The Tolerance Workshop

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SESSION 8: MECHANISMS OF TOLERANCE - PART II
HOW DO THE CELLS REACT TO TOLEROGEN?

2) Is T cell help involved in establishment of tolerance?
Theory and experiments

MEL COHN: We have a very interesting program with six speakers. Let's begin with our first one, Dr. De Boer.

ROB DE BOER: My intention is not to speak about tolerance at first. I will simply consider the normal T lymphocyte proliferation process. I will show that in this proliferation model, which was not designed to account for tolerance, clonal anergy develops in some circumstances. The most essential substance which drives T lymphocyte proliferation is IL-2. If we make a very simple formula, which says that IL-2 is produced by helper T cells that see antigen, we have a model of the IL-2 production process. It's sensible to assume that there is some saturation function that limits the production of IL-2 by helper T lymphocytes (HTL).

$$\text{IL-2 production} = \frac{HTL \cdot AG}{K + AG}$$

If we plot antigen and the IL-2 production of single helper T cells, the function looks like this (Figure 1).

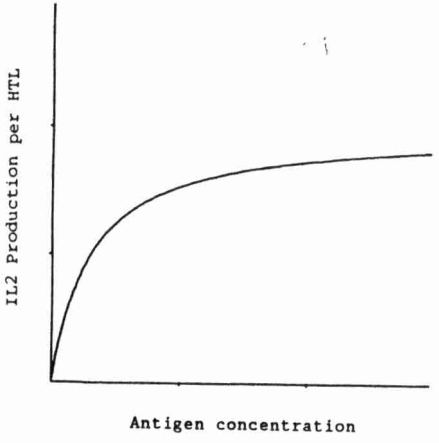

Figure 1

This is a simple Michaelis-Menten function. Now we have a model of IL-2 production, we can make a model of the response of helper T cells to IL-2. That's easy again. One can say that the proliferation rate is a function of IL-2, and again let's assume that this is a Michaelis-Menten function.

$$\text{HTL proliferation} = \frac{\text{IL-2}}{\text{KI} + \text{IL-2}}$$

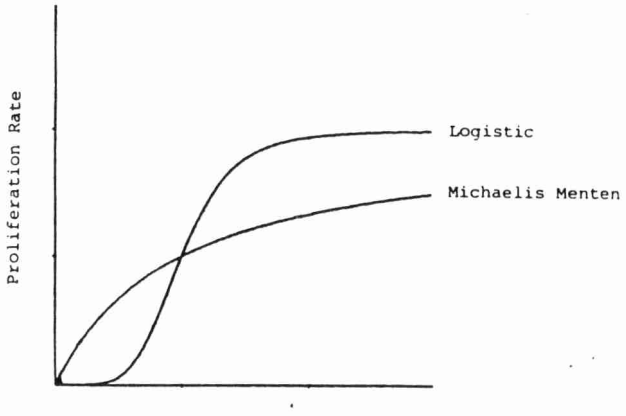

Figure 2

This function was actually described in the literature to be logistic (S-shaped, Figure 2). The parameters reported for the slope are, however, very close to a straight increase. So I'll just take the most simple model and assume that the dose response curve looks like the Michaelis-Menten function. If you incorporate both IL-2 and the response to IL-2, we can make a model of the helper T cell proliferation process. The differential equation, specifies the changes of the helper T cells in time.

$$\frac{dHTL}{dt} = p \cdot \frac{HTL \cdot IL\text{-}2}{KI + IL\text{-}2} - D \cdot HTL$$

Helper T cells proliferate if they see IL-2, so there is a proliferation constant (P), times the number of helper T cells, times the IL-2 function, which is the same as above. Moreover, we use the above estimate of the IL-2 concentration. Helper T cells do not only proliferate, they also have a turnover rate, so I have to incorporate a decay term. This is the simple D•HTL term in the equation. It is a very simple model because it omits all things not necessary for showing you the results. I can make this model

far more realistic and have exactly the same results. So please do not ignore this as being too simple; I can talk about a more realistic model but then it's very difficult to get the message across, so I've made it as simple as possible.

