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DOES THE SIGNAL FOR THE ACTIVATION OF T CELLS ORIGINATE FROM THE ANTIGEN-PRESENTING CELL OR THE EFFECTOR T-HELPER?

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Abstract

The present view is that the Antigen-Presenting Cell (APC) processes and presents simultaneously on its surface several different antigens that are displayed randomly (with respect to their being Self or Nonself) as peptide-MHC complexes. The naive T-cell interacting with its ligand on the APC is activated by “co-stimulation,” the first step on the pathway to effectors. This view ignores the requirement for Associative Recognition of Antigen (ARA) in mediating both the Self-Nonself discrimination and the regulation of effector class. The introduction of ARA as a requirement for these two decision functions highlights a critical role for the effector T-helper (eTh) and necessitates rethinking the contribution of the APC.

Keywords

APC; effector T-helper; Self-Nonself discrimination; Associative Recognition of Antigen; co-stimulation

Introduction

This essay is an attempt to bring to the fore a central question concerning the origin of the specificity of an adaptive immune response. Because of its centrality, this question is being analyzed here at a theoretical level. In this way any model or view can be directly confronted by an agreed upon principle and the design of discriminating experiments. Only *antigen-specific interactions* will be under analysis here. The antigen-unspecific interactions that affect the sensitivity or kinetics of the response do not determine the inactivation-activation decision. Only the TCR contributes an antigen-specific element. The conclusion will be that the effector T-helper (eTh), not the Antigen-Presenting Cell (APC), is the source of the activating Signal [2] for T-cells. It is the absence or presence of Signal[2] that distinguishes the inactivation from the activation pathway.

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Background

The adaptive immune system is defined by the somatic generation of a large random paratopic repertoire that functions when coupled to a set of biodestructive and ridding effector mechanisms. This adaptive paratopic repertoire recognizes a portion of the antigenic universe to which the innate immune system is blind. The consequence is that the decision steps must have been selected to be dependent on the antigen-specific contribution of the adaptive repertoire itself [1].

The protective efficacy of this system depends on two decision processes [1–4]. First, the repertoire must be sorted into those specificities (anti-Self) which, if expressed, would result in the death of the host by autoimmunity and those specificities (anti-Nonself) which, if not expressed, would result in the death of the host by infection. Decision 1, the sorting of the repertoire, can justifiably be described as the Self-Nonself discrimination.

Second, the sorted repertoire must be coupled to the effector mechanisms in a coherent and independent manner for each antigen. Decision 2, the coupling to effector mechanisms, must be regulated so that ineffective mechanisms do not inhibit the effective mechanisms and the biodestructive and ridding response does not overshoot to kill innocent bystanders (immunopathology).

The normal functioning of both Decisions requires Associative Recognition of Antigen (ARA). ARA means that the regulatory cell interacting with one determinant derived from an antigen dictates to every other defensive cell interacting with that antigen what its response should be. ARA is required both to minimize the breaking of tolerance at the level of Decision 1 and to give the immune response to each antigen, coherence and independence at the level of Decision 2 [5–12]. Without ARA the immune response would be ineffective.

The problem then boils down to which cell is the regulator, APC or eTh, what does it regulate and how does it carry out its function in ARA? In order to deal with these questions a brief recalling of the ARA model is warranted.

The essentials of the ARA model

Antigen-responsive cells are born as initial state cells (referred to as i-cells) that 1) express a single paratope, 2) are without effector function and 3) have two pathways open to them, inactivation and activation. This applies to all classes of i-cell, iTc (cytotoxic), iTh (helper), iTsu (suppressor), and iB. Paratopes interact with epitopes, not antigens. Antigens are collections of linked epitopes. The interaction of the antigen-receptor on the i-cell with an epitope results in its eventual inactivation.

The signal delivered to the i-cell via its antigen-receptor, TCR or BCR, is designated Signal [1], the consequence of which is differentiation to the anticipatory or a-state. The a-cell interacting with an epitope cannot tell if it is on a Self- or Nonself-antigen. It must be told. The decision as to which pathway, inactivation or activation, is taken depends squarely on the functional presence or absence of Signal[2]. This is the role of Signal[2] which must be antigen-specific. As a consequence Signal[2] must only be delivered to those a-cells that are interacting with epitopes linked on or derived from that antigen (i.e., Associative Recognition of Antigen (ARA)).

