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A Conversation with Cohn on the Activation of CD4 T Cells

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Abstract

Despite an agreement on most issues surrounding models for how lymphocytes are activated and inactivated, and arising out of the 1970 Two Signal Model of lymphocyte activation, Cohn and I have different perspectives on two critical issues concerning the activation of CD4 T cells. One issue is the origin of the first effector T helper (eTh) cells, postulated by both of us to be required to optimally activate precursor Th (pTh), that is naïve CD4 T cells, to further generate eTh cells. The second issue arises from our agreement that the antigen-dependent CD4 T cell cooperation, that we both postulate is required to activate naïve CD4 T (pTh) cells, most likely is mediated by the operational recognition of linked epitopes. Although agreeing on the centrality of this operational mechanism, we disagree about how it might be realized at the molecular/cellular level. I respond here to issues raised by Cohn concerning these two mechanistic questions, in his response to my recent article on the activation and inactivation of mature CD4 T cells.

Preface

Cohn, in response [1] to my recent article on the activation and inactivation of CD4 T cells [2], has expressed his view that 'very significant additions, changes and precisions in the 'Original Two Signal' model [that Cohn and I had proposed in our 1970 Science article [3]] have been made'. In his 1994 leading article for Annual Reviews of Immunology [4], Cohn says: 'The 'two signal' model had a rocky intellectual history; but, as formulated today, it is highly likely to be correct. In essence, there is no validly competing model'.

I have been aware of most of Cohn's proposals over the years post-1970. I have had and have reservations concerning the plausibility of several of the proposed changes and additions to the 1970 Two Signal Model that he has envisaged. When I read today our 1970 proposal, I feel there is nothing conceptually faulty. Naturally, with the enormous amount of information gathered in the last 44 years, it is possible to make more detailed and testable proposals as to what are the mechanisms by which antigen activates and inactivates lymphocytes, including CD4 T cells. I tried to achieve this with my 1999 Two Step, Two Signal Model [5]; however, this 1999 model is consistent with the propositions of the 1970 model, and so the 1999 model is just a more detailed proposal for the nature of the underlying mechanisms. In addition, my colleagues and I have experimentally tested predictions of the models over the years [6–10].

To my mind, much information, gained subsequent to the 1970 formulation, is naturally accommodated within its framework. For example, at a time when T helper cells were generally envisaged to merely present a repetitive array of antigenic epitopes to the B cell [11, 12], we suggested that signal 2, postulated to be required to activate lymphocytes, would likely be mediated by the delivery of short-range, antigen non-specific molecules, and/or by membrane/membrane interactions. These possibilities were supported by the subsequent discovery of interleukins and costimulatory systems. A currently less-accepted proposition of our 1970 and my 1999 model is that, in addition to there being a requirement for helper T cells in the activation of virtually all B cells and CD8 T cells, the activation of CD4 T helper lymphocytes themselves also requires the action of CD4 T helper cells. This proposition is central, as it is envisaged that such antigen-mediated CD4 T cell cooperation allows CD4 T cells not only to be activated, but prevents their antigen-mediated inactivation. Studies by others [13, 14] and by us [6–10] support the proposal that CD4 T cell activation requires, or is at least facilitated by, CD4 T cell cooperation.

Naturally, I was aware of these different perceptions by Cohn and myself when I wrote my recent article on the activation and inactivation of CD4 T cells [2]. I deliberately started with the essence of our 1970 model, to bring back what I consider to be clarity to the basic issues.

Cohn introduces, in passing, comments as to the history of how concepts arose. For example, Cohn states [1] that 'No viable model of the primer source of signal 2 appeared until

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1983, when I proposed an antigen-independent pathway for the derivation of primer effector T helpers (eTh)'. I note, in view of this statement, that I discussed various solutions to the priming problem in 1972, in a 50 page article in Transplantation Reviews [15]. For example, I proposed that precursor helper T cells might possess the same effector activity as effector T helper cells, but at a considerably lower level, so that, when present in sufficient numbers, they could allow antigen to initiate immune responses through lymphocyte cooperation. This proposal is close to the one I currently still favour, as discussed below.

A context for the discussion

I consider it useful to start by outlining two of our studies [6, 7] that provide reasonably strong support for two ideas concerning the activation of CD4 T cells and proposed in the scheme outlined in our 1970 paper. As these ideas are critical to points I wish to make, this outline will provide an appropriate context for the discussion that follows.

We showed, expressed in contemporary terms, that radiation-resistant eTh cells, specific for an antigen F, could facilitate the activation of unprimed CD4 T cells specific for an antigen G, where F and G were chosen as non-cross-reacting antigens. However, F-specific CD4 T cells would only facilitate the activation of G-specific CD4 T cells if both F and G were present and *linked* to one another [6]. These observations illustrate what we mean when we say that CD4 T cell activation requires/is facilitated by CD4 T cell cooperation mediated by the operational recognition of *linked* epitopes. These experiments also show that the helper activity of primed cells was radiation-sensitive for a couple of days after *in vivo* priming with F, but became radiation resistant by day four, observations consistent with the idea that radiation-sensitive precursor Th (pTh) cells are activated by antigen to multiply first before some of their progeny differentiate, around day 3, into radiation-resistant eTh cells. Further experiments support the idea that the generation of eTh themselves is also facilitated by T cell cooperation [7]. It thus seemed, and still seems to me, that CD4 T cell activation is facilitated by a cascade of CD4 T cell interactions. In summary, these observations provide support for the idea that the activity of pTh and eTh cells, in helping the activation of other pTh cells, can be, respectively, distinguished by their radiation sensitivity and insensitivity, as activated precursor cells have to divide before they optimally give rise to effector T helper cells; in addition, they also illustrate that the CD4 T cell cooperation, involved in CD4 T cell activation, is mediated by the operational recognition of linked epitopes.

Issues for discussion

In reading the substantial response that Cohn wrote, I believe the subjects of disagreement, that I should address, can be distilled into two areas: issues surrounding the priming problem, as far as the activation of CD4 T cells is concerned, and issues of how a satisfactory model of CD4 T cell interactions, involving the operational recognition of linked epitopes, might be realized at the cellular/molecular level. I find it helpful, for reference purposes, to make a point-by-point response addressing these two issues.

I. The priming problem for the origin of the first eTh cells

(i) Defining the context of the problem

Both Cohn and I propose that eTh cells are required to allow antigen to *optimally* activate pTh cells to generate more eTh cells. How are the first eTh cells generated in the context of this proposal? We refer to this question as the priming problem. I may not have been sufficiently clear about the context in which I envisage this problem to be pertinent. I therefore start by clarifying how I see this context.

