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CIRCUITS FOR REGULATING ANTIBODY RESPONSES

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INTRODUCTION

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-anti-idiotype (complementary V_H) recognitions[1]. This theory provided some extremely useful insights in that it stimulated the successful search for expression of immunoglobulin V_H regions (idiotypes 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, idiotype-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 non-responsiveness (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 type of systematic view of how the immune system is organized[1].

MATERIAL AND METHODS

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 non-responsiveness, 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 (CRC) that determines whether a given idiotype will or will not be produced and three auxiliary regulatory circuits (ARCs) that respond to antigen and serum antibody (idiotype) levels by switching the CRC 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 suppression and helper T cells, macrophages and soluble regulatory products). As an integrated unit, they constitute a detailed "working" model that depends on relatively few assumptions and is consistent with the known interactions between circuit elements and the known properties of humoral responses.

The CRC (core regulatory circuit) provides the basic on-off control in the system. Its derivation is guided by the following assumptions:

Assumptions for the derivation of the core regulatory circuit

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., idiotype-anti-idiotype 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.

The fourth assumption, that Ts do not attack their own Th, is basic to derivation of the circuits described here. 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 which 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 which 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 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[3-6] 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 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).

These triads, when overlapped, constitute an infinite chain (actually, alternate rings of Ts and Th in an annular network) 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 backwards 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

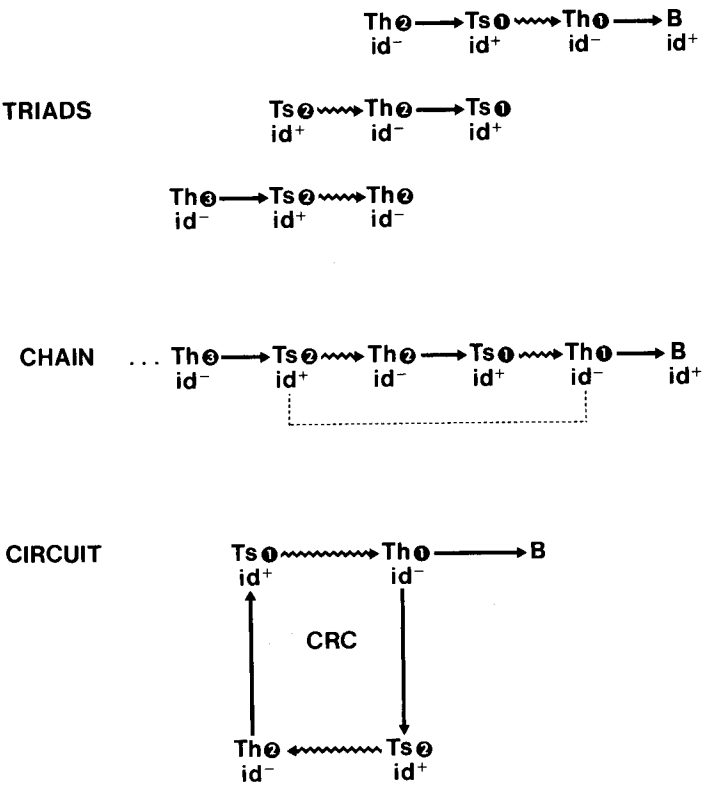

Fig. 1. Development of the Core Regulatory Circuit (CRC). $\underline{id^+}$ and $\underline{id^-}$ symbols represent complementary idiotypes.

the bottom of Fig. 1.

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 in (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 which 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 Fig. 1) if 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 T2(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 responses. This system, we suggest, consists of several auxiliary regulatory circuits (ARCs) which control CRC Th stimulations and thereby control affinity maturation, allotype and isotype expression, maintenance of tolerance, etc.

Summary of the Properties of the Core Regulating Circuit (CRC)

1. Tends to lock into suppression or help mode
2. Can be switched from suppression to help or vice-versa by pressures strong enough to reverse the ratio between the two Th
3. Auxiliary Regulatory Circuits (ARC's) 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.