The pathway then becomes:

[As an aside, I feel that the introduction of the precise, theoretically defined states, initial (i), anticipatory (a), activated (g) and effector (e) is essential to clarity. No competing equivalent nomenclature used routinely by immunologists substitutes to describe these states].

Signal[2], whatever its mechanism or modifying components, must be able to convert a Nonspecific a-cell receiving Signal[1] to the activated state leaving the Self-specific a-cell receiving Signal[1] to go on to inactivation. This is what confines the discussion to the antigen-receptors of the adaptive system and their ligands, that is, to antigen-specific events only.

The activated cell (symbolized g-cell) is not yet an effector (e-cell). It requires a series of steps of differentiation and proliferation defined by Decision 2 to become an effector (e-cell).

The ARA model has evolved over the years and has been analyzed in numerous papers to which the reader interested in the details is referred [1–5,13–15].

The APC is an obligatory platform for eTh-APC-aT interactions, but is it at the origin of Signal[2]?

The interaction between the eTh and an aT-cell requires an APC intermediate (i.e., eTh-APC-aT). Direct T-T interactions are excluded. This is what introduces the question as to the origin of Signal[2], eTh or APC.

While it is clear that Signal[2] is delivered by an eTh in order to activate the aTc (cytotoxic) [16,17] and aB-cells [18], the debate that is the subject of this essay is whether Signal[2] is delivered by an Antigen-Presenting Cell (APC), so-called “co-stimulation,” or by an effector T-helper (eTh) to activate anticipatory helper T-cells (aTh), namely those receiving Signal [1].

The ARA model defines the eTh as the regulatory cell functioning in ARA that delivers the activating Signal[2], the first step of Decision 2. The standard competing position is that the Antigen-Presenting Cell (APC), not the effector T-helper (eTh), is the regulatory cell delivering Signal[2] (“co-stimulation”). In sum then, under the ARA model, inactivation is driven epitope-by-epitope, whereas activation is driven antigen-by-antigen (ARA). Under the standard view both inactivation and activation are driven epitope-by-epitope (non-ARA) leaving questions of the specificity of response to the later steps of Decision 2 where ARA would be required.

The assumption that the eTh is the source of Signal[2] requires a solution as to its origin because the activation of aTh to gTh is dependent on eTh. This is referred to as the “primer problem.” One solution is that there exists a Nonspecific antigen independent pathway to effector T-helpers [11,12]. If the rate of inactivation of aTh receiving Signal[1] is rapid compared to the rate of conversion of iTh to eTh, a primer eTh anti-Nonspecific population sufficiently devoid of anti-Self reactivity will emerge. This is the source of the eTh anti-Nonspecific that primes the aTh to gTh conversion “autocatalytically.” *Important for our discussion here is that, if the primer pathway proves to be correct, then the T-helper populations used experimentally to analyze activation by APC are a mixture of iTh and eTh at a priming level.* As a consequence, it cannot be argued from the experiments to date that the APC, in the absence of eTh activates the aTh.

Associative Recognition of Antigen (ARA) is obligatory for a regulated response

The strength of the argument depends on the assumption that ARA is a requirement for the activation of all cells whether they be B or T. When dealing with APC function, ARA is rarely considered, the notable exceptions being Bretscher [5], Mitchison [19], and Zanetti [20].

The a-cell receiving Signal[1] has no way to know if it is interacting with a Self or Nonself ligand. Consequently, the a-cell population must either have been adequately presorted by an independent mechanism to be uniquely anti-Nonself or the a-cell must be instructed by an adequately presorted second signal that defines Nonself.

If the a-cell repertoire were somehow presorted to be uniquely anti-Nonself, then it would only need to be inducible-only (equivalent to being an effector) and activation (the g-state) would not be a required step in the response. Neither an eTh nor an APC would be required to provide a second signal. This would be, in essence, the Lederberg model [21] under which cells are born tolerizable-only and differentiate antigen-independently to inducible-only (equivalent to effectors).