As discussed [2], single cell assays allow one to demonstrate that there are, in the spleen of immunocompetent mice not deliberately immunized by an immunologist, low numbers of antibody-producing cells specific for most 'test' antigens [16]. These antibody-producing cells presumably reflect ongoing immune responses to environmental antigens that cross-react, often minimally, with the 'test' antigen. Similarly, and as previously described [2], assays for detecting single, antigen-specific cytokine-producing cells allow one to similarly detect, in the spleen of immunocompetent and 'unimmunized' mice, what are likely to be cytokine-secreting, CD4 T cells specific for diverse, foreign 'test' antigens. I suppose that the presence of these eTh cells specific for most foreign 'test' antigens also reflects ongoing immune responses to environmental antigens; their availability allows these 'test' antigens to initiate CD4 T cell activation, and thus induce immune responses, in immunocompetent mice. Thus, the 'priming problem' primarily exists in neonatal mice, in which there are no ongoing, established immune responses. The 'priming problem', as I see it, reflects how the first CD4T lymphocytes are activated in neonatal mice, and so how the neonatal immune system 'gets started'. (I refer explicitly to neonatal immune systems of mice as the immune system of some other animals can respond to foreign antigens half way through gestation). As I indicate later, Cohn suggests I think the priming problem is pertinent to the initiation of responses in mature, immunocompetent animals.

(ii) Two proposed solutions to the priming problem and some consequences

(a) Cohn's proposal. Cohn suggests [1, 17], as I understand it, that, at a particular time, around birth in mice, conditions arise such that there is a slow differentiation of pTh cells into

eTh cells in the absence of antigen. The obliteration of pTh cells specific for peripheral self-antigens is envisaged to occur following the generation of signal 1, due to the early and continuous presence of peripheral self-antigens. This proposal is illustrated in his Fig. 2 [1], reproduced here as Fig. 1. The envisaged consequence of this proposal is that a population of eTh cells is generated uniquely specific for foreign antigens. Cohn says [1] 'It is the sufficiency/insufficiency of eTh that determines responsiveness'. This is a radical change from the 1970 framework in two respects. In our 1970 framework, the number of lymphocytes specific for an antigen was critical in determining whether antigen activates/inactivates lymphocytes. According to Cohn's proposed solution to the priming problem, the sufficiency/insufficiency of eTh cells determines whether antigen can activate pTh cells to further generate eTh cells. The generation of these first eTh cells is envisaged to be independent of antigen and of lymphocyte cooperation. However, this first 'step' is also postulated, as I understand it, to critically determine whether, when an antigen impinges upon the immune system, pTh cells are activated, and in turn CD8 T cells and B cells. Thus, this basis of self- to non-self-discrimination is radically different from what was envisaged in the 1970 theory. The suggested basis does not depend on antigen-mediated cooperation between lymphocytes. I discuss below my thoughts on the plausibility of this potential solution to the priming problem, and illustrate problems, I believe, it leads to.

(b) My proposal. Figure 2 shows my proposal for how step two of the two step process, required to activate CD4 T cells, normally occurs in immunocompetent animals [2]. As discussed [2], I propose that the first CD4 eTh cells are neonatally generated in mice by an antigen-dependent, CD4 T cell cooperative process, in an inefficient 'step two', where step one primed CD4 T cells have similar but lower effector activity as fully activated effector CD4 T cells. I envisage this occurs when foreign antigens first impinge upon the immune system. This is possible as pTh cells specific for these antigens will have accumulated prenatally in the absence of these antigens. This proposal is in line with the 1970 framework. It is a slightly more precise

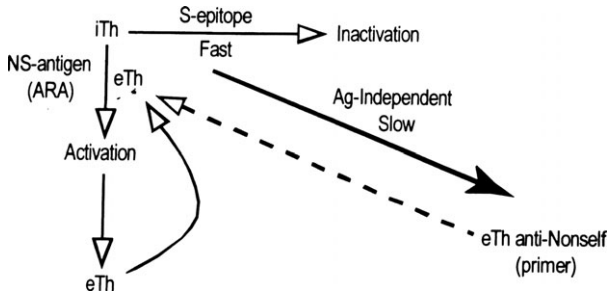

Figure 1 Cohn's proposal for the antigen-independent pathway for the generation of primer eTh cells.

formulation of one of the three proposals I discussed and one of the two I favoured in my 1972 article [15].

(c) Cohn's criticism of my proposal on the grounds of 'delayed responsiveness'. Cohn states: 'Bretscher's concept that every new antigen must first *de novo* induce primer eTh cells for it before it can proceed to induce an effector response, is unsettling because it makes the primer eTh pool behave either as a long-lived memory population useless for the induction of responsiveness to a new antigen or as very short-lived requiring for all antigens, the induction of primer eTh prior to responsiveness. Dependent on the number of divisions of the step one partially activated qTh-cells that are required to solve the putative scarcity problem, an additional delay in unresponsiveness is unavoidable. For example, a requirement for four divisions at step one would add a delay in responsiveness of 2–3 days, making the immune response, the other elements of which require 5–6 days, ineffectual against a rapidly growing pathogen'.

Of the various issues that Cohn touches upon in the above quotation, I shall respond to the two I think most important. Cohn seems to me to conflate the priming with the scarcity problem when discussing step one. Step one is primarily concerned with the scarcity problem. Firstly, with regard to the scarcity problem, Cohn states of this problem: 'If a scarcity problem exists, and that is not obvious, it needs a detailed quantitative calculation'. I disagree with this statement. I agree with some considerations entertained by the originators of the Clonal Selection Theory, as I understand them. They appreciated the need, if the immune system is to be effective, for the rapid generation of effective immunity, and realized the importance of multiplying the factories, that is, lymphocytes, that mediate immunity, upon antigen impact [18–20]. Step one is not wasted; the proliferation of CD4 T cells in step one both allows CD4 T cell cooperation to more readily and more rapidly occur in step two, and in there being more eTh cells to facilitate the subsequent activation of CD8 and B cells. Thus, the postulate of step one does not 'delay' the immune response as Cohn proposes it must inevitably do. On the contrary, the existence of a first step of CD4 T cell proliferation, that is not dependent on CD4 T cell cooperation, that would presumably be a slower process, favours a rapid initiation of the immune response.

Secondly, Cohn seems to suggest that my solution to the priming problem would result in 'sluggish' immune responses in *immunocompetent* mice. However, as described above, I consider the priming problem to be primarily pertinent to understanding how *neonatal* mice initially gain the ability to respond. I suggest an antigen-dependent process, in generating the first eTh cells upon neonatal infection by a rapidly growing pathogen, is likely to be much more rapid that an *intrinsically slow*, antigen-independent process, that Cohn envisages and as outlined in Fig. 1.