The auxiliary regulatory circuits we have drawn as controlling units for CRC function (Figs. 2-4) are derived in part with a view towards understanding both the all-or-none regulation of responses and the qualitative regulation inherent in the process of affinity maturation. 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 "auxiliary regulatory circuits" (ARCs) whose properties include sensitivity to V_H (idiotype) affinity and serum isotype (allotype) levels.

The basic design of each of the ARCs 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 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.

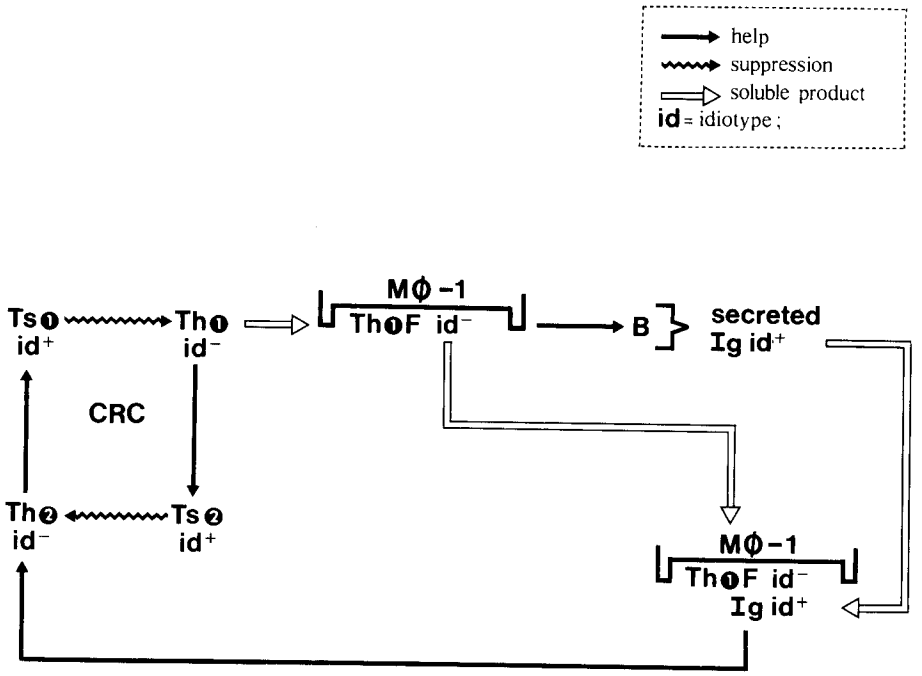

Fig. 2. Suppressive Auxiliary Regulatory Circuit (ARC). Secreted idiotype Id^+Ig combined with macrophage-bound soluble product from Th(1) stimulates the suppressive side of the CRC.

The inclusion of idiotype (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 idiotypic 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.

Summary of Basic Assumptions for Auxiliary Regulatory Circuits (ARC's)

1. Antigen and V_H regions on T cell factors and serum Ig create recognition bridges in the ARC's.

2. Accessory Cells (macrophages) carry factors and Ig between lymphocytes.
3. Either accessory cells or factors are responsible for distinguishing Th(1) from Th(2)
4. Either accessory cells or factors deliver signals to the appropriate Th.

The ARC that regulates Th(1) stimulation contains our most novel and contentious suggestion, i.e., that carrier-specific Th (CTh) regulate responses by regulating Th(1) activity rather than interacting directly with B cells. We make this suggestion more with a view towards exploring potential circuit interactions than presenting a definitive model; nevertheless, we feel some arguments can be made for the potential validity of the idea. This requires a brief review of helper T cell history and function.

For many years, carrier-specific Th (CTh) were the only T cells known to be required to help B cells respond to antigen[7,8]. 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 antibody forming cells 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[3-6,9]. 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[10,11]. Thus it became increasingly clear that the original view of how Th help B cells had to be expanded to provide non-overlapping 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 auxilliary 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 (see Fig. 3).

