLES OF CARRIER-SPECIFIC AND IDIOTYPE-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 macrophages) that enabled triggering of sufficient B cell expansion and differentiation to account for the large numbers of afc obtained in the response. This state of innocence, however, was shattered when allotype and idiotype 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 (Feldmann and Nossal, 1972; Schwartz *et al.*, 1978; Shevach, 1976; Paul and Benacerraf, 1977; Pierce *et al.*, 1976). The demonstration of two Th populations (CTh and IgTh) with clearly distinct specificities reinforced conclusions from earlier studies suggesting that two separate Th were active in supporting initial B cell responses in adoptive secondary assays (Janeway, 1975; Janeway *et al.*, 1977). 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 idiotype 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 Fig. 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 macrophage and may serve to activate the macrophage 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.

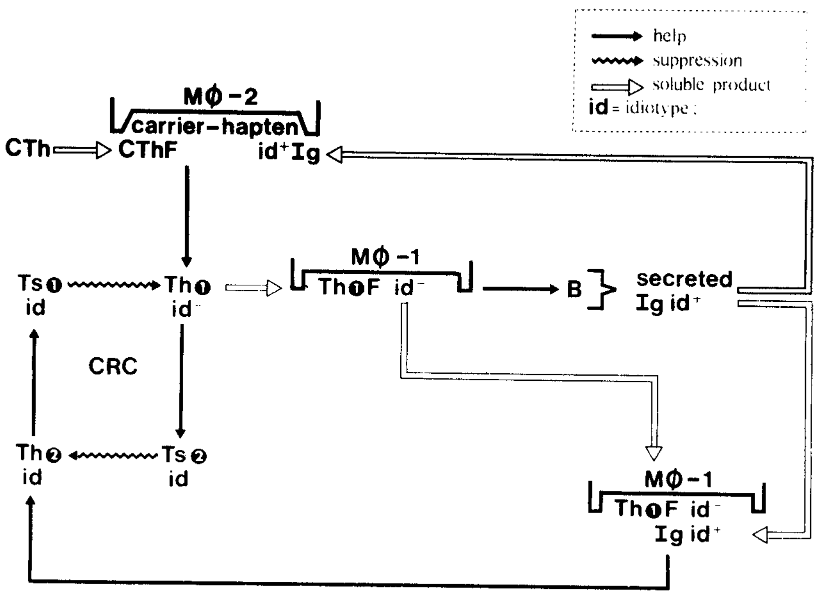

Fig. 3. Help-stimulating auxiliary regulatory circuit (ARC). Secreted idiotype (id^+Ig) combined with macrophage-bound complex of antigen and soluble product from CTh stimulates the help side of the CRC.

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 on-going response; however, at the beginning of a response, antibody could instead be "stolen" from B cell surface Ig. In either event, once antibody binds to the hapten, the macrophage will carry idiotypic determinants that can be recognized by the Th(1) in an idiotypic-specific CRC.

If we now assume that the macrophages that receive CThF can stimulate Th(1) but not Th(2), 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 idiotypic 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 Th(1) supply. 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.

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 idiotype-specific Th. 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.

REGULATION OF AFFINITY MATURATION OF ANTIBODY RESPONSES: COOPERATION BETWEEN TWO ARC's

In describing the first ARC, which shifts the CRC from help to suppression when serum idiotype levels are elevated, we pointed out that the tendency for antigen to selectively remove high-affinity idiotypes from circulation would tend to favor suppression of lower affinity idiotypes. Such selective suppression could 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, could favor expansion of higher-affinity clones. However, 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. Thus it could be expected to be regulated by circuits that increase help from these clones. The CTh ARC described in the previous section provides this capability.

In the CTh ARC, macrophage-CThF-antigen complexes bind antibody from circulation (or possibly from B cell surfaces). The bound antibody then creates a recognition bridge between the macrophage and the idiotype-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. Thus it is likely (if these circuits are real) that affinity maturation involves operations of both ARC's as they regulate individual CRC within the response.

MAINTENANCE OF OSCILLATING INTERMEDIATE RESPONSE LEVELS: AN ALLOTYPE SUPPRESSION ARC

Circuits such as the idiotype-specific CRC 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 oscillate 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 (see Fig. 4), is integrated with the previous circuits; however, we include it in outline in this discussion because it illustrates a circuit capable of oscillating indefinitely around a fixed response level, be it high, low, or intermediate.