If the aT-cell repertoire is only partially presorted centrally in the thymus, then when it encounters the APC in the periphery, an antigen-specific second signal would be required for activation and this signal must be delivered in ARA. Even a centrally totally presorted aT-cell repertoire does not obviate the requirement for ARA at later steps of Decision 2 where an effector response must be induced that is coherent and independent for each antigen.

The two models for the source of Signal[2] for the activation of T-helpers

The majority position is that activation of the T-helper simply requires an interaction with a so-called “activated” APC presenting the appropriate processed ligand. It is assumed that the APC is activated to “costimulate” either by interaction of microbial products with a variety of germline-encoded receptors, or by products of necrosis and inflammation or by effector T-helpers (eTh). Costimulation in the eyes of most immunologists is innocent bystander activation, not ARA. Of course, if the repertoire were purged centrally of all anti-Self reactivities, an activating antigen-specific Signal[2] delivered in ARA would not be required, but that is equivalent to saying that the APC is also not a decision component in making the Self-Nonself discrimination. It takes a response to one Self-antigen to debilitate the individual making it reasonable to assume that a mechanism of peripheral tolerance exists, not to mention the experimental evidence [22].

Over the years both Bretscher [5] and I [4] have addressed this question of the role of the APC as a putative source of Signal[2]. We have pointed out that the APC cannot be the platform for or the decisive determinant of the inactivation-activation decision because, as viewed today, the APC presents simultaneously several antigens both Self and Nonself and, therefore, without further assumptions, would have no way to mediate Associative Recognition of Antigen (ARA). ARA is what establishes the relationship between what is to be ridded and what is to be induced.

Given that ARA is an obligatory requirement for activation of T-helper cells, we might compare the two solutions by asking:

1. What assumptions would be required to make the eTh the source of Signal[2] (Model 1)?

2. What assumptions would be required to make the APC the source of Signal[2] (Model 2)?

The eTh recognizes peptide (P) presented by MHC-encoded Class II restricting elements (RII). The APC processes antigen and presents it as a [P-RII] complex, as do B-cells. The difference is that the APC presents several antigens simultaneously whereas the B-cell presents only one. Given that the APC cannot be the platform for ARA, Bretscher [5] proposed that the B-cell, not the APC, is the sole antigen-presenter for eTh-B-aTh interactions in ARA. As B-cells are known to function in this capacity [23–25] it is a crafty viable solution because one can easily envisage that the T-helper repertoire has been purged in the thymus of recognition of B-cell Self-antigens. However, the T-cells of B-less mice respond normally making B-cells a possible but not the only antigen-presenters. This requires that we confront the role of the APC in the activation of T-helper cells.

Associative Recognition of Antigen (ARA) and the APC

It is because the APC is a participant in the interactions under both models that we can begin by addressing elements common to them.

First, there must be a relationship between what is to be eliminated and what is induced. This has led to the coining of a word more precise than “antigen” to describe the unit of this relationship, namely, the *Eliminon* [6]. The Eliminon can be a monomeric protein (e.g. a toxin), a virus, a bacterium, a protozoan, a piece of necrosing cell, etc. The Eliminon, the unit of elimination, is taken up by an APC to be presented in such a way that it becomes the unit of induction. The mechanism of uptake and of presentation must, in the end, permit Signal[2] to be delivered in ARA.

Second, the APC has a limited number of germline-selected (“innate”) receptors that permit uptake of a proportion of the universe of Eliminons. As pointed out earlier, this germline-selected innate repertoire is unable to recognize a large part of the antigenic universe that the somatically generated immune repertoire sees. Therefore, it is expected that the APC would have an uptake mechanism that engages the somatic repertoire, and this implies uptake of an Eliminon-Antibody complex [26]. Such a complex would only contain one Eliminon due to the specificity of antibody. Of course any Eliminon seen by the germline-encoded (“innate”) repertoire, would also be recognized by the somatically generated (“adaptive”) repertoire.