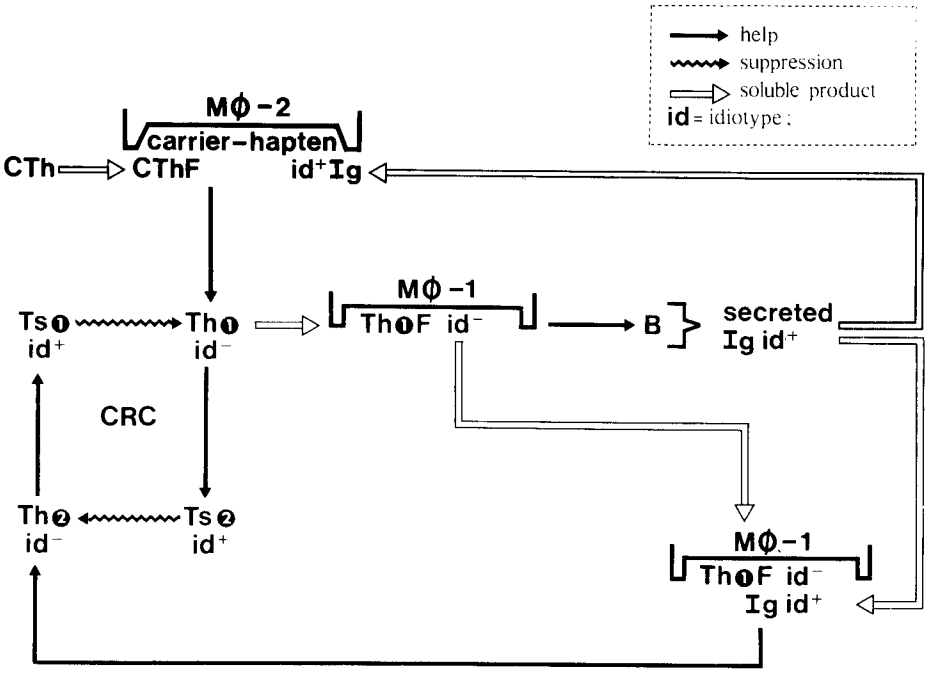

Fig. 3. Help-stimulating Auxiliary Regulatory Circuit (ARC). Secreted idiotype (id+Ig) combined with macrophage-bound complex of antigen and soluble product from CTh stimulates the help side of the CRC.

The subordination of Th(1), i.e., IgTh, to CTh has several practical consequences. It makes the stimulation of Th(1) that are specific for individual idiotypes dependent on the binding of antibody carrying those idiotypes to the CThF-antigen complex on macrophages. 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 would also favor 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 idiotypes.

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. 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 idiotype-specific suppression or help can be demonstrated, since results from these studies indicate that responses fail unless both CTh and IgTh are present[3-6,9].

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 concommitant loss of low affinity representation in the response. Thus it is likely (if these circuits are real) that affinity maturation involves operations of both ARCs as they regulate individual CRC within the response.

Affinity maturation should be favored by the operation of the (suppressive) idiotype-specific ARC which shifts the CRC from help to suppression when the serum level of the circuit-regulated idiotype becomes high. In this ARC (see Fig. 2) macrophages 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 macrophage 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 idiotypes with high antigen-binding affinities are preferentially removed by sequestered or circulating antigen, the serum idiotype spectrum will tend to become biased toward idiotypes with lower antigen-binding affinities. This bias will in turn tend to induce more rapid shifting of the CRC regulating the low-affinity idiotypes 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.

In the CTh ARC, CThF-antigen complexes bound to macrophages 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 the 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.

Summary of Arc Control of Affinity Maturation.

1. 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.
2. 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's for low affinity antibodies from the help to the suppression mode.

In addition to the ARC's described above, we have also constructed an ARC that will provide for the oscillating regulation seen in allotype suppression (see Fig. 4). The derivation of this ARC and justification of the principles assumed to govern its operation are described here only in outline.