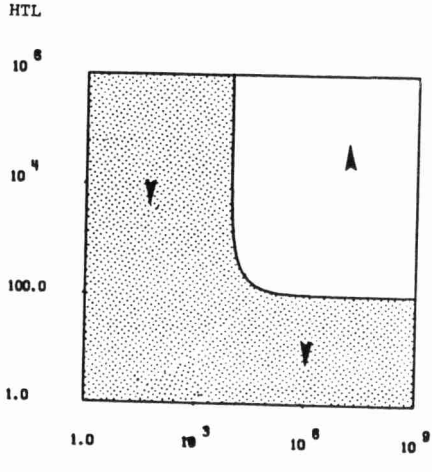

Figure 3

If we examine the formula graphically (Figure 3), an interesting thing happens: we plot what happens for a number of different antigen concentrations and a number of different helper T cell populations. It's clear from the formula that, if there is a lot of antigen and many helper T cells, then a lot of IL-2 is produced, and the proliferation term will be large, i.e. there will be a net increase of helper T cells. So if antigen is in high amounts and there are many helper T cells, we get proliferation (the upward arrow). But if the proliferation term is small due to a limited production of IL-2, because antigen concentrations are low and/or helper T cell numbers are low, the proliferation term may be smaller than the decay term, so there is the decrease of the helper T cell population (indicated by the shaded region in Figure 3). If there is almost no antigen and many helper T cells, there is a decrease in helper T cell populations. The most interesting case is when there are only a few helper T cells and a lot of antigen, which leads to a decrease of the helper T lymphocyte population. In between there is a line in which the helper T cell population remains constant, we have called this line the proliferation threshold. Thus, although we are considering an autocrine process, i.e. a self reinforcing process, this figure shows that the process has difficulties in starting up. The helper T lymphocytes first have to cross the proliferation threshold before proliferation can continue as an autonomous process. The interesting thing is that we have omitted from the model all sorts of downregulatory processes, e.g. suppressor T cells. The helper T cells simply follow the above dose response curves, but nevertheless we find

conditions in which the helper T cells fail to proliferate. So I would conclude that we can have large helper T cell populations in that high antigen concentration zone which fail to proliferate: such populations are anergic. This situation is a form of T cell tolerance without any form of downregulation. I agree this is counterintuitive and difficult to understand, but my argument is that it simply follows from this simple differential equation, which, in turn, simply follows from the two dose response curves.

The model is, however, a bit too simple because there is no way that these helper T cells can ever start to proliferate, unless they already begin somewhere in the region in which you have a lot of helper cells and a lot of antigen. What we have omitted from the model, however, is the influx of precursor cells. We assume a constant influx (I) from the thymus. Such cells may become activated if they see antigen:

$$\frac{dHTL}{dt} = I \cdot \frac{AG}{K + AG} + P \cdot \frac{HTL \cdot IL\text{-}2}{KI + IL\text{-}2} - D \cdot HTL$$

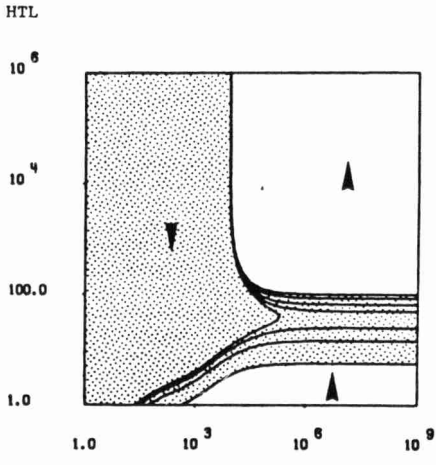

Figure 4

If we incorporate influx (Figure 4) there is a zone at the bottom in which the helper T cells increase again. This means that if a sufficient amount of antigen is present, precursors arriving from the thymus become activated, generate helper T cells, and there is a small zone at low cell numbers where helper cells increase. If we increase the influx paramater, that means if we assume that more precursors are arriving from the thymus, we have a larger clone, this line rises and eventually they coincide, and we get the following picture (Figure 5). Here we see that a helper T cell population can only decrease if the antigen concentration is low. If the

antigen concentration is high enough, proliferation always occurs.