Third, the uptake of the Eliminon by the APC must result in the formation of a site where ARA can occur. If the peptides from several processed Eliminons were displayed randomly mixed on the surface, ARA would not be possible. The APC can only be the platform for ARA, if it were to display no recognized Self and only one Eliminon at a time (Model 2). A model for the delivery of Signal[2] in ARA by an eTh via an APC requires a proposal as to mechanism. In addition to Bretscher’s [5] suggestion that the B-cell, which processes only one Eliminon, is the sole platform for ARA, Mitchison & O’Malley [19] proposed that the APC processes and presents only one antigenic molecule or particle at a time (Model 2). As this seems unlikely, a tweak in their model can be introduced, namely that the Eliminon taken up either by the germline-encoded receptors or as an [Ag-Ab]_n complex, is processed and presented as a unit in a >“*signaling patch*” on the APC surface. The eTh-APC-aTh interaction can only occur in ARA between cells engaging ligands expressed in the signaling patch [6]. If ARA is an obligatory component of the regulation of responsiveness and if the APC is an obligatory cell mediating the interactions between eTh and aTh, then a solution of this type (Model 1) seems unavoidable. The Bretscher [5] and Mitchison-O’Malley [19] models require the additional, albeit reasonable, assumption that the recognition of intracellular Self presented by the B-cell and APC be efficiently purged in the thymus. As long as intracellular or extracellular Self is presented by the APC or B-cell, then every Nonself antigen processed to peptides presented

randomly distributed on the surface would be converted to a Nonself antigen that shares determinants with Self. This would be a recipe for autoimmunity.

Consider the following conundrum. A Nonself antigen that shares no determinants with Self, under the present view, is processed by the APC and converted into Nonself that shares epitopes with Self. Yet this results in a low probability of breaking tolerance (i.e., establishing a self-sustaining autoimmunity). By contrast a Nonself antigen that shares epitopes with Self has a much higher probability of breaking tolerance (e.g., torpedo acetylcholine receptor in mice/rabbits results in *Myasthenia gravis*). How can epitopes linked on an antigen processed by an APC be distinguished from epitopes derived from independently processed antigens? Keep in mind that the breaking of tolerance requires that the effective level of effector T-helpers anti-Self be brought to a self-sustaining level. This implies a mechanism for ARA on an APC.

The consequence of APC uptake requiring an [Ag-Ab]_n complex

If the processing by an APC of a significant proportion of the universe of Eliminons requires uptake antibody, then we must face the question of its origin. The priming uptake antibody repertoire must be as large as the virgin B-cell repertoire [27] if it is to accomplish this role. This implies an antigen-independent secretion of antibody by B-cells that are born as IgM/IgD producers. The rules for this process must be the same as those for the antigen-independent generation of eTh discussed earlier [11,12]. In brief, the rate at which anti-Self B-cells are inactivated must be rapid compared to the rate at which they differentiate to secretors of primer uptake antibody. This would result in anti-Nonself uptake antibody sufficiently devoid of anti-Self activity to safely prime the uptake of Nonself-antigen only.

In order to be functional the concentration of the primer uptake antibody must be maintained high enough to form sufficient aggregate with the Elimion to prime the uptake by interaction with appropriate Fc-receptors on the APC. Such a model would define IgD or monomeric IgM as the primer uptake isotypes.

The assumption that a major uptake route by the APC is [Ag-Ab]_n complexes must face the fact that immunoglobulin-negative mice appear to respond normally in the T-helper class. It is possible that these observations are the result of uptake and presentation of the test antigen via the germline-encoded uptake systems. It is quite likely that the favorite antigens of immunologists like serum albumin, gammaglobulin, lysozyme, ovalbumin, cytochrome C, etc. are non-immunogenic without aggregation or adjuvants because of a failure of uptake. The level of priming uptake antibody specific for monomeric or close-to-self antigens might be too low to permit adequate uptake. The injection of these antigens as [Ag-Ab]_n complexes would predictably render them immunogenic without adjuvant.

The only way that the APC could act as a platform for the delivery of Signal[2] (“costimulation”) in ARA (Model 2) would be if it presented one Elimion for a time long enough to rid the invader. As this appears unlikely, it is reasonable to consider the alternative, namely that the Elimion is processed by the APC to a signaling patch that acts as the platform for the delivery of Signal[2] in ARA by an eTh (a possible mechanism for Model 1).