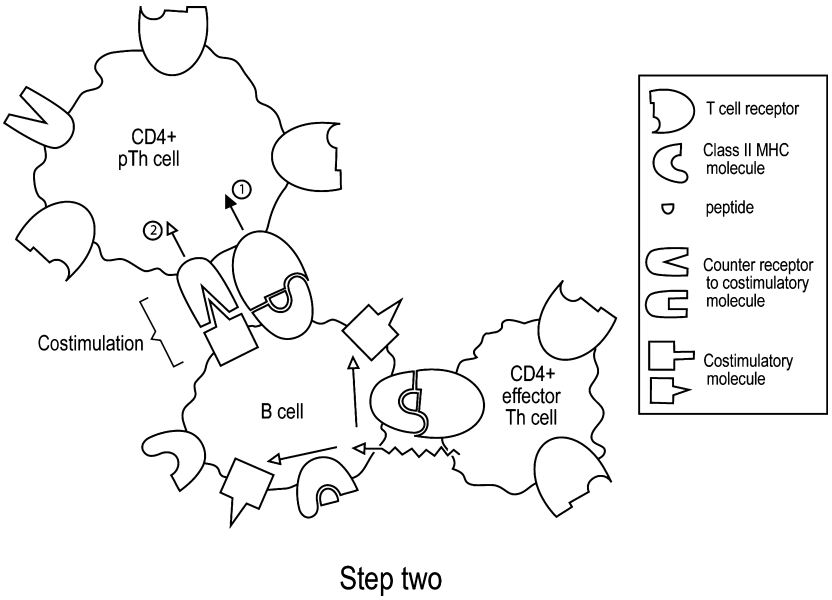

Figure 2 Step two of the two step, two signal model.

(d) The experiments of Anderson and colleagues. Cohn expressed surprise at my initial and considerable concern over the Anderson experiments [21] that appear, at face value, to be inconsistent with the Historical Postulate. Cohn accounts for Anderson's observations on the basis of his model directed at solving the priming problem. Cohn describes the Anderson experiments well and in some detail, so I will just try to encapsulate their essence here. Immuno-incompetent female mice were grafted with male skin, and subsequently reconstituted with female wild-type stem cells, allowing the immune system to regenerate. The male skin was rejected, showing that the presence of the male skin, as the immune system is generated, does not induce tolerance, violating, at face value, the Historical Postulate. I indicated in my recent article [2] how I was inclined to deal with these observations in a manner that maintains the validity of the Historical Postulate, but rather demonstrates a limitation under which the model I favour holds. My purpose in revisiting these very interesting observations is to further consider Cohn's proposals.

Cohn suggests that the rate of pTh inactivation, by peripheral self-antigens, is generally fast compared with the antigen-independent generation of eTh cells from pTh cells, see Fig. 1. However, this proposal seems a bit forced to me, if one anticipates that the rate of inactivation of pTh cells, by peripheral self-antigens, *is likely to depend on the concentration/availability of the peripheral self-antigen.* In discussing the observations of Anderson and colleagues, Cohn indeed invokes such a *dependence*. He suggests the male skin graft employed in these studies is 'small', so that the rate of inactivation of pTh cells is now not fast but slower than the antigen-independent generation of male skin-specific eTh cells. Thus, these eTh cells accumulate and the male skin graft can then be rejected. Cohn suggests that if the grafts were larger, then the rate of pTh inactivation could be fast, and rejection will not take place, a finding that would be in agreement with the Historical Postulate. In other words, it seems Cohn's interpretation, based upon his proposed mechanism, is that the Historical Postulate holds for large grafts (conjecture) but not for small grafts (fact). This seems an unsatisfactory state of affairs to me.

An important feature of the 1970 Two Signal Model was that the activation signals (signal 1 and signal 2) included the inactivation signal (signal 1). We thought this was neat, as it appeared to provide a guarantee that precursor cells, unable to be inactivated by antigen due to faulty antigen-specific receptors, could not be activated. A distinct but related quantitative principle would be that a low level of a peripheral self-antigen, insufficient to inactivate its CD4 T cells at a significant rate, should also not be able to activate these CD4 T cells. It would seem, if this *Antigen Threshold Principle* did not hold true, that autoimmunity would often be generated against peripheral self-antigens that are present at low levels. Indeed, Cohn's explanation of the Anderson observations is to my mind unsatisfactory. It violates the Antigen Threshold Principle and leads one to anticipate, if valid, that autoimmunity would be naturally generated against some peripheral antigens expressed at low levels.

II. How can CD4 T cell cooperation be mediated by the operational recognition of linked epitopes?

(i) Three proposals

All three proposals have some common assumptions. It is useful to state upfront what these commonalities are, so we can focus on the differences.

As the receptor of CD4 T cells recognizes peptides derived from the nominal antigen and bound to class II MHC molecules of the host, and as class II MHC molecules are not expressed by mouse CD4 T cells, an interaction between CD4 T cells must be mediated by a cell expressing class II molecules that presents the antigen. Thus, all three models envisage an antigen-presenting cell (APC) as central in mediating the cooperation between the CD4 T cells.

These three proposals have been recently outlined [1, 2], so I only highlight those features pertinent for a discussion of the relative merits of the proposals. I find the two possibilities outlined by Cohn implausible. Cohn has outlined many criticisms against a proposal that has pleased me as a solution to this central problem that I had struggled with over several years.

Proposal 1, first outlined I believe by Langman and Cohn in 2000 [17], and supported by Cohn until fairly recently, considers DC and macrophages to be the APC-mediating CD4 T cell cooperation. Cohn considers the situation where these APC can take up antigen/antibody complexes of different antigens. Consider two non-cross-reacting antigens, F and G. Cohn has proposed that antibody/antigen complexes of these distinct antigens are processed by a single APC as separate units and that, once the G- and F-derived peptides, bound to class II MHC molecules, are expressed on the surface of the APC, they exist in different 'signalling patches'. In addition, it was envisaged that a pTh cell can only interact with an eTh cell via this APC if both Th cells are interacting with peptide/MHC complexes in the same signalling patch. This proposed mechanism has always seemed to me somewhat implausible. I understand that antigen taken up by these APC, by mechanisms not involving antibody, are envisaged to be unable to present antigen to pTh cells in a manner that leads to their activation. This seems somewhat arbitrary. I also wonder how processing and presentation is envisaged to occur with particulate antigens, such as xenogeneic red blood cells and bacteria, when they activate pTh cells. This proposal seems not to acknowledge, or address, the fluid and dynamic nature of cell membranes. One would think the signalling patch would have to be of a considerable size to allow two synapses, by the pTh and eTh cells, to form around it. I did not understand the appeal of this mechanism when first proposed, and Cohn appears now to be not so keen on it either [1]. I do not understand the reason for the change of view. Cohn has formulated another possibility.