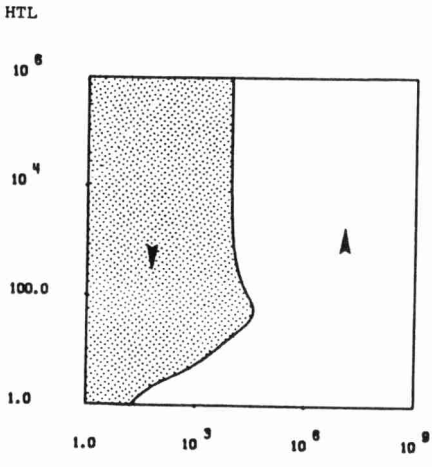

Figure 5

My conclusion from this part of the talk, which was the definition of the proliferation threshold, is that although the helper T cell proliferation process is an autocrine process, there is a difficulty in starting up, which may account for a stable tolerance state. In addition we have shown that the influx of precursor cells may determine whether proliferation begins. There must be a sufficient influx of precursors to overcome the proliferation threshold, and proliferation starts. So let us incorporate an additional population in this model: memory T cells. Until now I have only considered the active cells which produce IL-2. There are, of course, resting helper T cells, and it's clear that if resting helper T cells are activated you get an enormous influx of precursors which may influence the position of the proliferation threshold.

We obtain a model like this: a helper T cell produces IL-2 if it sees antigen, and, secondly, if that helper T cell sees IL-2 it generates a new helper T cell. There is some influx of virgin precursors, which, if they see antigen, become activated cells. A helper T cell may return to a resting state, I refer to it as a memory (MEM) cell, but it could just be in a resting state. The only assumption is that these memory cells are long lived. An activated cell returning to the memory stage fails to see antigen, that is if the restimulation of helper T cells is sufficiently low. A memory cell is reactivated if it (again) sees antigen. If we also incorporate an equation for the antigen we get a complete system consisting of helper cells and memory cells (Figure 6). The complete model of differential equations describing this system will soon be published in the J. Theoretical Biol.

Now that we have an immune system we can study an immune response, just by introducing, for instance, a large dose of antigen (Figure 7A). On a time scale we see that virgin precursors are activated and produce helper T cells. No memory cells will be formed because all helper cells are highly restimulated by antigen. This only yields a population of helper T cells. If the influx of virgin precursors is sufficiently low, the proliferation threshold will inhibit the initiation of proliferation. So we get an accumulation of helper T cells, but proliferation will fail to occur. We conclude that this system is tolerant. If we now consider a low antigen dose (Figure 7B), suppose it's a replicating antigen, you get activation of virgin precursors that generate helper T cells. But because the antigen concentration is still low, those active helper T lymphocytes will return to the resting stage. So we have an accumulation of memory cells, as long as the antigen conconcentration is sufficiently low. Because the memory cells live longer than activated cells, the clone increases. There is a transition in life time from short-lived cells to long-lived cells which means that the average turnover time of the cells decreases and that the clone size must increase. In conclusion, we get a larger memory population than the original population of effector cells we had in the previous system. Once the antigen concentration is large enough for reactivating the memory cells, the memory population will decline and give rise to activated cells (Figure 7B). If the number of activated cells is larger than the proliferation threshold, and that may well be the case because we are at higher levels here, proliferation initiates, and those helper T cells will expand exponentially. The effectors will decline because they all return to the memory stage. We have seen that the antigen, introduced in a low dose may initiate an immune response, whereas the same antigen introduced in a large dose will initiate a form of high zone tolerance.