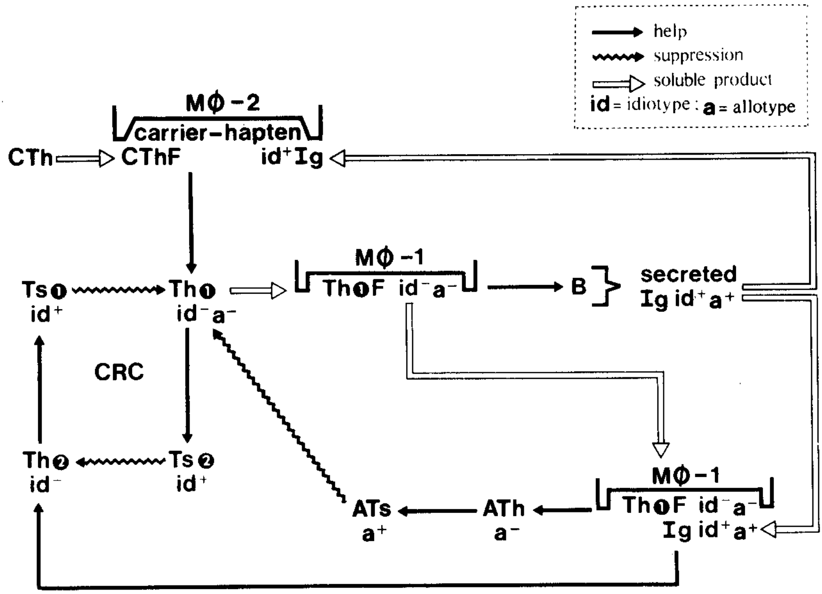

Fig. 4. Allotype-suppressive auxiliary regulatory circuit (ARC). Secreted allotype-bearing immunoglobulin (Ig id^+a^+) combined with macrophage-bound soluble product from Th(1) stimulates increase in allotype suppressor T cells that deplete Th(1).

For this ARC, we assume that: (a) Th(1) is responsible for delivering both allotype-specific and idiotype-specific help. (b) Th(1)F has two recognition sites for B cell Ig: an id^-V_H receptor site complementary to the id^+V_H on the Ig molecule and an a^- Th(1)F-constant region site complementary to an (a^+) allotypic determinant in the IgH chain constant region. (c) Delivery of help (and suppression) signals by T cell factors requires engagement of the id^- V_H receptor on the factor. (d) Secondary interactions between factor and B cell Ig constant regions improve help delivery (e.g., by decreasing binding affinity required between id^+ and id^-V_H) but are insufficient, in and of themselves to trigger transmission of the help signal.

These assumptions govern construction of the allotype ARC shown in Fig. 4. In this circuit, Th(1) carrying a^- constant region determinants are targets of allotype suppressor T cell (ATs) that have a^+V_H receptors. These two cells, plus another Th (ATh) that has a^-V_H receptors and helps the ATs, constitute the initial links of a Th-Ts-Th chain similar to the chain from which the CRC is derived. Rather than closing into a CRC, however, this chain remains open-ended because, according to the assumptions stated above, Th(1) cannot use its a^- constant region determinants to deliver help to a Ts further on the chain, i.e., Th(1) can be a Ts target but not a Ts helper in this circuit. This means that the only regulation Th(1) can exert on ATs and ATh will be through an ARC that closes because the A^-V_H receptors on ATh recognize a^+ serum Ig whose production is regulated by Th(1).

An ARC constructed in this manner has certain distinct properties. Its operation results in an oscillation of the serum allotype levels it regulates because increases in serum Ig stimulate ATh and, therefore, increase ATs activity. An increase in ATs then reduces allotype levels by depleting the Th(1) that help allotype producing B cells and the reduced serum allotype levels reduce ATh activity. Finally, ATs activity decays without continued help from ATh and the cycle can start again.

The serum level around which oscillations occur will depend in part on the affinity of the ATh a^-V_H receptor for the regulated allotype. A high-affinity ATh receptor will bind the macrophage-Ig complex more easily and will thus be stimulated and provide help for ATs at low serum allotype levels while a low-affinity ATh receptor will require higher serum allotype levels to trigger the suppressive cycle. The initial ratio between ATs, ATh, and Th(1) will also influence the fixation of the average level; therefore, Ts induction in neonate could result in chronic suppression, especially if there is a genetic predisposition toward limiting Th(1) activity. Strong antigenic stimuli 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 provides a specificity system that allows for simultaneous regulation of all isotypes associated with individual H chain isotypes or allotypes.

CARRIER-SPECIFIC REGULATORY CELLS: A TRIAD IN SEARCH OF A CIRCUIT

Ironically, although the demonstration of two types of T cell operative in regulation of carrier-specific help (Herzenberg *et al.*, 1976; Black *et al.*, manuscript in preparation) led in part to the development of the ideas presented here, we find it inappropriate to propose a circuit design for the regulation of this function. The constitution 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 in that both CTh and CTs have V_H receptors that recognize carrier determinants on the antigen. This introduces an entirely new cognitive principle into the circuit, i.e., antigen bridges between regulatory T cells and places such circuits outside the scope of the discussion of the idiotype-anti-idiotype based circuits presented here.

The reassignment of carrier-specific CTh, however, 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's we have drawn and is worthy of some comment. 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 must be on the same molecule for effective stimulation of anti-hapten responses; but our proposal that the hapten-carrier-conjugate combined with antibody connects CTh and Th(1) puts a different interpretation on this requirement, and presents, we believe, 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 CTh-Th(1) ARC stands as an example of how consideration of circuit-based immunoregulatory interactions can lead to the development of new perspectives that 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 discussion 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.

Although some of the guesses we have made may prove correct, others may 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 idiotype-, allotype- and carrier-specific suppression; for the induction and regulation of macrophages and other accessory cell populations; for the regulation of immune responses other than antibody production. Thus we have ignored major blocks of information that potentially could allow more accurate shaping of the circuits we described.

However, 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.

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