Proposal 2 appears to be recent and is briefly outlined towards the end of Cohn's response [1]. I think it proposes that eTh cells specific for peripheral self-antigens are extremely rare, and so the operational recognition of linked epitopes, in the collaboration between CD4 T cells, may not be so important. Cohn suggests this new approach may become more plausible once it has been explored to a greater extent by computer-aided modelling. I am uneasy I may have misunderstood the model, in which case I apologize. If I did understand it, I have to say I feel it merely avoids the problem, which is of concern on several accounts. Importantly, our experiments have clearly shown that the operational recognition of linked epitopes underlies CD4 T cell cooperation [5], and so I am inclined to insist on both its conceptual importance and on its reality.

Proposal 3 is the one embedded in my 1999 Two Step, Two Signal Model. As I explained elsewhere [2, 5], the wish to find a plausible means, by which CD4 T cells could interact by a mechanism involving the operational recognition of linked epitopes, was a driving force in the creation of this model. Cohn raises various arguments against the model, which I will now discuss.

(ii) Responses to Cohn's concerns

(a) 'Experimental challenges {from the literature} to Bretscher's proposed mechanisms'. Cohn lists four such challenges.

1. Immune responses in B cell-deficient mice: Cohn cites three papers showing that immunization of B cell-deficient mice can result in T cell activation. These studies, Cohn suggests, demonstrate the invalidity of step two of my model, in which B cells play a critical role in the activation of CD4 T cells. I am glad to have the opportunity to address this concern.

There are many papers in the literature addressing whether B cells are required to activate CD4 T cells. I must have read close to thirty. A major reason for this effort is that the employment of different systems leads to different conclusions. It is perhaps injudicious, under these circumstances, to quote three papers as definitive evidence against a requirement for B cells in the activation of T cells. In fact, the three studies quoted by Cohn all appear to have employed certain B cell KO mice. Other studies employ other B cell KO mice. These studies with the autoimmune lpr/lpr mouse were directed at examining the role of B cells in the aetiology of the autoimmunity. B cell KO lpr/lpr mice are no longer autoimmune [22]; most interestingly, these lpr KO mice, made transgenic for a μ gene that is intact apart from a section coding for a part of the μ chain required for IgM secretion, regain their autoimmune phenotype [22], as previously outlined [2]. The tentative conclusion of these studies is that B cells have a central role in the autoimmune response that is distinct from their production of antibody. These studies [22], at face value, seem to invalidate proposal 1, according to which specific antibody is needed for antigen presentation to CD4 T cells, and to support proposal 3, according to which B cells have a central role in step two, a role that does not involve the secretion of antibody. Cohn has called for an agreement upon a specially designed experiment that would settle the matter of the role of B cells once and for all. However, the virtue of not

relying on just one system, that may have unanticipated features, is, I think, illustrated by these diverse and, at face value, inconsistent reports.

To be honest, we have come with time to realize that the properties of KO mice are both very useful as a guide to physiological mechanisms and that their physiology is sometimes so abnormal that their properties may be misleading as to what normally occurs. This is why, when it comes to a crunch, I urge my colleagues to discount conclusions drawn from observations made with KO mice, if in contradiction with observations made in less artificial systems. It is for this reason that I laid such emphasis [2] on the finding, by Janeway and colleagues [23], that the provision to normal mice of mouse cytochrome C-specific, activated B cells, allows mouse cytochrome C to activate mouse cytochrome C-specific CD4 T cells. In addition, it appears, as previously outlined [2], that depletion of B cells in patients can ameliorate cell-mediated autoimmunity. These observations, made in more natural systems, make me question Cohn's unequivocal ruling out of my proposal for the reasons he indicates.

2. The role of B cells as APC: Cohn says 'B-cells may not always be more single-minded that dendritic cells'. The description of this challenge has no references to the literature.

This and other comments are made as an argument against relying on the specificity of the B cell to achieve CD4 T cell cooperation, mediated by the operational recognition of linked epitopes, as envisaged in step two of the model. I wish to make two points in response.

It seems important, given the envisaged role for B cells in mediating CD4 T cell cooperation, that there are not substantial numbers of B cell-specific peripheral self-antigens, denoted as pS-B; if there were such antigens, and if consequently pTh cells specific for the nominal antigen pS-B were exported out of the thymus into the periphery, one can readily imagine circumstances under which they would be activated. Consider a B cell, specific for a foreign antigen, and presenting peptides derived from this foreign antigen and from pS-B; this B cell could mediate the interaction between the eTh cells specific for the foreign antigen and a pTh cell specific for pS-B, resulting in B cell autoimmunity. A way of preventing this from happening is if B cells are present in the thymus, and so efficiently cause central tolerance, so pTh cells specific for pS-B do not emigrate to the periphery. Some observations are consistent with this possibility [24].

Secondly, I think it is helpful in the context of Cohn's concern to reconsider what is known in another, related context. It is generally accepted that the activation of hapten-specific B cells can be efficiently helped by Th cells specific for the a nominal antigen Q, when h-Q is present, but normally not under other conditions where the two antigens Q and h-R are present, R being an antigen chosen not to cross-react with Q [25, 26]. Thus, an effective B cell/eTh cell interaction is normally mediated by the operational recognition of linked epitopes. There are circumstances where non-linked recognition does occur. It was shown in the 1970s that the *in vitro* activation of hapten-specific B cells could be facilitated by Q-specific Th cells, when both h-R and Q are present [26]. To achieve such an interaction, the presence of very high concentrations of the antigen Q is required. It was interpreted that, under such unusual circumstances, sufficient Q could be taken up by the B cell, through non-specific means, so that the B cell could present Q peptides. These circumstances probably represent a pathological rather than a physiological situation. They are most probably related to the hyperproduction of antibody that occurs in some parasitic diseases. In these cases, there is not only a lot of antibody directed at the parasite, but hyperproduction of 'non-specific' antibody of the same isotype as the anti-parasite antibody. Such hyperproduction of antibody occurs when the parasite load is very great. It is likely that parasite antigens are non-specifically taken up by B cells and that parasite-specific Th cells can help these B cells to produce antibody that is not specific for the parasite but for other antigens [27]. Such observations do not invalidate the idea that, for the most part, the interaction between B cells and eTh cells requires the operational recognition of linked epitopes.

3. The unlikelihood of B cell involvement in cell-mediated responses: Cohn says 'In general, B cells do not encounter internal cell constituents that are targets for T cells', with the implication they cannot be involved in cell-mediated responses as antigen-specific APC. Cohn again cites no references to substantiate this challenge.

Cohn's comment is similar to one brought up by a reviewer of my first submitted draft that, in revised form [2], led to this discussion. The point made by a reviewer was that cell-mediated responses are often made against internal antigens that B cells are unlikely to have access to. My reply [2] was along the lines that, if B cells have the central role postulated, these antigens must be sufficiently available. I pointed out that the generation of Th1 cells in such responses suggests that internal antigens from cells (other than from APC) must be available for processing by APC and presented if Th1 cells are indeed generated. I do agree it is important to test whether B cells, perhaps in cooperation with other APC, can cross-present, as I would anticipate, in generating CD4 T cell-dependent CTL specific for tumours that do not express class II MHC molecules.