Summary of Assumptions for an Arc that Regulates Allowtype Production.

1. Th(1) delivers both allotype-specific and idiotype-specific help.
2. 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 a^- Th(1)F-constant region site complementary to an (a^+) allotypic determinant in the IgH chain constant region.
3. Delivery of help and suppression signals by T cell factors requires engagement of the V_H receptor on the factor.

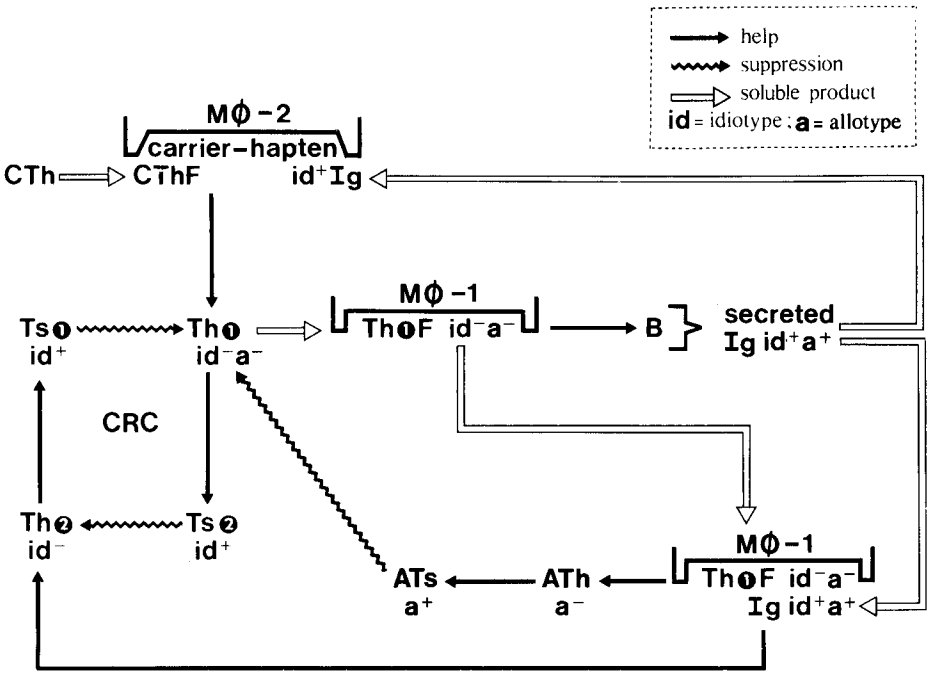

Fig. 4. Allotype-suppressive Auxiliary Regulatory Circuit (ARC). Secreted allotype-bearing immunoglobulin (Ig Id^+a^+) combined with macrophage-bound soluble product from Th(1) stimulates increase in allotype suppressor T cells that deplete Th(1). $\underline{a^+}$ Determinants are IgH chain constant region (Fc) allotypes or TsV_H region determinants that mimic the $\underline{a^+}$ allotype structure. $\underline{a^-}$ Determinants are complementary to a^+ and found on the Th(1) F "constant" region and the ATh $V_H$region. Signals can be received through all a^+ to a^- interactions; signals can be delivered only when the V_H region of the "signaling" molecule is engaged, i.e., is a a^+ or a^-.

4. 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.

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 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 immunoglobulins 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.

Thus, in sum, we have drawn four integrated circuits that together could account for some of the basic regulatory phenomena in the immune system. In designing these circuits, we have attempted to incorporate cell and cell product interactions that we believe are consistent with current evidence; but we have also repeatedly emphasized the tentative nature of the detail with which we embroidered the basic circuit structures. Essentially, we have made a number of educated guesses, some of which may prove correct, other not. But whether or not the circuits we have drawn are representative of real immunoregulatory processes, we think it highly likely that the basic principles of circuit interactions they embody are valid and 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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