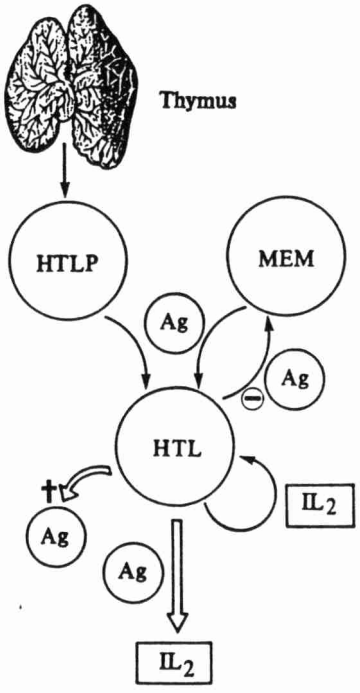

Figure 6

High Antigen Dose

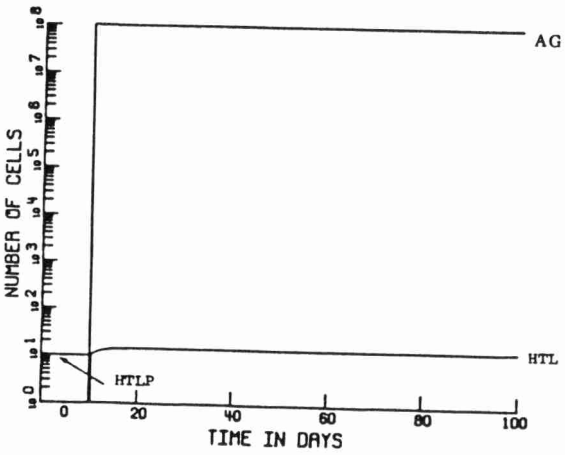

Figure 7A

Low Antigen Dose

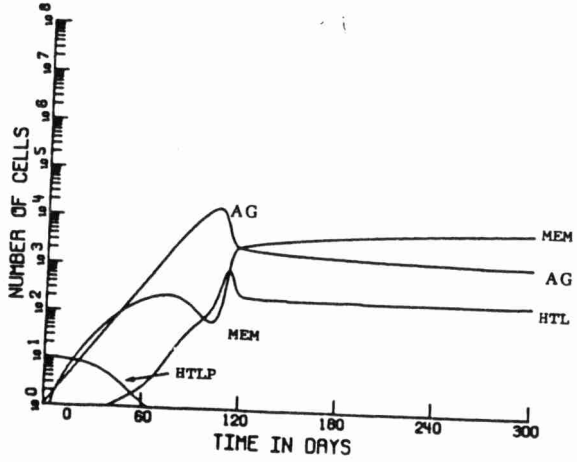

Figure 7B

To influence the initial population size we needed the accumulation of memory cells. The model assumes that memory cells accumulate if stimulatory conditions are poor. When antigen concentrations are low, stimulatory conditions are indeed poor and memory cells accumulate. Stimulatory conditions are also poor if the affinity of the T cell clone for the antigen is low. So if we make a plot of affinity against the number of memory cells that accumulate we get an optimum curve (Figure 8).

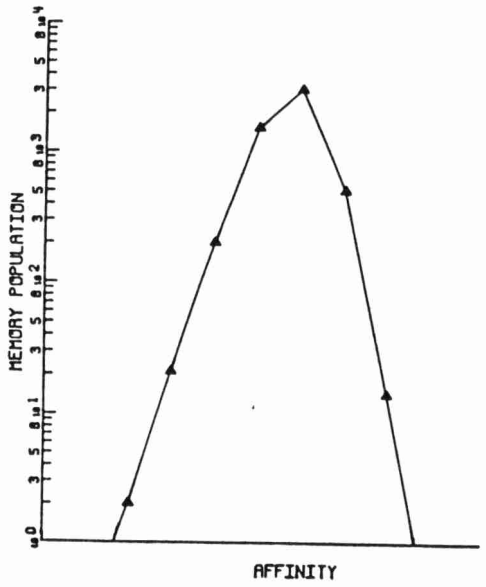

Figure 8

If affinity is high, effectors won't return to their resting states and no memory cells are produced. If affinity is too low, virgin precursors will not get activated and you again get no memory cells. Thus, if we consider the repertoire of T cells during, for instance, the neonatal development phase, this optimum function predicts that those clones that have intermediate affinity to self antigens will accumulate as memory cells. Those clones will expand if you compare them with clones that have high affinity to self antigens, or have low affinity to self antigens. You thus get a shift in the repertoire and, importantly, only clones with intermediate self affinity can reach a clone size large enough for initiating proliferation. Only clones with intermediate affinity to self thus become responsive. Thus we have self/nonself discrimination. The clones that recognize self with high affinity are in anergy because they don't cross the proliferation threshold. Clones with low affinity to self never get activated.

The main problem with this process is its sensitivity to distant IL-2 production: if there is an infection going on somewhere in the body, you get IL-2 production which will be able to push every clone over the proliferation threshold and that will give rise to autoimmunity. Thus, for this system to work, we have to assume that the communication between clones at the IL-2 level is poor and the justification is of course the rapid turnover time of IL-2 in the blood. My model assumes that IL-2 remains confined to the local site of production.

I would like to close with an interesting point considering APC which were omitted from the model (for clarity). APC and IL-1 production increase the expression of the IL-2 receptors on the responding T cell. The number of receptors on the responding T cell determines the IL-2 concentration which is sufficient for initiating proliferation, thus the magnitude of antigen presentation will determine the height of the proliferation threshold and hence will determine whether a clone will become responsive or will remain tolerant.