4. Distinct roles of B cells: Cohn states: 'Every B cell acting as APC for step one T helpers via uptake of antigen by its BCR would also be induced to produce antibody when primer eTh arise as a consequence of interaction with them, whether or not the response is appropriate. A problem in regulation is posed'. There are again no cited references.

I think I understand what Cohn means. I think he suggests here and elsewhere that a given cell, in this case a

B cell, *cannot* be given different signals under different circumstances and subsequently land up with different functions. In contrast, I see no reason why a B cell cannot be given one set of signals under one set of circumstances and land up as an APC in step two, involving the generation of a Th1 response, and under other circumstances receive a different set of signals and land up as a B cell that divides and its progeny produce antibody. We know that a B cell can receive different signals under different circumstances to give rise to cells producing different classes of antibody.

(b) Concern over dying cells having a pivotal role. Cohn suggests that the interactions postulated in step two are paradoxical. 'Paradoxical, because the pTh cell and the B cell, both on signal 1 driven pathway to death, are postulated to be required ..... for all Th-Th interactions that are at the nub of immune responsiveness'.

In our 1970, two-signal paper, we envisaged that the generation of signal 1 cannot inactivate B cells over a short period of time. Antigen would certainly interact with B cell receptors, and so Signal 1 would be generated, before eTh cells could engage with the B cell under circumstances where the B cell is eventually activated. We suggested it would take one or two days to cause irreversible B cell inactivation when signal 1 alone was generated. If the generation of signal 1 irreversibly inactivated B cells over minutes, before any B cell/Th cell interaction could take place, B cells would never be induced. Our suggestion that B cell inactivation takes of the order of a day or two is consistent with subsequent findings of, among others, Norman Klinman and colleagues [28]. Similarly, there is no harm in various cells being on the pathway to death and being rescued by events determined by the environment they are in, and performing significant functions, is there?

(c) Implausible signalling pathways. Cohn says, in discussing my suggestion that step one primed CD4 T cells can mutually interact via a B cell to result in the generation of eTh cells: 'It is questionable whether a reasonable signalling pathway ....can be envisaged ...' It would be helpful to have a justification of this statement.

(d) The gratuitous nature of eTh cells. Cohn says: 'It might be pointed out here that a role for eTh needs rationalization as it seems gratuitous in his Two step model. Signal 2 originating from the B-cell in the absence of eTh might have been viewed, in principle, as equivalent'.

The B cell may indeed be able to deliver the critical signal 2, without being currently engaged with the eTh cell, if it has been shortly engaged before and so is 'licensed' to deliver the critical signal 2. However, this is not, I think, what Cohn is driving at. Cohn's comment makes me outline again the reasons for my current proposals. Could a naive B cell deliver the critical signal 2? I go back to the core postulates. Yes, the two core postulates of the 1970 Two Signal Model could in principle allow a formulation where naïve B and naïve T cells interact in an antigen-dependent way to initiate an immune response, by mutually helping each other. Why not then adopt this minimal premise as the axiom for understanding how the immune system functions? The answer is because it is not a unique means of realizing our two core postulates. Other models, consistent with the 1970 framework, take account of further observations and considerations. One consideration is posed by the scarcity problem. I respond to what I understand is Cohn's question by stating: The Two Step, Two Signal Model satisfies the two core postulates of the 1970 framework and is also consistent with a variety of observations, viz: (1) the activation of CD4 T cells is facilitated by CD4 T cell interactions, mediated by the operational recognition of linked epitopes; (2) naïve B cells can present antigen to inactivate Th cells, for example [29], and they are known not to express a variety of costimulatory molecules; (3) B cells, activated in an antigen-dependent manner by eTh cells, express costimulatory molecules, that facilitate the activation of pTh cells. In summary, my response to Cohn is that the proposal he questions is entertained for three reasons: it satisfies the two core postulates, takes account of further considerations such as those related to scarcity and non-interference, and in addition accounts for diverse observations.

(iii) My concerns over Cohn's view of the critical role of eTh cells

Critical to the formulation of the 1970 framework were observations, particularly those of Weigle, on breaking the unresponsive state. Subsequent findings have supported the significance of such studies for understanding the generation of autoimmunity. It seems to me important then to look at such circumstances carefully, to see whether our envisaged mechanisms too readily explain how pathology might arise. I would like to examine Cohn's view on the pivotal role of eTh cells, in controlling responsiveness, in this context.

As previously described [2], the phenomenon of 'epitope spreading', namely that the specificity repertoire of CD4 T cells in responses to antigens in general, but especially evident in autoimmune responses, increases in time, is to be anticipated on the basis of CD4 T cell collaboration in the activation of CD4 T cells. 'Epitope spreading' explains how cross-reacting antigens, for example virally infected self-cells, can induce autoimmunity to peripheral self-antigens expressed by the virally infected, and the corresponding normal, cells. In this case, some eTh cells specific for peripheral antigens are likely to be generated and, according to Cohn's vision that they determine responsiveness, irreversible autoimmunity would be anticipated. I think both observations and these considerations prompt me to go back again to the 1970 framework.

Once an acute, viral infection is cleared, what determines whether the anti-self-CD4 T cell response is sustained? Observations suggest that not only pTh cells,

but eTh populations, can be inactivated, when they interact with antigen under conditions where CD4 T cell cooperation is unlikely to occur, as I recently reviewed [2]. It seems to me that a physiological insight from this fact is that the induction of a few anti-self-eTh cells, upon a temporary exposure to a viral infection, will not be sustained if the viral antigens are effectively cleared and the population of anti-self-CD4 T cells is insufficient, in size, to sustain their own, peripheral self-antigen-mediated propagation, that would require CD4 T cell collaboration. Thus, the autoimmune response would often be curtailed under these circumstances. These considerations are based upon the premise that CD4 T cell collaboration is required for the sustained activation of CD4 T cells, and that, in the absence of such collaboration, even populations of eTh cells peter out on continual exposure to antigen. It seems to me that Cohn's current view would be that once eTh cells, specific for a peripheral self-antigen, have been generated, the peripheral self-antigen will inevitably maintain the further activation of eTh cells. I feel I should point out, for reasons of clarity, that it is not exactly clear, at the cellular level, how the population of cells, expressing eTh activity in the form, for example, of cytokine-producing CD4 T cells, is inactivated. It may be that these eTh cytokine-producing cells are themselves inactivated; more likely, these cells, whose activity is radiation-resistant, may be short-lived, and the generation of further eTh cells, from activated 'stem Th cells', that requires CD4 T cell collaboration, is not sustained under circumstances where CD4 T cell cooperation does not occur.