A proposed tweak to save the costimulatory APC model

The essence of the proposal is that a tolerigenic-only APC (immature, resting, naïve, newborn) presenting Self-only competes with an activated inducible-only APC presenting both Self and Nonself to prevent the breaking of tolerance. Given this, the inducible-only APC could use an antigen-unspecific costimulation signal for activation because the level of iT-cells anti-Self would be maintained below a threshold that permits autoimmunity. This model is untenable.

If the tolerigenic-only APC presents only those Self-antigens that it produces, then it would not participate in a major segment of peripheral tolerance. If it uses an uptake mechanism, then it would present both exogenous Self and Nonself. If it effectively carries out its tolerigenic-only role then the individual would die of infection. If it is activated by Nonself and it effectively carries out its inducible-only role, then the individual would be debilitated by autoimmunity.

The immune system is under a steady state Nonself antigenic load that engages up to 10% of its adaptive repertoire. The APCs are quite busy all the time. An uptake and processing mechanism that is indiscriminate with respect to Self and Nonself rules out the APC as the source of Signal [2].

If logic be a guide, the activating Signal [2] must be initiated by an antigen-specific interaction functioning in ARA that involves a donor cell (eTh is proposed) that has independently made a Self-Nonself discrimination.

Who is going to put Humpty-Dumpty together again?

As long as iTh leave the thymus with a repertoire that contains specificities directed against peripheral Self, then a Decision 1 mechanism to sort that repertoire must exist. Further, the response to Nonself must be coherent and independent for each Elimino (Decision 2) and this requires Associative Recognition of Antigen (Elimino). ARA is what establishes the relationship between what is to be eliminated and what is to be induced. ARA makes the effector T-helper (eTh) the key regulator in the inductive pathway.

The present view of the APC is that it processes simultaneously several Self and Nonself antigens to peptides that are presented distributed on its surface. This makes ARA between an eTh and aTh impossible. Having taken several Eliminos apart by processing to peptides and displaying them intermixed for interaction with iTh on an epitope-by-epitope basis, the gTh/eTh generated by costimulation would have to be subsequently gathered or regrouped antigen-by-antigen (i.e., ARA) to determine the class of the response (e.g., eTh1 vs eTh2). This is the Humpty-Dumpty problem. To date no proposal for the coherent and independent effector response to each antigen in the absence of ARA has surfaced.

No signal via the TCR (Signal[1]) can tell the aTh if it is interacting with a Self or Nonself ligand. The aTh must be told by a second signal that can come either from an eTh or an APC that has itself made a Self-Nonself discrimination.

If the APC ("costimulation") were the source of Signal[2] then it would have to present only one Nonself antigen at a time and the aTh repertoire would have to be deleted of recognition of Self specificities expressed by the APC. As discussed, in order to do this the Self-Nonself discrimination would *perforce* become a property of the humoral repertoire which would have to be presorted by a Nonself antigen-independent pathway.

If the eTh is the source of Signal[2], then its repertoire would also have to be presorted by a Nonself antigen-independent pathway[11,12]. In order to accomplish ARA in the delivery of Signal[2] to the aTh on an APC that processes simultaneously several Nonself-antigens, each one would have to be displayed in separate compartments referred to as "signaling patches."

In sum, either Associative Recognition of Antigen (ARA) is obligatory for the inactivation-activation decision and for the coherent and independent regulation of effector class or it is not. If ARA is obligatory then the present day view of APC function as a "costimulator" is lacking and the effector T-helper becomes the source of Signal[2]. If ARA is not obligatory, then the present day view of the costimulating APC becomes a mere standby diversion until a theory of the two decision processes, the inactivation-activation discrimination (Decision 1)

and the regulation of class (Decision 2), in the absence of ARA, is presented. If logic be our guide, no meaningful theory that ignores ARA is likely to emerge and, therefore, the role of the APC as the source of Signal[2] justifiably needs rethinking. Hence, this commentary.

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List of abbreviations

ARA	Associative recognition of antigen
i-cell	initial state cell (antigen-responsive, naive)
a-cell	anticipatory cell
g-cell	activated (“galvanized”) cell
e-cell	effector cell
Th	helper
Tc	cytotoxic cell
RI/II	MHC-encoded restricting element (R) Class I or II
P	peptide