IVAN LEFKOVITS: I liked the presentation, I liked the mathematics, but I have to be quite cynical about the overall impression it made on me, because every few years there's a mathematical model, which, based on different parameters, tries to explain the whole system. I don't think that we have all the necessary parameters to describe the immune system. If we had them all, we would be able to make a model, but it would not be a simple model.

MEL COHN: In other words, Ivan, you're against any attempt to formulate a quantitative model!

IVAN LEFKOVITS: No, I'm not against a quantitative model, but explaining the system piece by piece, to expand our horizon, and not to narrow it down by oversimplification.

ROB DE BOER: You say do it piece by piece. My answer is that I just made a model of the IL-2 proliferation process and the only data I had to incorporate were the two dose response curves, and I got the tolerance as a present and I think that's interesting. Nobody realises that an intrinsic element of the self reinforcing proliferation process may be a nonresponsive state and I think that's an important conclusion.

HANS-GEORG RAMMENSEE: When T cells from a normal mouse, strain A, are negatively selected for B reactive cells and transferred to a mouse of strain B, which is irradiated and thymectomized, would you expect an accumulation of memory cells specific for the original A antigen? Would you predict a response to A antigen from these A T cells after immunization with A antigen? The question is whether a normal A mouse contains self reactive T helper cells at a low number and whether you get accumulation of memory cells upon removal of the antigen.

ROB DE BOER: No, you don't get accumulation of memory cells because you need stimulation of the virgin precursors to get accumulation of memory cells. The influx of virgin precursors was eliminated from your experiment by thymectomizing the animal.

HANS-GEORG RAMMENSEE: If you don't accumulate the memory cells but still come with the antigen at a moderate concentration, do you then get the response against self?

ROB DE BOER: If the self antigen itself is removed?

HANS-GEORG RAMMENSEE: Yes, first you remove the self antigen and later you come back with an optimal concentration of self antigen.

ROB DE BOER: Well, that's a normal immune reaction, there is no self antigen, and you introduce a new antigen which used to be a self antigen.

HANS-GEORG RAMMENSEE: Did you do that experiment?

ROB DE BOER: I don't do experiments. And it is not clear what to predict from your experiment. A number of variables are changed; it will depend on the number of transferred cells and on the thymectomy.

POLLY MATZINGER: I know of an experiment that has been done and I would like Rob to make a prediction. You do an adoptive transfer A into A, titrating the number of T cells. You either transfer a lot of T cells or a medium amount of T cells, or a small amount of T cells, and then you put in a large amount of antigen.

ROB DE BOER: I predict it would depend on the titration.

POLLY MATZINGER: Let us say you find that if you put in a small number of T cells and a large amount of antigen, you don't get a response. (That's one of the things that you would predict.) What would you predict from that experiment if you put in that same small amount of T cells, and dropped the amount of antigen?

MEL COHN: You're reducing the level of both the precursor and the antigen?

POLLY MATZINGER: Yes, with a small number of T cells and a large amount of antigen, you don't get a response.

ROB DE BOER: But you still need an influx from the thymus to get accumulation of memory cells.

POLLY MATZINGER: You have memory cells. In this case you're transferring memory cells.

ROB DE BOER: Yes, but they don't accumulate if there's no influx. If you reduce the antigen dose, it simply means that you activate fewer memory cells, you get no increase of the clone. The accumulation of memory cells depends on virgin precursors that are activated once in a while, because there is a low antigen concentration. Such a precursor becomes a helper T cell which is not restimulated because the antigen concentration is not high enough. So that cell will return to the resting state, which means that you transform virgin precursors, via an activated cell, into a long lived memory cell. And that means that the clone increases.

POLLY MATZINGER: Fine, you've got memory cells. This experiment's been done (by Jonathan Howard) so I want to know what you're going to predict and then I'll tell you the answer. You've transferred memory cells. They've been primed and you've allowed them to go into memory. When you transfer a certain small number of those memory cells and give a lot of antigen, you don't get a response. Now it seems to me that what you're telling us here is that, if you have that same influx of T cells (whether it's virgin or memory) and you drop the antigen dose, that you should then get a response. Is that correct?

ROB DE BOER: I'm not sure about one point. Is there still influx from the thymus or do you only have those transferred memory cells?

POLLY MATZINGER: There probably is influx from the thymus, but maybe not in the 7 days that you do the experiment. Whether this influx from the thymus occurs or not, it will be the same for all the experimental groups. Now you either put in a very large dose of antigen, or a smaller dose of antigen. With a large dose you get no response and when you put in a small dose of antigen you get a response. If you increase the number of T cells you put in, then you get a response with the large dose of antigen as well.