Responses to substantial comments of reviewers

For clarity and ease of reference, I will denote reviewer's comments I explicitly respond to with a number. I was given comments from three reviewers, Colin Anderson, Melvin Cohn and Alexandre Corthay. I understand from Zlatko Dembic, the Associate Editor, that at least Alexandre Corthay will make more extended comments later, so I will not respond to substantial comments made by Alexandre Corthay at this time. Some comments made by Anderson and Corthay were not intended to be substantial scientifically, but were made with the aim of increasing the clarity of my submitted paper. I am grateful for these comments, which I responded to by amending the text.

Comment from Mel Cohn

Comment 1

I feel that this paper would be strengthened if Bretscher made clear one point. An immunoglobulin negative animal should be unable to induce pTh to eTh given his hypothesis. This seems like a straightforward test of the theory. Among the 30 papers that he refers to, it would take only one that met his criteria of Ig-negativity and inducibility of pTh to eTh to disprove the hypothesis. The disprovability of his hypothesis is its strength.

Response

I take it that all immunologists, including both the reviewers of my manuscript and myself, are concerned by the conflicting evidence on whether B cells are needed to activate naïve CD4 T cells. I will give my thoughts at the end of this response to this predicament, as I regard it as central. However, Cohn's comment contains two statements, beyond this question, with which I disagree. I want to briefly comment on them, even though some might regard the ensuing points as more philosophical than scientific. Perhaps this is what is needed.

Difficult situations are always defined within a context. Cohn suggests that one paper showing that antigen can activate CD4 T cells in a B cell-negative mouse would invalidate my hypothesis. However, this suggestion ignores the complexity of the real situation. I argue that some of the other 30 papers show that B cells are required to activate naïve CD4 T cells. Why, *by the same logic* Cohn uses, would one such report not be regarded as sufficient to support the idea that there *is* an essential role for B cells in the activation of naïve CD4 T cells? The evidence is conflicting, as most of us perceive it, so I would take this as a warning. I would take heed. 'There is more in heaven and earth, Horatio, than is dreamt of in your philosophy'. It seems likely in these circumstances that some of the assumptions we employ in our deductions are wrong. We shall only have earned our rest when we understand the basis of these different observations. In the absence of such an understanding, we have to either give up or, with humility, consider which observations are more likely to represent physiological processes, a point to which I shall later return.

Cohn suggests that 'disprovability' of my hypothesis is its strength. I disagree. It is admittedly a virtue. However, I consider the main strength of this hypothesis is that it provides a solution to the conundrum of how antigen-mediated CD4 T cell cooperation can occur by the operational recognition of linked epitopes. I would require, because of the plausibility and neatness of this possibility, and because some evidence supports it, a very strong balance of evidence against it, to discard it, with regrets. I believe that aesthetics are a valid, but not an overriding, criterion contributing to a judgment on whether or not to entertain a proposition.

Comments from Colin Anderson

Comment 2

The presence of antibody-producing cells and cytokine-producing T cells in unimmunized mice is discussed in a

manner that suggests that Bretscher believes these to be related (interdependent?) phenomena; that is, this B cell antibody production is antigen and T cell help dependent. Such a conclusion would be questionable, as the level of spontaneous serum Ig is only modestly affected by lack of either MHC class II or T cells (e.g. athymic mice; MHC II knockout mice [25]).

Response

I understand Anderson's point. I cannot give a compelling response without a detailed understanding of how the antibody in athymic and MHC II KO mice is produced. I would point out that the level of Ig antibody in these mice may be subject to feedback regulation [26]. Thus, the level of Ig in athymic and class II MHC KO mice may not reflect so much the pathways of Ig production in normal mice, but rather a minor pathway of Ig production controlled by a feedback mechanism, resulting in 'almost normal levels of Ig'. Given that CD4 T cell-dependent antibody responses are almost completely abrogated in athymic and these KO mice, I suggest that the Ig produced in athymic and class II MHC KO mice may not accurately reflect the pathways of Ig production in normal mice. Secondly, though not formally published, I did refer [2] to unpublished observations, employing the ELISPOT assay for detecting single, antigen-specific, cytokine-producing CD4 T cells, that such cells can usually be found specific for diverse antigens in mice not deliberately immunized by immunologists. Thus, whatever their cause, their presence indicates, if they are functional, that there is less of a priming problem in immunocompetent than in neonatal mice, the primary point.

Comment 3

Bretscher limits the priming problem to neonatal mice. As for immunocompetent mice, he states there is no priming problem. Does he not mean much less of a problem? Would there not be some foreign antigens/pathogens yet to be experienced that do not have epitopes in common with those previously experienced? In that case there would be a priming problem.

Response

I agree with the general point made by Anderson. I was keen to make the distinction that the priming problem is *critical* in neonatal and less severe in immunocompetent mice, and so made the argument 'too clean'. Nevertheless, despite this qualification, I would like to bring up some quantitative considerations.

When I looked for the number of antibody-producing cells specific for six different kinds of xenogeneic red blood cells (RBC), in the spleen of mice not deliberately immunized by immunologists, I found that they were readily detectable, with the exception of antibody-producing cells specific for rat RBC. This made sense in that both mice and rats are rodents, whereas the donors of the other xenogeneic RBC employed belonged to other orders of species. Thus, rat RBC are less foreign than these other RBC as 'seen' by mice. It makes sense that the less foreign the antigen is, the lower is the number of antigen-specific eTh cells producing cytokines, and the number of other CD4 T cells. At some point, with antigens that are really minimally foreign, we cannot generate an immune response, even though we know some CD4 T cells exist specific for such antigens, as seen in some strains of mice with the antigen mouse cytochrome C [27], as previously discussed [2]. However, most pathogens are fairly complex chemically and very foreign, so I would expect that there usually are some eTh cells due to fortuitous, small cross-reactions between them and, for example, gut flora.

Comment 4

Bretscher states that Cohn describes the Anderson experiments well. This is mostly correct, however, given the discussion of the potential importance of B cells as APCs, it is important to note Cohn's description is incorrect when he states 'The only sources of PH-Y-RII are the APC in the graft and possibly some male B cells that might have contaminated it and survived the long healing period, but these would express PH-Y-RII, BCR-independently'. In the experiments referred to [18, 28], both the recipient and the skin graft donor were Rag deficient, and therefore, there was no possibility of male B cells contaminating the graft.

Response

I acknowledge the appropriateness of Anderson's comment.

