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HOW DOES THE IMMUNE RESPONSE GET STARTED?

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Abstract

An effective adaptive immune response requires the prior induction of the regulatory effector T-helper (eTh). There are two competing models of how this cell is induced to effectors. Under the Associative Recognition of Antigen (ARA) or "two signal" model, the T-helper requires eTh in order to be induced to eTh, an "autocatalytic" process. Under the "costimulation" model eTh are induced by an antigen-unspecific signal derived from an "activated" APC. Under the ARA model the problem of the origin of the primer eTh is posed. A nonself antigen-independent pathway to eTh is proposed as well as an experiment to reveal its existence. In the costimulation framework no primer eTh need be postulated but it lacks a mechanism that, in the absence of ARA, accounts for the self-nonself discrimination and the determination of effector class.

Keywords

Self-nonself discrimination; primer effector T-helper; costimulation; activation of T-helpers

Most readers will wonder why anyone would bother to ask this question. It has occupied the occasional thoughts of a handful of immunologists in the past 50 years and often inadvertently. Yet all conceptualizations of immune responsiveness end up having to face this question. The goal of this discussion is to trace its origins, show why it is important and evaluate the two categories of answers.

The "Stone Age" of immunology

Jerne, in 1955, postulated an embryonic or developmental time window during which the total paratopic repertoire was generated as secreted immunoglobulin that was then purged of anti-self reactivity. The window closed and the residue, anti-nonself, was now specifically replicated upon interaction with antigen [1]. Burnet, in 1957, put the paratopic repertoire on cells and had them sorted as did Jerne [2]. Lederberg, in 1959, rejected the assumption that the repertoire was generated 'big bang' during a developmental time window and then shut down [3]. He assumed that the generation of the repertoire was a steady state lifelong process, leading him to mistakenly abandon the assumption of a developmental time window operating at the level of the animal and, in its place, suggested sorting of the repertoire at the level of the differentiation of the cell. In essence he assumed that the cell is born tolerizable-only and then differentiates antigen-independently to inducible-only. This aspect of his paper was ignored by immunologists until 1968 when Bretscher and I [4,5] disinterred his idea as a starting point

for a competing theory on the sorting of the repertoire that at the time was termed the "two signal theory" but over the years has matured to what would be more inclusively described as the "Associative Recognition of Antigen (ARA) theory [6–9]."

The Lederberg model that cells are born inactivatable-only and mature to activatable-only required that the sorting of the repertoire be initiated during a self-only period and that self persist. It did not, therefore, obviate the requirement for a developmental time window (as has been assumed [10]) during which all and only self is expressed. The antigen, whether it be self (S) or nonself(NS), is inactivating for the newly arising cells and activating for the end cell. The system responds using the end cells that had accumulated in the absence of the nonself antigen. The persistence of self maintains the state of unresponsiveness to self. Newly arising anti-nonself cells in the presence of nonself(NS) would be inactivated as if it were self(S).

Although the Lederberg Model was consistent with the facts in 1959, it became clear by 1970 that it could not deal with all classes of antigen-responsive cell, T and B. This was the starting point for the "two signal model." In this model the initial state or i-cell as we now refer to it, has two pathways open to it, inactivation and activation. A choice between two pathways requires two signals as a matter of formal logic and we reasoned that the interaction between the antigen-receptor (BCR or TCR) and its ligand was inactivating (Signal [1]). This required that activation depend on a second signal that was related to the first by associative (linked) recognition of the antigen (ARA) (Signal ([1]+[2]). In other words, inactivation is mediated epitope-by-epitope, whereas activation is mediated antigen-by-antigen (ARA). Under the Lederberg model both inactivation and activation are driven epitope-by-epitope. There is no requirement for an activating Signal ([1]+[2]) or ARA.

When a role for the effector T-helper (eTh) became known [11], it was evident that this cell was the source of Signal [2] for the B-cell and the cytotoxic T-cell. These latter will be referred to as defensive cells, the effector T-helper (eTh) being regulatory. This left us with the problem of the origin of Signal [2] for the induction of the regulatory effector T-helper (eTh) itself.