ROB DE BOER: Yes, that's the same as the picture I have here.

POLLY MATZINGER: Right! That's why I was asking you to predict the result of this experiment. You didn't want to do that. Anyway that's the result. It fits your model.

ROB DE BOER: But it's exactly the same as the picture here, it's the low dose vs. the high dose.

POLLY MATZINGER: What I told you about was an experiment. What you have is a theory. I'm just giving you an experiment that fits.

LAURIE SMITH: I'm not sure if I understand either of the two experiments that were just talked about. Just to talk about the model, unless I've completely misunderstood it, what your model is attempting to explain, is why certain T cells will proliferate in the course of a response to antigen, depending on how much antigen there is, etc. and also predict certain things about which cells will become memory cells, and that talks about primary responses and also which clones will proliferate in a secondary response, but it seems to me that what it doesn't even address is the problem of why exposure to antigen at one time renders cells, which would otherwise respond to antigen, nonresponsive. What has that got to do with memory cells?

ROB DE BOER: The essence of the proliferation threshold is that the initial size of the clone determines whether it will be responsive or not.

LAURIE SMITH: What needs to be explained is how initial exposure to antigen changes the cells in such a way that at a later time when they otherwise would respond they now don't respond because they're "tolerant".

ROB DE BOER: They don't respond because they fail to produce enough IL-2 to outweigh their own decay rate. The cells themselves do not change.

MEL COHN: If you put in large amounts of antigen, the antigen responsive cell just decays. That's his point.

ROB DE BOER: There's always a balance between the turnover rate and the rate of proliferation, and the rate of proliferation depends on the IL-2 concentration, and the turnover rate is a constant. So if I drop the IL-2 concentration the clone will decrease.

HERMAN WALDMANN: I think the problem people are having is trying to work out how the clone size should get less than you started with, rather than to remain the same.

ROB DE BOER: It doesn't get less than what you started with.

MEL COHN: Is that what you're asking? How it decays?

HERMAN WALDMANN: If it's a model for tolerance then you should be able to come in with a high antigen dose and meet some of the requirements, so the first exposure must do something to inactivate the clone. It can't just be leaving it at the same number.

ROB DE BOER: What happens is that antigen is available in a large dose when the clone starts to influx from the thymus, so you start with a very small clone, there is no accumulation of memory cells, and the population remains at a low level just because proliferation doesn't start up.

MEL COHN: The point is that this is a kinetic model, and what it's showing is that you need very simple assumptions to generate something which looks like the immune system response. Whether it has reality is another question. You don't have to make complicated suppressor circuits or anything.

MARK SHLOMCHIK: How did you decide what decay rate to use? It seems like the whole thing is very dependent on a decay rate. If you have a decay rate of zero, then the clone size never can get smaller...this takeoff phenomenon, isn't it very dependent on the decay rate? How did you figure out what decay rate to use?

ROB DE BOER: The data show that activated cells have a high turnover rate.

MARK SHLOMCHIK: What data show a turnover rate?

ROB DE BOER: As an activated cell, for instance,

MARK SHLOMCHIK: What experiments were they?

ROB DE BOER: Remove antigen and you always see the effector level dropping at a high speed. You need a high turnover rate for that.

MEL COHN: In this model if the effector returned to the antigen responsive state, that would be equivalent to a decay. There would be no more effectors. What we haven't done, we haven't discussed the pathway of induction of T cells, and this is a suggestion for the beginning of a discussion of the induction pathway.

ROB DE BOER: You're right, a proliferation threshold always exists, the point is only at which level. There must be a decay rate for helper T cells, so there's always an IL-2 concentration that generates a proliferation rate smaller than the decay rate. That's evidently true. The point is, if the proliferation threshold lies at the single cell level, then it's of no significance.

MARK SHLOMCHIK: But single cells could never get started there?

ROB DE BOER: No, that's a prediction from the model. You can do experimental tests with single cells in physiological conditions and this model would predict that a single cell needs additional IL-2 to initiate proliferation.

MARK SHLOMCHIK: But with small numbers of cells the threshold level would be extremely dependent on what you thought the decay rate was? Not single cells, but can two cells get started?

ROB DE BOER: Yes, in this model you need about 50 cells to get started. But that, indeed, is an arbitrary value.