Comment 5

Bretscher's reservations about Cohn's interpretation of rejection of skin grafts given pre-immunocompetence are clear and valid. However, it would be useful to also address Cohn's criticism that such H-Y expressing skin grafts should not be rejected in Bretscher's model not only because the rejection violates the historical postulate but also because B cells appear unable to present H-Y via BCR-mediated uptake. Does Bretscher postulate that in such a system, the female B cells do in fact pick up (via the BCR) H-Y and present it for T cell activation but are themselves unable to be activated to make antibody to H-Y? In order to maintain the position that B cells were necessary for the rejection, one could speculate that H-Y protein is presented by B cells but in a manner that directs exclusively a cell-mediated response. Why H-Y would

induce only cell-mediated immunity would need justification. Our findings, discussed by Cohn and Bretscher, were that male skin grafts ('single minor' mismatch) given pre-immunocompetence were nevertheless rejected [18]. In contrast, pre-immunocompetence internal male grafts (islets or heart) induced tolerance, but if the mismatch was increased to multiple minors even the internal grafts failed to induce tolerance and were mostly rejected [28]. I have drawn two primary conclusions from these studies. Firstly, the rejection of pre-immunocompetence grafts, particularly single minor grafts, is a disproof of the historical postulate (i.e. the postulate that timing of antigen exposure is the central determinant of self-/non-self-discrimination). Neither Cohn nor Bretscher have provided a viable interpretation to the finding of rejection of pre-immunocompetence male skin grafts that would allow the historical postulate to survive. Cohn's interpretation (quantity of antigen) violates his own previously well-reasoned conclusion that 'As classes, self cannot be distinguished from non-self by any physical or chemical property' [29]. Rejection of male skin grafts but not male heart and islets given pre-immunocompetence indicates that it is context, not time, that is central to the immunity/tolerance decision step. The second conclusion I have drawn from the pre-immunocompetence experiments is similar to that proposed by Bretscher. Tolerance of pre-immunocompetence internal single minor but not multiple minor grafts indicates there is a limit to what peripheral tolerance can handle in terms of frequency of responding cells. Bretscher proposes that the increased frequency of cells allows too much cellular collaboration, preventing tolerance and promoting immunity. There has been a recent challenge to the view that these experiments should be interpreted to indicate a generalizable limit on the capacity of peripheral tolerance. Fadi Lakkis and colleagues have provided evidence that cells of the innate immune system can specifically recognize cells with certain multiple minor mismatches [30, 31]. This is a testable alternative explanation for the lack of tolerance of pre-immunocompetence multiple minor grafts.

Response to comment

Comment 5 requires multiple responses.

(a) Firstly, I agree with Anderson's guess of what I would say about the role of B cells in this system: 'female B cells do in fact pick up (via the BCR) H-Y and present it for T cell activation but are themselves unable to be activated to make antibody to H-Y'.

(b) Anderson further asks why would no anti-H-Y antibody be produced? I would suggest H-Y is not sufficiently foreign, and so, according to the Threshold Hypothesis, only Th1 cells are generated and antibody is not produced [32]. I should emphasize in this context that an exclusive Th1 response in mice does not result in IgG_{2a} antibody production, as often suggested, but rather that no IgG antibody is produced [33].

(c) 'Bretscher [has not] provided a viable interpretation to the finding of rejection of pre-immunocompetence male skin grafts that would allow the historical postulate to survive'.

I do admire the thought and care underlying the series of experiments Anderson has outlined [18, 28]. I tried to suggest [2] in my first article that the one lymphocyte/multiple lymphocyte model for the inactivation/activation of lymphocytes, that provides an explanation of peripheral tolerance consistent with the historical postulate, has numerical limitations. I feel it would be helpful if I enlarge here upon what I meant. If one accepts there are limitations to the applicability of the one lymphocyte/multiple lymphocyte model in extreme experimental situations, but it holds under normal physiological conditions, then it seems to me one can in this sense 'rescue the model and the historical postulate' from observations that seem inconsistent with it. For example, if 'single' lymphocytes are generated much more rapidly than normal, then they might not be regarded as single anymore. I would suggest this is likely what happens in individuals with AIRE mutations, such that many 'peripheral' antigens are no longer expressed in the thymus and so the corresponding specific CD4 T cells are not even partially eliminated by central tolerance [34]. The T cells specific for peripheral self-antigens are then generated and exit the thymus in this case at such a rate that they can no longer be 'inactivated as they are generated one, or a few, at a time'. I suggest this finding of peripheral autoimmunity does not show the Historical Postulate to be invalid, but rather quantitative limitations on the validity of the framework, envisaged to operate under normal physiological conditions. I will argue along similar lines that the studies by Anderson and colleagues may not necessarily demonstrate the invalidity of the Historical Postulate, as Anderson suggests.

Anderson says: 'Rejection of male skin grafts but not male heart and islets given pre-immunocompetence indicates that it is *context, not time* [my emphasis], that is central to the immunity/tolerance decision step'. I would like to outline a different view that acknowledges that context can have a significant, but not the critical, role sometimes accorded to it.

When I first came across what I call the first Anderson paper [18], I considered the idea that the extreme lymphopenic environment existing upon initial reconstitution with stem cells might favour both rapid generation of lymphocytes and somehow lead to a readjustment of the threshold, required for lymphocyte activation through lymphocyte cooperation, so that immunity could be generated against foreign insults, despite the extreme sparsity of lymphocytes, as perhaps occurs in a newborn mouse. However, I could not convince myself of the plausibility of this possibility, as H-Y is not a strong antigen, and I accepted

there was something here that I simply could not understand. However, subsequent experiments from Anderson's laboratory [28], changed my stance somewhat.

This second Anderson paper [28], discussed by Anderson in comment 5, and published in a not very prominent journal, came to my attention through a personal discussion with Anderson. The critical point for me was that foreign tissue containing only H-Y as a foreign antigen, grafted at internal sites of female mice, could induce tolerance, but that H-Y tissue with an additional foreign minor histocompatibility antigen, could not. I agree with Anderson that these observations probably demonstrate the importance of lymphocyte numbers specific for the graft in determining whether or not tolerance is induced. I would tentatively suggest these observations are consistent with the one lymphocyte/multiple lymphocyte model for the inactivation/activation of lymphocytes and the Historical Postulate. I put this proposal forward as an idea for accommodating the incisive observations made in these interesting systems with the views I favour on general grounds. Lack of tolerance against H-Y grafts, bearing in addition a foreign minor histocompatibility antigen, supports our model. This attempt to account for these observations does not readily explain why such a weak antigen as H-Y is immunogenic when the mice receive a male *skin* graft. It is possible that in these limiting circumstances, 'danger', such as that arising from skin flora, plays some role. I have and never have had anything against PAMPs/'danger' sometimes playing a role, and in certain limiting cases, such as this, a critical role. Indeed, I have always thought PAMPs and danger have roles, but what I think implausible is that PAMPs/'danger' has an obligatory, and therefore generally pivotal, role in determining whether antigen activates or inactivates naïve CD4 T cells, the positions developed by Janeway [35] and Matzinger [36]. I have to finally add that my take on the observations made in this system is affected in part by the fact that this system is a pretty extreme experimental model, rather far removed from normal physiology, so that we might anticipate that rather unanticipated observations may be made!