Although couched in an entirely different language in 1968, the "two signal model" pinpointed for the first time the question that is the subject of this essay. At the Brook Lodge Meeting September 20, 1968 where the model was first presented [12], the participants saw immediately that there was a problem. As an understanding of the role of T-helpers was in its infancy at the time, we had assigned the delivery of Signal [2] to "carrier antibody" postulated to be the specific element derived from the eTh that cooperated in ARA with the initial state or i-cell receiving Signal [1].

Dr. Ceppellini ([12], pg 288): "*And where does the carrier antibody* (eTh) *come from*?"

Dr. Edelman ([12], pg. 299): "*The cell that makes carrier antibody* (eTh) *does it have to be induced too? Because in that case you are going to have an infinite mess.*" [My note: The "court" stenographers who recorded his comment, incorrectly transcribed the word "mess" instead of "regress" which is what he actually said. I know because I put into my notes to look the word up as I was not sure what he meant.]

Dr. Smith ([12], pg. 302); "*What is the postulated origin of the carrier antibody* (eTh)? *I am not clear on that.*"

At the time, I presented several solutions to the primer question, none of which have withstood the test of time. In our laboratory we referred to the primer question as the "chicken and egg problem."

The "Star Trek" era of immunology

Under today's ARA model, the primer question is a problem only for the T-helper (Th) lineage. This cell, when induced, is required to be the effector for the Signal ([1]+[2]) activation of all defensive i-cells, iB and iTc. This raises the question, "how are the iTh themselves activated?" or "how does the immune response get started?" There are two facets to this question.

What determines when the developmental time window closes?

During fetal life when the immune system arises, it is unresponsive, in fact tolerizable-only, due to a lack of eTh (the source of Signal [2]). The i-cells are always born with two pathways open to them, inactivation and activation. When the developmental time window closes, the system becomes responsive because a priming population of eTh anti-NS have accumulated to a steady state level. In the presence of NS-antigen, the priming population initiates an autocatalytic induction of iTh to eTh that, in turn, determine the effector biodestructive and ridding response to nonself.

What is the origin of this primer eTh?

Given the ARA model, all i-cells including the iTh require Signal ([1]+[2]) delivered in ARA by an eTh in order to be activated. Bretscher proposes [9,13] that iTh are born with "weak" effector activity for delivery of Signal [2] and upon induction to effector T-helpers (eTh), this activity is greatly amplified. We have proposed [14–17] taking a cue from Lederberg that there is a nonself antigen-independent pathway to the production of primer eTh (Figure 1). Once present, the primer eTh "autocatalytically" convert $\mathrm{iTh} \xrightarrow[\mathrm{ARA}]{\mathrm{eTh}} \mathrm{eTh}$. The homeostatically maintained steady state level of primer eTh must be high enough to permit rapid initiation of a response but low enough to keep autoimmunity acceptably infrequent.

The immunological community treats Signal [2] as "costimulation" taking its cue from Lafferty and Cunningham [18]. Costimulation as a source of Signal [2] is antigen-unspecific as it is not connected to the epitope on the antigen delivering Signal [1]. ARA is required for an appropriate effector response to nonself. Costimulation leaves us with the need to provide a mechanism for the sorting of the T-helper repertoire (i.e., a self-nonself discrimination). If ARA is not a part of activation by costimulation then it must be introduced at a later step before the T-helper becomes an effector (e.g., [9]).

The elements of a general solution

The general principle driving any priming model would be that the rate of inactivation of the iTh anti-self be rapid compared to the rate at which it is driven to differentiate to an effector T-helper (eTh). In other words the sorting of the repertoire of the primer eTh must be independent of the requirement for eTh as an "autocatalyic" inducer of iTh.