MARK SHLOMCHIK: Because it depends a lot on the decay rate?

ROB DE BOER: Yes, those 50 cells depend on that decay rate, so if I change the decay rate, I get another level.

CHARLIE JANEWAY: At a meeting some of us were at recently which dealt with questions like this, one of the points made which I thought was a good one, was that mathematical models are far better at explaining what were called local rules; you can make a nice mathematical description for one well-characterized cell which undergoes activation, proliferation, and so on. But when you try to iterate that individual cell into a multifaceted system with multiple different states of activation and so on, by iterating these local rules it gets very difficult very quickly. The question I have is this: starting with a local rule that is based on in vitro results, because I think in vitro you can model things quite nicely with mathematics, are you trying to extrapolate from that to a global picture? Then it gets very difficult.

MEL COHN: But it shouldn't be impossible.

BRUCE ROSER: This is a local prejudice, this is an immunologist's prejudice really hanging out in public. We all think the immune system is fantastically complicated because we don't understand it, and I think if you would talk to a physicist about mathematical modelling of physical processes they could well say the same thing if they didn't understand enough mathematics, but the mathematicans who do understand the mathematics can model physical processes and model them accurately. I think we've all got a terrible prejudice that these sort of simple mathematical models are just intuitively too simple to explain something as fiendishly complicated as the immune system, but I believe we're wrong. I believe it's all going to turn out to be a lot simpler than you think it is.

MEL COHN: I don't think it's important whether it's wrong, I'm not defending it, and I don't think the question lies there. The question is whether this model poses the questions that you have to ask to understand the observed working of the system. We have no other way of asking those questions, except to say that if a T cell divides and requires interleukin, given a certain affinity for the IL-2 receptor and a certain number of IL-2 receptor molecules on the surface, at what rate would it be expected to divide and what would control that rate? Then we can say, well that isn't enough, as a matter of fact you need something else. That's the only reason for presenting this model.

BRUCE ROSER: This mathematical model is grotesquely simple and yet it predicts things that otherwise are intuitively not obvious. That's the important point.

ROB DE BOER: Yes, you can get things you wouldn't expect.

BRUCE ROSER: But which are true, nevertheless. We've already heard about at least two or three of the things here that aren't intuitively obvious.

CHARLIE JANEWAY: But can you ever test them?

POLLY MATZINGER: We've already discussed one test which you didn't know about. One of the important things about a model is what kind of tests you would like us to do to see if your model fits. Okay, your model fits with what has been done in the past. Well, that's easy enough to do. How about asking us to do something to see whether it would fit with your model.

ROB DE BOER: To try and get a single helper T cell in a physiological condition and see whether it can proliferate.

POLLY MATZINGER: How about something we can actually do?

CHARLIE JANEWAY: I don't know what you call "physiological condition", I have a cloned helper T cell (but that's not physiological). This cell, you put one in a well, by itself, and IL-1, and anti-receptor antibody, and it grows, all of them grow, but you only have one cell sitting there. So one cell, which may not be a typical cell, can start itself, and it can proliferate for a good long time with just those ingredients.

ROB DE BOER: Just the initiation, if it starts?

POLLY MATZINGER: But that's nothing terribly physiological.

CHARLIE JANEWAY: Yes, that's the question, I don't know what he means by physiological.

RON SCHWARTZ: But a resting cell couldn't do it.

NICK COHEN: I think what one would define as physiological is an impossibility to set up. For example, lymphoid organs containing lymphocytes are well innervated by the sympathetic nervous system. Now how are you going to model that?

ROB DE BOER: What you could do (I'm not an experimentalist so perhaps my suggestions are mad) is take a very weak antigen for which you expect that there are only a few helper T cells distributed in the periphery. If you take a weak antigen to which you cannot induce an immune response then you can see if adding IL-2 assists them. If you can induce an immune response in those circumstances, you would show that IL-2 was the limiting factor in that system. My conclusion would be that there is such a thing as a proliferation threshhold.

NICK COHEN: So within the artificial construct that immunologists use you could find something that's physiological? You're talking about an in vitro model; it's not physiological. The immune system exists within an organism; in response to antigen, lymphocytes make a variety of factors which are not only the interleukins. They produce factors which are "received" by the brain. This, in turn, modulates the neuroendocrine system. The complexity of physiological reactions should be a prevading theme when we think about in vivo tolerance.