(d) Anderson brings up the interesting observations of Lakkis and colleagues [30,31]. My thoughts on these reports mirror those just made under (c). It appears innate mechanisms can in some instances influence immunogenicity. I accept the interpretation of the authors. The statement that recognition of antigen by innate mechanisms of defence can in particular cases influence immunogenicity is different from the statement that recognition of antigen by innate defence mechanisms is always required to activate CD4 T cells.

Comment 6

While the arguments Bretscher provides for B cells as a central APC in CD4 responses are well reasoned, his postulate that B cells cross-present antigen for CTL responses needs further consideration. Cross-presentation by B cells taking up Ag via the BCR would make them targets for CTL killing, an outcome lethal for the host if the B cell is specific for a deadly virus. Why not restrict the role of B cells as APCs to CD4 responses? It seems an error to extend their role to induction of class I restricted responses.

Response

Cytotoxic T cells are deadly effector cells, important in containing many viral infections and cancer, and in inflicting autoimmune damage in, for example, autoimmune diabetes. It is important that their generation be exquisitely controlled.

I have argued that the facilitation of the specific activation of both B cells and pTh cells by antigen and CD4 T effector helper cells requires the operational recognition of linked epitopes in the pertinent B cell/CD4 T cell and the pTh cell/CD4 T cell interactions. I suggest this is essential to minimize the induction of autoantibodies and autoimmune CD4 T cells. I think there is a similar requirement, for the operational recognition of linked epitopes, in the facilitation by CD4 T cells of the activation of CD8 pCTL cells. Without such a requirement, effector CD4 T cells specific for a foreign antigen may facilitate the activation pCTL lymphocytes specific for a peripheral self-antigen that does not cross-react with the foreign antigen, resulting in the too ready generation of autoimmune CTL.

There is classical evidence for the operational recognition of linked epitopes in the priming of CD8 T cells [37]. I cannot see how such operational recognition can be reliably achieved without there being a critical step in the activation of CD8 T cells involving B cell cell-mediated cooperation with CD4 T cells. I also recognize the basis of Anderson's concern. Might there be some additional factor that allows precursor CTL (pCTL) to interact with B cells during their activation, without the B cells being in effect susceptible to the CTL generated? Could this perhaps be achieved by the chemokine gradients present and the differential expression by different cell types of chemokine receptors, making it difficult for effector CTL to be in the right place to attack antigen-presenting B cells, that is at the sites where the precursors CTL are activated? It seems to me this proposal is relatively readily susceptible to experimental tests.

Comment 7

General comment: in a number of places, Cohn assumes that Bretscher is postulating that B cells only present antigen taken in via the BCR; a clear response to this assertion is needed. I would anticipate that Bretscher does

not in fact propose that only BCR-attached antigens are presented and that instead antigens made internally in the B cell are also presented on MHC class II (e.g. H-Y in male B cells). Central tolerance of CD4 cells specific to B cell intrinsic antigens would prevent the generation of eTh to these antigens. Bretscher makes a similar argument to explain tolerance for peripheral self-antigens taken in via the BCR and presented by B cells (pS-B). It would be important to state in his model that this central tolerance extends to B cell intrinsic antigens. If antigen-specific cellular collaboration (T cell help) were to be the central mechanism of self-/non-self-discrimination, I agree it would be extremely appealing to have the APC be an antigen-specific B cell, in order to increase specificity in the decision, as Bretscher proposes. The alternative view I have favoured is that cellular collaboration and the role of the B cell as APC is central to immune class control rather than self-/non-self-discrimination in T cells. This view does not exclude an important contribution from antigen-specific cellular collaboration in promoting and reinforcing a proper self-/non-self-discrimination established by the primary context-dependent mechanisms.

Response

Anderson's interpretation of my position is almost correct. I defined pS-B as a B cell-specific antigen, and so intrinsic to B cells, not as a peripheral antigen from other sources taken up by the BCR. I would just point out that antigens intrinsic to the B cell can be classified into two extreme types: those presented by the B cell and those not presented. Those CD4 T cells specific for 'presented' antigens would, by the mechanism I propose, be eliminated in the thymus by central tolerance by the presence of B cells in the thymus. Those not presented would not normally cause a problem.

A response on the role of B cells in CD4 T cell activation

We all seek to find rules that are generally applicable in all (or most) situations. This means that, if we are to have consistency within a proposed framework, all the pertinent observations must be correctly inferred, otherwise we may have apparent and not real inconsistencies. I have to say again, in this context, that if most observations are consistent with a framework, and the framework is otherwise attractive in that it makes physiological sense, I am inclined to be sceptical of observations that are inconsistent with the framework: either in the correctness of the observations, or in the way they are interpreted. I do not anticipate total consistency, as no description we have is anywhere near complete, and so I look for inconsistencies as a starting point for further considerations.

I have tried to express my thoughts and position on the contradictory evidence bearing on the role of B cells in the activation of CD4 T cells. I would summarize by saying that, given apparently contradictory evidence, I would trust observations made in experimental systems that are minimally altered from normal physiology. Given Cohn's challenge that one observation, apparently inconsistent with a model means the model must be wrong, I would like to *illustrate*, in a gedanken manner, why I disagree. Cohn cited various studies, employing B cell KO mice, demonstrating that CD4 T cells can be activated by antigen in the absence of B cells, thereby ruling out my proposed model. Consider what we surmise happens from the studies of Jenkins and colleagues [38, 39] when naïve CD T cells are activated. These T cells first interact with antigen presented by a non-B cell APC in a T cell area of the lymph node and, after a couple of days, the partially activated CD4 T cells express chemokine receptors on their surface and, responding to a chemokine gradient, move to the B cell/T cell boundary, where they now interact with antigen-specific B cells. If antigen-specific B cells are not there, I suggest the CD4 T cell is not further stimulated and dies. In this particular envisaged scenario, the B cell does not have to give any different signals to the step one activated CD4 T cells than would a non-B cell APC; the B cell's presence is just required for the full activation of the CD4 T cell in order to obtain the required *sustained* stimulation of the CD4 T cells. It is known that B cells can affect the development of lymphoid architecture [40]. Suppose, for these reasons of altered architecture that occurs in some B cell KO mice, there is *sustained* activation of CD4 T cells by professional, non-B cell APC. In this envisaged scenario, CD4 T cell activation would take place in these B cell KO mice, and yet, in normal mice, there would be a requirement for antigen-specific B cells to fully activate CD4 T cells. I do not for a moment think the proposal I have just put forward is correct. It is perhaps useful, however, in illustrating how dangerous it is to be adamant that one piece of evidence, taken in isolation, is indisputable.

Postscript

I am very grateful to have had the opportunity to respond in this way to important conceptual issues. I thank Mel Cohn for his response and comment, and Colin Anderson and Alexandre Corthay for their comments.

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