The Bretscher Model [9] is a good starting point as it confronts all of the questions that we have posed over the years. In order to assure ARA in the induction of eTh, he proposes that the B-cell is the sole APC for the induction of eTh. As each B-cell processes only one NS-antigen, ARA is assured. However, the problem of how three rare cells, one B and two T manage to find themselves in a *menage à tois* is raised. In order to solve this "scarcity problem" Bretscher includes "costimulation" in Signal [1], the consequence of which is several divisions before death. Whether this division step solves the "scarcity problem" needs further analysis. For our discussion here this step is an aside. His second step faces the primer eTh question. While not detailed by him, we might assume that the proliferating Th-cells that have received Signal [1] dock on a B-cell that has processed the cognate antigen. The two Th-cells might be assumed to express "weak" effector activity and mutually exchange a "weak" Signal [2] to

become a fully active primer eTh that initiates the "autocatalytic" induction of iTh to eTh. Whether or not the details of mechanism are correct is less important than the fact that Bretscher has confronted the questions as to how the system gets started and how it maintains an interaction of ARA at the level of the T-helper. Further, his model is eminently testable (disprovable).

Under the Langman/Mata/Cohn Model [16,17] of a nonself antigen-independent differentiation to eTh, the rates of inactivation and activation are sharply kinetically defined to provide a complete primer population at a steady state level (Figure 1).

Under the various versions of the "Costimulation" Model, inactivation by self is rapid due to its being dispersed as a large target on APCs whereas activation by nonself is slow because it is localized to APCs receiving a "danger," "pathogenicity," "cytopathicity," "harm," etc. signal thereby providing a small target. There are all manners of possible variation on this theme in which regulation in time is substituted for by regulation in space but the principle remains the same, namely to establish differential rates between inactivation of iTh anti-self and activation of iTh anti-nonself.

Once a sufficiency of eTh is achieved, the activation of all other defensive cells, iTc or iB are eTh-dependent as required under the ARA model. The disagreement involves the pathway of activation of the iTh itself. Under the ARA model the primer eTh acts autocatalytically to activate iTh. Under the standard "costimulation" model no primer eTh population exists unless, of course, one assumes that an APC-eTh interaction is required to give the APC costimulating potential (i.e., primer eTh is required). The functional level of eTh is induced directly epitope-by-epitope by interaction of iTh with an "activated" APC; no necessity for ARA is envisioned. An immune system that sees only epitopes, not antigens cannot regulate its effector output. The "costimulators" had better settle down and give us a meaningful model for ARA on the APC platform or deny its necessity.

A suggested experiment to settle the "primer" question

Consider a female RAG^{-} mouse expressing a unique transgenic TCR anti-[A^{b}-$P_{H\text{-}Y}$]. As far as is known, the only source of peptide recognized by this TCR is derived from the male antigen H-Y. The iTh are positively selected and leave the thymus without effector activity. They have two pathways open to them, inactivation and activation.

Does this animal express eTh anti-[A^{b}-$P_{H\text{-}Y}$]?

If this TCR were in a male RAG^{-} mouse it would be negatively selected (the inactivation pathway) implying that the eTh level is maintained by tolerance at a level that is insufficient to act as a primer. The animal does not manifest autoimmunity.

In a female RAG^{-} mouse, this TCR is positively selected in thymus, and the iTh cells are exported to the periphery. If, in the female, eTh are detected, then follow up questions might be asked such as, what is its steady state level and what is the course of eTh expression as a function of developmental time, fetal to neonate to adult? Given the absence of negative selection, implies that, in the female, this TCR sees no self-ligand as signaling. Consequently, positive selection must be peptide-unspecific and "costimulation" as being at the origin of the observed eTh is ruled out.

If the iTh-cells can be induced to effectors by immunization with male cells from Bless mice or male non-B cells, the Bretscher assumption that B-cells are the sole APC for activation of iTh, would be ruled out.

If there were an endogenous or exogenous H-Y peptide mimotope derived from self, one would expect negative selection. If derived from nonself, the eTh anti-[A^b-$P_{H\text{-}Y}$] would be expected to be induced, and therefore, be expressed in the range of >50% of $CD4^+$ Th cells compared to an antigen-independent priming level of <10%.

Failure to detect eTh, if sensitive enough, would rule out the antigen-independent pathway, leaving the "costimulation" pathway as a default model for the origin of eTh. No primer population is envisioned under this latter model but as pointed out earlier the model is incomplete in the absence of a mechanism for ARA or for bypassing it.

What might be used as a functional assay for eTh anti-[A^b-$P_{H\text{-}Y}$]?

Irradiated $CD4^+$ T-cells can be placed in a limit dilution assay with appropriate antigen and purified male transgenic H-2^b B-cells expressing a known easily assayed Ig when secreted (e.g., anti-phosphoryl choline or anti-SRBC). The wells in which secreted Ig is detected would assay the eTh in the $CD4^+$ population. Assuming acceptable controls, this assay would be extremely sensitive, certainly capable of detecting a primer eTh level in the range of $\geq 10^{-3}$.

Interpretation of results with this RAG^- female transgenic expressing the TCR anti-[A^b-$P_{H\text{-}Y}$]:

If eTh are detected in the range of $\leq 10^{-1}$ of the total $CD4^+$ T-cells, then the antigen-independent pathway is highly likely.

If eTh are detected in the range of $\gg 10^{-1}$ a cryptic source of $P_{H\text{-}Y}$ mimotope is implied and B-cells are ruled out as the sole APC for induction of eTh.

If eTh are not detected ($<10^{-3}$), the antigen-independent pathway would be highly unlikely. Should the "costimulation" pathway become a default model, we would have to consider the question, how, in the male (RAG^- or RAG^+), is this transgenic TCR negatively selected without causing autoimmune disease given that the animal is under a steady state microbial load (APC activators)?

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List of abbreviations

i-cell
: initial state of virgin antigen-responsive cell

e-cell
: effector

S
: self

NS
: nonself

Th
: helper T-cell

Tc
: cytotoxic T-cell

B-cell
: antibody producing lineage

TCR
: T-cell antigen-receptor

BCR
: B-cell antigen-receptor

eTh
: effector T-helper

APC
: antigen-presenting cell

RAG
: recombination activating gene

ARA
: Associative Recognition of Antigen

A^b-$P_{H\text{-}Y}$
: peptide derived from H-Y presented as ligand on Class II MHC Ab

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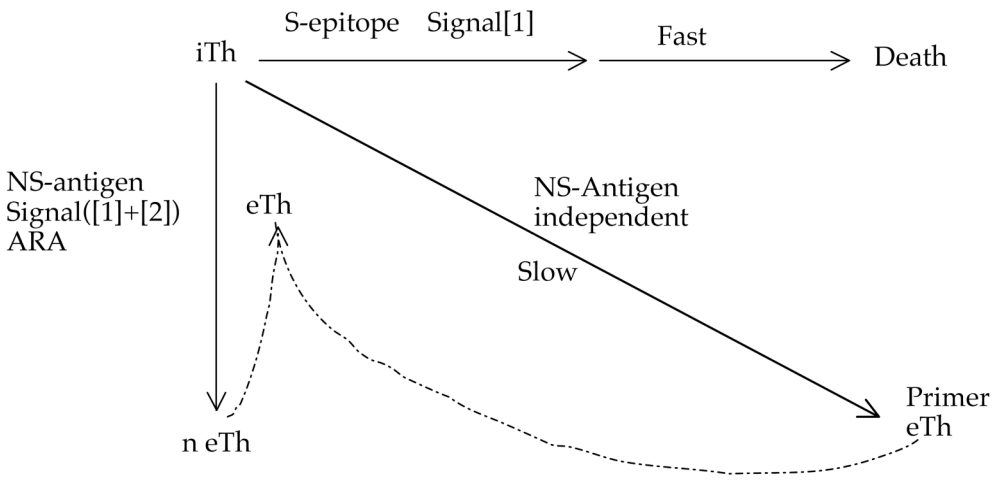

Figure 1.
The NS-antigen-independent pathway for the generation of primer eTh and its role in the "autocatalytic" activation of iTh (see List of abbreviations).